A review of factors influencing the implantation of euploid blastocysts after in vitro fertilization

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This is a comprehensive review of the current literature addressing factors that are involved in the successful implantation of euploid blastocysts. It includes a comprehensive analysis of published studies on laboratory factors that may impact the performance of euploid blastocysts, including morphology, day of biopsy, and rebiopsy. Preexisting underlying patient factors that may impact the outcome of the embryo transfer (ET) of euploid blastocysts are also explored, including body mass index and endocrine abnormalities. The role of the uterine environment and its potential impact on the successful implantation of euploid blastocysts are reviewed, including endometrial thickness and pattern, uterine architecture, and adenomyosis. Finally, published studies on the stimulation environment and methods of endometrial preparation for frozen ET are analyzed. Euploid embryos appear to have similar outcomes regardless of maternal age (up to the age of approximately 40 years), frozen-thawed ET protocol (in ovulatory women), stimulation or trigger type, or culture conditions. Decreased implantation rates may be expected from euploid blastocysts with poorer morphology, those biopsied on day 7, those twice biopsied/frozen, and those after a difficult ET. Patients with very advanced age, high body mass index, adenomyosis, polycystic ovary syndrome, and possibly methylenetetrahydrofolate reductase carriers may be at higher risk of euploid implantation failure or early pregnancy loss. This analysis only applies to those who are able to produce euploid blastocysts. There is a lack of evidence to support any interventions that subsequently increase implantation. (Fertil Steril Rev® 2022;3:105–20. ©2022 by American Society for Reproductive Medicine.)

Key Words: Euploid, blastocyst, implantation, PGT-A, embryo

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ESSENTIAL POINTS

- To choose the optimal euploid blastocyst for single embryo transfer, the embryo's morphological grade, status of the zona pellucida, and day of blastocyst development should be considered.
- Synchrony between the endometrium and embryo appears to play a key role in the implantation of euploid blastocysts.
- Patient factors such as optimal weight and thyroid hormone status should be addressed to optimize a healthy pregnancy after the transfer of a euploid blastocyst.
- The choice of in vitro fertilization stimulation and frozen-thawed embryo transfer protocols does not appear to have a significant impact on the successful implantation of euploid blastocysts.
- The timing, size, and number of embryo biopsies appear to have an impact on the implantation of a euploid blastocyst.

Live birth after in vitro fertilization (IVF) is dependent on the successful implantation of an embryo into a receptive endometrium. In general, 3 factors contribute to embryo implantation: embryo quality; endometrial receptivity; and embryo transfer (ET) technique. Of these factors, embryo quality is arguably the most important and perhaps the least well understood at this point in time. With the advent of preimplantation genetic testing for aneuploidy (PGT-A), the ploidy status of a blastocyst can be determined with high accuracy. Having a correct complement of chromosomes is a necessary, but not sufficient, criterion for resulting in a healthy live birth and, thus, a good proxy for determining whether an IVF treatment will be successful. However, roughly one third of euploid blastocysts deemed high-enough quality to transfer do not implant successfully.

Received January 5, 2022; revised and accepted March 8, 2022.
E.A.R. has nothing to disclose. A.R. has nothing to disclose. J.S.H. has nothing to disclose. J.M.T. has nothing to disclose. E.J.F. has nothing to disclose.
E.A.R. and A.R. should be considered similar in author order.
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Fertil Steril Rev® Vol. 3, No. 2, May 2022 2666-5719

https://doi.org/10.1016/j.xfnr.2022.03.001

A variety of factors have been investigated to predict IVF success (Table 1). Some of the previous studies have been biased by varying embryo quality in the transfer of multiple untested embryos, where embryonic aneuploidy cannot be ruled out as a factor that limited success. By controlling for ploidy, these variables can systematically be studied to determine which factors may better predict whether a euploid blastocyst successfully implants. At an embryonic level, the morphology, blastocyst expansion stage, and timing of blastocyst development may be predictive. Even if a blastocyst has the potential to result in live birth, other factors can impact its ability to implant, including maternal factors (age, body mass index [BMI], and general health status), paternal factors (age and sperm quality), uterine factors (endometrial thickness, pattern, and compaction and uterine pathology), medication protocols, and laboratory conditions. This review explores published retrospective and prospective studies that have included only euploid blastocysts after trophectoderm biopsy and PGT-A and examines the many other factors that may impact implantation. By studying these factors, clinicians may be better able to counsel patients on success and optimize selection for elective single embryo transfer (eSET).

EMBRYONIC FACTORS

Morphology

Blastocyst grading/morphology is a widely used marker to assess embryo quality and can assist in determining the single best embryo to transfer. However, blastocyst morphology alone is not accurate enough to reliably exclude aneuploid blastocysts from being transferred. A study by Capalbo et al. (1) suggested that morphology was not associated with the chance of ongoing pregnancy; "poor"-quality euploid blastocysts fared just as well as high-graded blastocysts. It is important to remember that even the "poor"-quality blastocysts were of sufficient quality to undergo trophectoderm biopsy and survive vitrification and warming to be transferred. This finding was challenged in a study by Irani et al. (2). They found a significant difference in the chance of ongoing pregnancy on the basis of the morphology of the transferred blastocysts (2). Similar to the study by Capalbo et al. (1), blastocysts were grouped into 4 categories (excellent, good, average, and poor). Although only 38 excellent embryos were transferred, these resulted in ongoing pregnancies significantly more often than the average- and poor-quality embryos (ongoing pregnancy rate, excellent, 84.2%, vs. average, 55.8%, vs. poor, 35.8%). Of note, patients were not randomized. Patients who had average- or poor-quality embryos transferred did not have an excellent or good-quality embryo available. Hence, it is possible that some other underlying factors, such as diminished oocyte and/or sperm quality, may have contributed to the lower success rates with poor-quality, euploid blastocysts.

Another criticism is the potential role of mosaicism or segmental imbalance. The study by Irani et al. (2) included embryos transferred after testing with array comparative genomic hybridization (aCGH), which is not as well validated for the detection of these embryonic abnormalities as next-generation sequencing (NGS). Next-generation sequencing is a deoxyribonucleic acid (DNA) sequencing technology that allows for rapid, low-cost, and high-throughput testing of a genome. In a study using only NGS, Gonzalez et al. (3) found no statistically significant difference in the implantation, pregnancy, or live birth rates on the basis of morphology after 179 thawed transferred euploid blastocysts. However, this study may also be biased because euploid blastocysts with higher morphological scores were prioritized over euploid embryos with lower morphological scores in cases where >1 euploid was available for transfer.

In the largest retrospective cohort study to date, Nazem et al. (4) investigated 2,236 euploid ETs from 1,629 couples. Embryonic ploidy was determined based on 1 of 2 different PGT platforms, including NGS or quantitative polymerase chain reaction (PCR), which is relatively insensitive for the detection of mosaicism or segmental imbalances. The likelihood of pregnancy and live birth rate was not affected by the type of PGT-A platform used. Grading of the blastocyst inner cell mass (ICM) grade was the most reliable predictor of pregnancy outcomes. Grade A ICM had a live birth rate of 55.6% compared with 32.3% of embryos with grade C ICM ($P< .001$). Their results suggest that blastocyst morphological grading, particularly ICM grade but also composite grade, is predictive of ongoing pregnancy/live birth after single frozen euploid ETs. These investigators concluded that morphological grading should be used to guide selection among euploid embryos.

Future studies with a larger sample size of embryos only undergoing PGT-A by NGS may further elucidate the role of morphology in predicting IVF outcomes. Other important factors to consider are the inherent variability and subjectivity of blastocyst grading. A good-quality blastocyst in one laboratory may be considered a poor-quality one in another. To determine the true effect of embryo morphology on IVF outcomes, a randomized controlled trial (RCT) would be needed in which embryos were transferred at random vs. "best" morphology. This would control for the possibility that patients who generate AA embryos are more likely to carry a better prognosis than those who generate embryos with lower grades. As of now, morphology can still provide guidance for the optimal selection of supernumerary euploid embryos to maximize the likelihood of clinical pregnancy after frozen-thawed embryo transfer (FET).

Expansion Stage

Extended culture media and PGT-A, which are used to enhance implantation rates, result in the transfer of a more developed and often fully hatched embryo. Without protection of the zona pellucida, a fully hatched embryo may be more vulnerable to trauma during biopsy, cryopreservation, warming, and transfer. Concerns regarding the survival of fully hatched blastocysts have been extrapolated from mouse studies, in which hatched blastocysts were more likely to bind to the inner surface of the cryostraw (5). However, this has not been seen in human embryos. Rodriguez-Purata et al. (6) compared catheter retention and implantation and pregnancy rates of 808 PCR-tested euploid embryos, of which 46% were

TABLE 1

Summary of evidence by subcategory for factors influencing implantation of euploid blastocysts.

Category	Subcategory	Summary statement
Embryonic factors	Blastocyst morphology	There is fair evidence to suggest that an increasing morphological grade of the ICM and trophectoderm has a positive impact on the implantation potential of euploid blastocysts.
	Blastocyst expansion stage	There is fair evidence that nonfully hatched euploid blastocysts have a higher chance of implantation than fully hatched euploid blastocysts.
	Timing of blastocyst formation	There is fair evidence that the implantation rates of euploid day 7 blastocysts are lower than those of day 5 and 6 euploid blastocysts.
	Mitochondrial DNA	There is insufficient evidence to determine whether testing mitochondrial DNA content in euploid blastocysts has an impact on the implantation potential.
Uterine factors	Endometrial appearance	There is insufficient evidence to determine whether endometrial compaction after the start of progesterone in embryo transfer cycles impacts the implantation potential of euploid blastocysts.
	Endometritis	There is fair evidence that the treatment of chronic endometritis can increase the likelihood of successful implantation in patients with a history of recurrent implantation failure, although further studies are needed in euploid blastocysts.
	History of cesarean section	There is fair evidence that a history of a prior cesarean lowers the implantation rate of euploid blastocysts after single embryo transfer.
	Presence of adenomyosis and endometriosis	There is insufficient evidence to suggest whether the presence of adenomyosis or endometriosis impacts the implantation of euploid blastocysts.
	Arcuate uterus	There is fair evidence that a diagnosis of an arcuate uterus does not impact the implantation rate of euploid blastocysts.
	Ease of transfer	There is fair evidence that a difficult embryo transfer does not lower the implantation rate of euploid blastocysts.
	Endometrial disruption	There is fair evidence that endometrial disruption before the transfer of a single euploid embryo does not improve the implantation rates.
IVF protocols	Ovarian stimulation and trigger	There is fair evidence that ovarian stimulation and trigger type do not impact the implantation rate of euploid blastocysts.
	FET protocols	There is good evidence that the type of FET preparation protocol does not impact the implantation rate of euploid blastocysts.
	Timing of transfer	There is insufficient evidence to determine whether adjusting the timing of the embryo transfer based on endometrial receptivity testing impacts the implantation rate of euploid blastocysts.
	Progesterone level	There is fair evidence to suggest that the implantation of euploid blastocysts is improved when the progesterone level is >20 ng/mL on the day of embryo transfer.
	Being from a previously vitrified egg	There is insufficient evidence to determine whether the implantation rates are altered if euploid blastocysts are derived from previously vitrified oocytes.
	Fresh vs. frozen transfer	There is fair evidence to suggest an improvement in the implantation rates of vitrified-warmed blastocysts compared with those of fresh blastocysts.
Patient factors	Maternal age	There is fair evidence that extremes of maternal age can negatively impact the implantation potential of euploid blastocysts.
	Paternal age	There is fair evidence to support that an advanced paternal age of 41–50 years impacts the fertilization rates but not the implantation or clinical pregnancy rates of euploid blastocysts.
	Sperm DNA fragmentation	There is fair evidence to suggest that sperm DNA fragmentation index does not impact the euploidy rates or pregnancy outcomes.
	BMI	There is good evidence that an increased maternal BMI leads to worse pregnancy outcomes after the transfer of euploid blastocysts, including increased miscarriage rates and decreased live birth rates.
	MTHFR gene mutation	There is fair evidence to suggest that MTHFR homozygosity in euploid blastocysts may negatively impact the implantation rates.
	Vitamin D level	There is fair evidence that low vitamin D levels do not negatively impact pregnancy outcomes in patients undergoing euploid blastocyst transfer.
	TSH	There is fair evidence that the TSH levels of <2.5 mIU/L do not impact pregnancy outcomes after a euploid blastocyst transfer.
Embryology protocols	Timing of embryo biopsy	There is good evidence that cleavage-stage biopsy negatively impacts the euploid embryo clinical pregnancy rate, whereas blastocyst-stage biopsy does not appear to have the same negative impact.
	Size of biopsy	There is fair evidence that a larger biopsy size can negatively impact the euploid embryo pregnancy rates.
	Type of culture media	There is good evidence that culturing euploid blastocysts in sequential media over monophasic media does not improve the implantation rates, although blastocyst progression is improved.
	Culture temperature	There is good evidence that lowering the embryo culture temperature to 36°C from 37°C does not improve the embryo implantation rates.
	Dynamic vs. static embryo culture	There is good evidence that dynamic embryo culture does not yield better blastocyst or implantation rates when compared with static embryo culture.
	Number of vitrification cycles	There is fair evidence that double vitrification and double biopsy of a blastocyst can negatively impact the implantation potential of euploid blastocysts.

Note: BMI = body mass index; DNA = deoxyribonucleic acid; FET = frozen-thawed embryo transfer; ICM = inner cell mass; IVF = in vitro fertilization; MTHFR = methylenetetrahydrofolate reductase; TSH = thyroid-stimulating hormone.

Reshef. Implantation of euploid blastocysts. Fertil Steril Rev 2022.

fully hatched. Fully hatched euploid blastocysts had a similar rate of being retained in the transfer catheter, a similar survival rate after warming, and similar implantation rates. The implantation, biochemical pregnancy, and early pregnancy loss rates were similar, but a trend toward poorer results was observed in fully hatched embryos compared with those in nonfully hatched embryos. A larger retrospective review by the same center included 2,236 euploid blastocysts (720 fully hatched, PCR- and NGS-tested embryos) and found a significantly higher likelihood of ongoing pregnancy or live birth in blastocysts within the zona pellucida than in fully hatched embryos (odds ratio [OR], 1.6; 99% confidence interval, 1.2–2.2) (4). This is consistent with other previously published data (7, 8, 9) and provides further evidence that when selecting among euploid blastocysts, the ones with an intact zona pellucida should be prioritized.

Timing of Blastocyst Formation

The relatively recent application of culture through day 7 in some centers increases the pool of transferable embryos for patients who would otherwise have no usable embryos if culture was terminated on day 6. This is particularly true for patients aged >35 years, whose embryos take longer to blastulate and, therefore, are more susceptible to cycle cancellation. While day 7 culture will benefit several patients, day 7 blastocysts have a higher likelihood of aneuploidy than day 5 and 6 blastocysts. In a retrospective study by Tiegs et al. (10) of 229 NGS-tested euploid day 7 blastocysts, the pregnancy rates were slightly but not significantly reduced compared with those for day 5 and 6 blastocysts. The sustained implantation rate for day 7 euploid blastocysts was 52.6% compared with 68.9% and 66.8% for day 5 and 6 blastocysts, respectively ($P=.29$ and $P=.14$) (10). A separate retrospective study by Hernandez-Nieto et al. (11) found a significant reduction in the euploidy and implantation rates for day 7 blastocysts compared with those for day 5 and 6 blastocysts. The euploidy rate was 40.5% for day 7 blastocysts compared with 54.7% and 52.9% for day 5 and 6 blastocysts, respectively ($P<.0001$). In this study, 116 day 7 euploid blastocysts (by PGT-A) were transferred, resulting in a significant decrease in the implantation (OR, 0.32; $P<.001$), clinical pregnancy (OR, 0.28; $P<.001$), and live birth (OR, 0.28; $P<.001$) rates. In the study by Tiegs et al. (10), day 5 embryos had higher euploidy rates than day 6 embryos, whereas in the study by Hernandez-Nieto et al. (11), day 5 and 6 blastocysts had similar euploidy rates. These data support the selection of day 5 and 6 blastocysts over day 7 blastocysts when available.

Mitochondrial DNA Level

Mitochondrial function and adequate energy production in the early stages of development are considered crucial to successful implantation and pregnancy. The predictive value of the mitochondrial DNA copy number (mtDNA) from a trophectoderm biopsy as a biomarker for viability has been explored. Prior studies have suggested that the higher amounts of mtDNA in euploid embryos are linked to decreased implantation potential (12, 13). However, a study of 69 cases where both a euploid male and female sibling from the same cohort were transferred simultaneously and resulted in a singleton delivery found no difference in mtDNA from delivered vs. nondelivered embryos (14). A separate study that applied a correction factor for aneuploid chromosomes also found no predictive value of mtDNA for ploidy or implantation potential (15).

Nonetheless, others argue that there is a threshold of mtDNA above which successful implantation does not occur. In a retrospective review that included 33 transferred embryos with elevated mtDNA quantities, none resulted in pregnancy (16). Thus, the negative predictive value of mtDNA assessment in this cohort was 100% (33/33). These findings were confirmed in a blinded prospective study where 9 morphologically good, euploid blastocysts with unusually high mtDNA levels were transferred. Again, the ongoing pregnancy rate for embryos with unusually high mtDNA levels was 0 (0/9) (17). Given the variability in mtDNA assays and associated findings, further study is still needed to determine whether an assessment of mtDNA will prove to be clinically useful in the selection of embryos.

UTERINE FACTORS

Endometrial Appearance

As one half of the requisite embryo-endometrium pairing, the endometrium is clearly implicated in implantation and pregnancy failure. Pelvic ultrasound is the most accessible and least invasive means of evaluating the endometrium. Thus, endometrial appearance, specifically thickness and pattern, has been proposed as a marker for IVF success. To assess the utility of pelvic ultrasound in predicting endometrial receptivity, Gingold et al. (18) evaluated the endometrium on the day of trigger and at the time of ET in 356 fresh and frozen cycles. Endometrial thickness, analyzed both continuously and categorically (≤ 8 or >8 mm), had no effect on the implantation, pregnancy, or clinical pregnancy rates, either at time of trigger or on the day of transfer for both fresh and frozen cycles. Physiologically, the endometrium is relatively hypoechoic in the early proliferative phase. As the endometrium thickens, it becomes echogenic relative to the myometrium, reflecting the formation of glands, blood vessels, and stroma during this time. The endometrium thickens even further and becomes more echogenic during the secretory phase. In the study by Gingold et al. (18), women with a mid-late secretory endometrial pattern at the time of trigger had lower implantation rates than women with an early secretory endometrial pattern after fresh ET. Furthermore, women with a mid-late secretory endometrial pattern had significantly higher progesterone (P4) levels at the time of trigger. The effect of the endometrial pattern type on the implantation rates was no longer significant when controlling for elevated P4 levels, implying that an increased P4 level causes the asynchrony between the embryo and endometrium that leads to decreased implantation rates in fresh ETs. Of note, this study was limited by a lack of patients with a very thin endometrium (<6 mm).

Although absolute endometrial thickness does not appear to affect pregnancy rates, the investigators have hypothesized

that endometrial compaction, which reflects the absolute change in endometrial thickness between the end of the proliferative phase and the day of transfer, may be a marker for reproductive success. Zilberberg et al. (19) evaluated the effect of endometrial compaction in 225 frozen NGS-tested euploid ETs and found significantly higher ongoing pregnancy rates with all levels of compaction compared with those of cycles without compaction. The pregnancy rates ranged from 44.3% in the 5%–10% compaction group to 54.9% in the ≥20% compaction group vs. 30.5% and 31.0%, respectively. A more recent study by Gill et al. (20) found similar results, with an endometrial compaction of ≥10% as measured by transvaginal ultrasound leading to higher clinical pregnancy rates in frozen euploid ETs.

Conversely, a prospective cohort study of 259 medicated frozen euploid ETs found no increase in the clinical pregnancy or live birth rates with endometrial compaction, defined as a ≥5% decrease in endometrial thickness (21). This study differs from that of Zilberberg et al. (19), mainly in the timing and nature of ultrasound assessment; the current study used sequential transvaginal ultrasound measurements with follow-up scan on the day before ET to calculate compaction as opposed to transabdominal scan at the time of transfer, resulting in a much lower rate of compaction (16.6% vs. 43.1%). The investigators postulate that inaccuracies in transabdominal measurement led to this discrepancy and falsely elevated the rate of compaction, thus leading to improved pregnancy rates in the compaction group.

Based on the current limited data, the influence of endometrial compaction on IVF outcomes after euploid ET remains unclear. While it seems physiologically plausible that a decrease in endometrial thickness in response to P4 exposure indicates optimal endometrial receptivity, the data do not support altering ET cycles in response to the presence or absence of endometrial compaction on the day of transfer.

Endometritis

Although generally asymptomatic, chronic endometritis, or inflammation of the endometrium, has been associated with poor reproductive outcomes. Approximately one third of women with recurrent implantation failure are diagnosed with chronic endometritis based on immunohistochemical staining for the cell marker CD138, indicative of the presence of plasma cells in the endometrial stroma (22, 23). Moreover, women with recurrent implantation failure successfully treated for chronic endometritis have improved implantation, clinical pregnancy, and live birth rates similar to those without endometritis (24). There are currently no data specifically assessing the effect of chronic endometritis on IVF outcomes after euploid ET, although there is little biologic plausibility to suggest that euploid vs. aneuploid embryos would react differently in such an inflammatory milieu.

Previous Obstetric History

Multiple studies have established decreased implantation and live birth rates in patients with a previous history of cesarean delivery. Studies specifically investigating the IVF population have included patients undergoing fresh ETs, a combination of fresh and frozen ETs, transfers at the cleavage stage, and transfers at the blastocyst stage. In a recent study, Friedenthal et al. (25) attempted to account for numerous variables other than the impact on the uterus that previous cesarean deliveries may have on the implantation rates. This study only examined patients with a history of 1 vaginal delivery or 1 cesarean delivery who subsequently underwent single euploid ET at the blastocyst stage. This study verified what had previously been postulated that women who have had a previous cesarean delivery have lower implantation rates than those who have delivered vaginally. Even in patients undergoing single euploid ETs at the blastocyst stage only, the implantation rates were decreased among women with a history of cesarean delivery. This study found a statistically significant difference in the implantation and ongoing pregnancy rates (68.0% vs. 55.5% (P=.004)) as well as live birth rates (59.1% vs. 49.0% (P=.02)) between the controls (the vaginal delivery group) and cases (the previous cesarean delivery group). These data further validate the notion that cesarean delivery should be limited when possible to reduce downstream effects on fertility, even when embryonic aneuploidy is taken out of the equation.

Presence of Adenomyosis and Endometriosis

Adenomyosis has been implicated in poor IVF outcomes, including lower implantation, clinical pregnancy, and live birth rates, along with higher miscarriage rates (26). However, as adenomyosis is associated with other factors related to infertility, namely, age, and obesity, it is uncertain whether this effect can be attributed to adenomyosis itself. In an attempt to answer this question, Neal et al. (27) performed a prospective study evaluating the effect of adenomyosis, as diagnosed by 3-dimensional (3D) ultrasound, on reproductive outcomes after euploid blastocyst transfer. The cohort included 648 women with euploid embryos confirmed via NGS who underwent a transvaginal 3D ultrasound on the day before scheduled transfer. The clinical pregnancy (80.0% vs. 75.0%), miscarriage (10.5% vs. 7.7%), and live birth (69.5% vs. 66.5%) rates were similar between the groups with and without adenomyosis with no change when controlling for confounders, including age at transfer and BMI. Additionally, no differences were noted based on the number of sonographic markers of adenomyosis identified, and no single sonographic marker was associated with worsening IVF outcomes. The investigators admit that the results of this study are limited by poor interobserver agreement for the diagnosis of adenomyosis, with raters agreeing on the presence of specific sonographic markers in only 11% of positive cases. This suggests a possible overdiagnosis of adenomyosis, which would bias the data toward a null result, as is seen here. Alternatively, the investigators hypothesize that asymptomatic adenomyosis, as was the case for most of their cohort, is a different clinical entity with a lesser effect on reproductive outcomes.

Contrary to these findings, a retrospective cohort study by Stanekova et al. (28) found higher miscarriage rates in women with adenomyosis. This study evaluated 171 women who

underwent euploid ET as determined by aCGH and successfully conceived. The overall miscarriage rates were higher in those women with adenomyosis (53.0% vs. 19.7%, $P<.0001$), with most of them falling into the biochemical category. The investigators also note lower beta-human chorionic gonadotropin (hCG) values at 16 days after ovulation in the adenomyosis group, suggesting a deleterious effect of adenomyosis on embryonic trophoblast function and its ability to maintain an early pregnancy. Unfortunately, by including only women with confirmed pregnancy, this study is unable to evaluate the effect of adenomyosis on the implantation and clinical pregnancy rates.

Current data are unclear regarding the effect of adenomyosis on euploid ETs. While it appears that adenomyosis does not significantly impact the implantation and clinical pregnancy rates in asymptomatic women, there is still a possibility of an increased risk of miscarriage, specifically before ultrasound verification of the pregnancy. Routine screening for adenomyosis before ET is not currently indicated, although some have recommended interventions such as gonadotropin-releasing hormone (GnRH) suppression before transfer, which is not supported by prospective data (26). Further studies are needed to evaluate reproductive outcomes in women with symptomatic adenomyosis.

Endometriosis has also been implicated in worse laboratory and clinical outcomes than in other patients undergoing IVF. One of the proposed mechanisms of the effect of endometriosis is impaired endometrial receptivity. It has also been proposed that oxidative stress and increased free radicals may result in impaired embryo development. A study by Bishop et al. (29) sought to determine whether subfertility in patients with endometriosis is due to impaired endometrial receptivity by comparing the pregnancy and live birth rates in women with endometriosis vs. 2 control groups without suspected endometrial factors. In this study, there was no statistically significant difference in live birth rates in patients with endometriosis compared with those with male factor infertility or noninfertile patients undergoing PGT for monogenic disorder. In 459 frozen euploid ET cycles in 328 patients, the results showed that the aneuploidy rates in the patients with endometriosis were similar to those in patients with male factor infertility despite the proposed mechanisms that endometriosis may contribute to oocyte structural instability that could result in aneuploidy. In this study, only patient age was found to be a factor contributing to increased aneuploidy rates in the study population (29).

Arcuate Uterus

An arcuate uterus, defined by convex fundal contour with shallow endometrial indentation, is the most common congenital uterine anomaly in the general population and among women with recurrent pregnancy loss (30). As a variant of normal, there is debate as to whether surgical intervention is necessary before ET. To address this question, Surrey et al. (31) compared IVF outcomes after a euploid ET in 78 women with an arcuate uterus vs. 354 controls with a normal uterine cavity. All patients underwent routine 3D ultrasound followed by confirmatory hysteroscopy to diagnose the condition. An arcuate uterus is defined as an endometrial indentation of 4 to <10 mm in size and a myometrial angle of $>90^{\circ}$. Euploid status was determined by aCGH. There was no difference in the implantation (63.7% vs. 65.4%), live birth (68.7% vs. 68.7%), chemical pregnancy (8.4% vs. 7.7%), or miscarriage (4.8% vs. 4.3%) rates between women with an arcuate and those with normal uterus. While there is wide variation in the definition and diagnosis of arcuate uterus across the literature, this study suggests that surgical intervention is not necessary before euploid ET in women with this mild uterine anomaly.

Ease of Transfer

Anecdotally, several reproductive endocrinologists suspect that the ease, or difficulty, of ET may contribute to live birth rates. This hypothesis was tested by Alvarez et al. (32) in a single-center retrospective study of 370 frozen transfer cycles using euploid embryos as screened by aCGH. This center uses a standardized stepwise approach to transfer whenever resistance is encountered: use of an outer catheter sheath; use of a malleable Wallace stylet; need for tenaculum; and insertion of uterine sound. A "difficult" transfer was defined as the need for a Wallace stylet or beyond. The investigators found a trend toward lower live birth rates for difficult (40.5%) vs. easy (54.5%) transfers, but no statistical significance was reached after adjusting for confounders. The study was limited by the small sample size, specifically in the more difficult transfer categories; only 3 transfers required the use of a tenaculum, and no transfers required the insertion of a uterine sound. While confirmatory studies are needed, the current evidence suggests a deleterious effect of difficult ET on live birth rate, and all efforts should be made to anticipate challenges and optimize transfer leading up to the procedure itself.

Endometrial Disruption

Initial research suggested that endometrial disruption, otherwise known as endometrial scratch, could be an effective intervention for women with implantation failure via recruitment of cytokines, growth factors, and other inflammatory molecules to the endometrium (33). More recent data have brought into question the benefit of endometrial disruption in the general IVF population (34–36). A multicenter RCT of endometrial scratching (n = 690) vs. no intervention (n = 674) in both fresh and frozen transfer cycles showed no difference in the live birth rates between groups (36). There were also no significant differences in the clinical pregnancy, ongoing pregnancy, or miscarriage rates. To isolate the possible endometrial benefit of such disruption while controlling for the embryonic component of implantation failure, Werner et al. (37) examined the outcome of this procedure in the high-risk group of patients with prior euploid ET failure. This retrospective analysis included 290 patients who failed their initial euploid ET and completed a second euploid ET cycle. Of these women, 39 (13%) underwent single-pass endometrial biopsy within the 2 cycles immediately before their second transfer. The clinical implantation and sustained implantation rates were

equivalent between groups: 43.6% vs. 55.0% ($P=.13$) and 38.5% vs. 42.6% ($P=.60$), respectively. This study is notably weakened by a lack of randomization with endometrial disruption performed at the treating physician's discretion, introducing the possibility that the procedure was performed in the subgroup of patients with the worst prognosis. However, the only difference in demographics and cycle characteristics between groups was maternal age, with slightly younger women in the intervention group. As age is primarily related to increasing aneuploidy, this difference was likely corrected by including only women with euploid ETs. Despite promising initial data, endometrial disruption does not appear to have a beneficial effect on the implantation rates in women with prior euploid ET failure.

PATIENT FACTORS

Patient-related factors must also be taken into consideration when analyzing the success of euploid ETs. Several of these factors have been reviewed extensively, including maternal age, paternal age, BMI, sperm DNA fragmentation index (DFI), vitamin D levels, and thyroid-stimulating hormone (TSH) status. What effect, if any, do these have on the success of euploid embryo implantation and pregnancy rates?

Maternal Age

Increasing maternal age is the most significant factor contributing to the inability of generating chromosomally competent embryos. Several women of advanced reproductive age who undergo IVF do not have euploid embryos available for transfer. However, maternal age may not be as big a factor if embryonic aneuploidy is controlled for. A 2013 retrospective study by Harton et al. (38) showed that selective transfer of a euploid embryo demonstrated equivalent implantation and pregnancy rates among all women between the ages of 35 and 42 years. The overall implantation rates ranged from 40%–54%. Of note, this study only included 18 patients aged >42 years. Another observational study from 2017 by Ubaldi et al. (39) included a slightly larger cohort of women aged ≥44 years. Although the rate of euploid was low (11.8% of 187 embryos), women aged 44 years had a live birth rate of 57% in 21 frozen ET cycles.

A more recent study performed in 2020 in a much larger cohort demonstrated that maternal age does have a negative impact on implantation beyond ploidy status (40). Reig et al. (40) retrospectively reviewed 8,175 single euploid ETs to determine if age remained a significant risk factor contributing to reproductive senescence. This study included 319 women aged 41–42 years and 243 women aged ≥43 years. The implantation rates were negatively correlated with age, with an OR compared with the youngest group (women aged <35 years) of 0.85 at the age of 38–40 years, 0.69 at the age of 41–42 years, and 0.51 at the age of >42 years. While early data indicated that the use of PGT-A could potentially abrogate the effects of maternal age on IVF success rates, very advanced age likely remains an important factor in euploid embryo success.

Paternal Age

Although the impact of advanced paternal age on reproductive outcomes is less significant than advancing maternal age, it is possible that sperm from older men could produce embryos with lower reproductive potential. Prior studies have investigated the effect of paternal age on IVF success rates using donor oocyte cycles, which were summarized by a systematic review by Sagi-Dain et al. (41). In this review, 7 studies examined the clinical pregnancy rates, and most of them found no statistically significant relationship between paternal age and achieving pregnancy. One study did find a decrease in the clinical pregnancy rate in men aged ≥39 years compared with that in younger men (54.7% vs. 46%, $P=.01$) but only when the donor egg recipient age was >38 years. The study by Luna et al. was also included in this systematic review that showed a trend toward lower clinical pregnancy rates with men aged >60 years, but this result was not statistically significant. The only study in this review that demonstrated a clear statistical impact of paternal age on achieving pregnancy was that of Girsh et al., which found that men involved in the group that achieved pregnancy were younger than men in the nonpregnant group (43.2 vs. 46.8, $P=.003$). Of the 7 studies examining the association of live birth rate with paternal age, 5 showed a correlation that was not statistically significant. The study by Frattarelli et al. demonstrated a lower live birth rate in men aged >50 years than in younger males (56.0% vs. 41.3%, $P<.01$) after controlling for female age. Moreover, the retrospective study by Robertshaw et al. demonstrated lower odds of live birth in older men. Using logistic regression analysis, this study found 26% lower odds of live birth with each 5-year increase in paternal age ($P=.01$). Although the studies included are overall of suboptimal quality, there is some available evidence to suggest that advanced paternal age is associated with adverse reproductive outcomes in oocyte donor cycles, possibly due to higher aneuploidy rates in older sperm.

To control for ploidy status, Tiegs et al. (42) examined whether increasing paternal age had any adverse effects on IVF outcomes in cycles that used only euploid embryos. They found that the fertilization rates were negatively impacted with increasing paternal age; however, the implantation and pregnancy rates were not significantly different when maternal age was controlled for. The implantation rates ranged from 59%–71%, whereas the clinical pregnancy rates ranged from 45%–61%. This is the largest study of its kind, including 140 men between the ages of 41 and 45 years and 94 men aged >45 years. More data are needed to clarify the impact of an advanced paternal age of >50 years on the performance of euploid blastocysts.

Sperm DNA Fragmentation

Advanced paternal age has also been linked with increased sperm DNA fragmentation (SDF), which has emerged as a new diagnostic tool for evaluating semen quality in men with infertility. Although paternal age is directly proportional to sperm DNA damage, the clinical significance of these changes is not well defined. In addition, SDF can vary

significantly within the same patient (43). A 2020 prospective cohort study by Green et al. (44) assessed the direct impact of SDF on IVF outcomes by determining the DFI of the actual sperm used for fertilization in the IVF cycles analyzed. They also controlled for embryonic euploidy to isolate any observed changes to the DFI of the sperm used. The study included 179 men with low DFI ($\leq$15%) and 55 men with high DFI (>15%). As expected, men in the high DFI group were older and had lower total motile sperm and lower sperm concentration than those in the low DFI group. There were no clinically significant differences in the implantation or ongoing pregnancy rate in either group. The euploidy rates were also the same among both groups. These data indicate that SDF on the day of fertilization may not impact the euploidy rates or pregnancy outcomes in single euploid ETs.

Body Mass Index

Of all patient factors, BMI appears to have the most significant impact on the euploid ET success rates. Most notably, obesity is a known risk factor for increased miscarriage rates in both natural reproduction and assisted reproduction (45). However, it does not appear that BMI is directly related to aneuploidy rates, indicating that adverse pregnancy outcomes may be related to other factors (46). Given that a large percentage of spontaneous abortions are associated with chromosomal abnormalities, Tremellen et al. (47) sought to isolate the effect of BMI on pregnancy outcomes by analyzing the transfer of euploid embryos. A total of 125 women were included in the study: 70 in the lean category (BMI, 18.5–24.9 kg/m^2), 24 in the overweight category (BMI, 25–29.9 kg/m^2), and 31 in the obese category (BMI, >30 kg/m^2). They found that the lean patients had significantly lower miscarriage rates than overweight and obese patients (14%, 29%, and 42%, respectively). These results held true after controlling for maternal age and obstetric history. These findings were also corroborated in a 2020 retrospective review of 707 patients by Boynukalin et al. (48). Patients who achieved a live birth had a significantly lower BMI than patients who did not (22 vs. 27 kg/m^2). Every increase in 1 unit of BMI decreased the probability of live birth by 20%.

Methylenetetrahydrofolate Reductase Gene Mutation

Studies have also investigated the effect of methylenetetrahydrofolate reductase (MTHFR) gene mutations on embryo viability. Methylenetetrahydrofolate reductase is an important enzyme for reproductive function because it helps in the metabolism of folic acid and DNA synthesis. Enciso et al. (49) analyzed the frequencies of MTHFR single nucleotide polymorphisms on euploid embryos as well as their reproductive outcomes. Interestingly, they found that euploid embryos homozygous for the 677T MTHFR gene variant had a lower chance of implantation than wild-type and heterozygous MTHFR embryos. While interesting, parental genotyping for MTHFR is not routinely tested or recommended by the American Society for Reproductive Medicine. Furthermore, these results have not been replicated in a larger cohort.

Vitamin D Levels

Vitamin D has also become a marker of interest in reproductive health. Prior observational studies have implicated vitamin D deficiency with preeclampsia and small-for-gestational-age infants (50, 51), but the effect of the vitamin D levels on pregnancy outcomes in patients undergoing infertility treatment is limited. Franasiak et al. (52) performed a retrospective review in 2015 to assess the impact of vitamin D levels on the implantation and pregnancy rates in patients undergoing IVF with PGT-A. A total of 517 patients were included in the study and subdivided into 3 groups: vitamin D deficient (<20 ng/mL), insufficient (20–29.9 ng/mL), and replete (>30 ng/mL). Only 18% of the patients included in the study were considered replete. There were no differences in the implantation, clinical pregnancy, or ongoing pregnancy rates after a euploid ET in either group. While vitamin D deficiency is not uncommon, low levels likely have no impact on pregnancy outcomes in patients undergoing euploid ET.

TSH Levels

It is generally accepted that there are separate TSH goals for pregnant patients compared with nonpregnant patients (53). The current recommendation is to maintain a TSH level of <2.5 mIU/L in women attempting conception and during early pregnancy. However, an optimal TSH level has not been established. Green et al. (54) sought to analyze if there is an optimal range of TSH below 2.5 mIU/L that could positively impact the IVF success rates with euploid embryos. They conducted a retrospective analysis of 1,599 euploid blastocyst transfers (fresh and frozen) with TSH measured 8 days after ET. No differences were observed in the implantation, live birth, or miscarriage rates by the TSH levels. In addition, there was no difference in the live birth rates for those on levothyroxine (69.9%, n = 584) vs. those not on supplementation (69.5%, n = 1,015). These data indicate that the variations of TSH levels of $\leq$2.5 mIU/L do not seem to have an effect on pregnancy outcomes after a euploid blastocyst transfer.

IVF PROTOCOLS

Wide spectra of IVF protocols have been developed to address the needs of different patient populations. Ovarian stimulation protocols vary when applied to high responders, low responders, previous IVF failures or suboptimal ovarian responses, patients with an increased risk of ovarian hyperstimulation syndrome (OHSS), patients in various age groups, polycystic ovary syndrome (PCOS), or diminished ovarian reserve. Endometrial replacement cycles for FETs may also vary depending on past experience or endometrial receptivity assessments. This section addresses the specifics of IVF protocols and what impact, if any, they may have on the success rate of euploid blastocyst ETs.

Ovarian Stimulation

Ovarian stimulation protocols are designed to meet the needs of each patient. The goal is to optimize the number of oocytes

retrieved while minimizing the risk of ovarian hyperstimulation. Often, the higher the ovarian response to stimulation, the higher the number of oocytes retrieved. Recent studies have examined whether ovarian stimulation length or gonadotropin dosage affect embryo euploidy or live birth rates. A prospective study by Hong et al. (55) compared the aneuploidy and implantation rates in 369 unstimulated IVF cycles with those in 2,846 conventionally stimulated IVF cycles with exogenous gonadotropins. They found that the aneuploidy rates were equivalent in both groups. Moreover, the implantation rates were similar in both the natural and stimulated IVF cycles. A more recent study by Irani et al. (56) corroborated these findings. Of 2,230 IVF cycles with nearly 12,300 embryos, this study found that higher doses of gonadotropins, more prolonged ovarian stimulation, higher estradiol levels, follicular size at time of trigger, and higher number of oocytes retrieved did not have a negative impact on the euploidy or live birth rates, regardless of a patient's age. Together, these data indicate that there is likely no toxic effect of gonadotropin administration on aneuploidy risk or euploid embryo reproductive potential.

Type of Trigger

Stimulation of final oocyte maturation before oocyte retrieval can be achieved by the administration of an hCG or GnRH agonist "trigger" either alone or in combination. While both trigger types will induce ovulation through a luteinizing hormone/hCG surge, the hCG trigger is known to increase the risk of OHSS in high responders due to its long half-life of 24 hours or more. The GnRH agonist trigger, which has a half-life of 60 minutes, induces rapid and reversible luteolysis compared with hCG, thereby decreasing the risk of OHSS (57). A study by Makhijani et al. (58) analyzed whether a GnRH agonist trigger impacts the implantation potential of euploid embryos. They found no statistically significant difference in the ongoing pregnancy and live birth rates in 263 frozen-thawed euploid ET cycles using either a GnRH agonist (n = 145) or an hCG trigger (n = 118) (58). At this time, there is no evidence that the type of trigger injection used for final maturation before oocyte retrieval affects the implantation potential of euploid embryos.

FET Protocols

Embryo implantation is a crucial element of IVF. The successful implantation of an embryo depends on the transfer of a viable embryo into a receptive endometrium. Several methods for endometrial preparation for embryo implantation are available, including the natural cycle (NC), modified natural cycle (modified-NC), and artificial cycle (AC). In the NC, the development of the endometrium occurs under endogenous hormone stimulation, and ET is timed after spontaneous luteinizing hormone surge.

In the modified-NC, the ET is planned after ovulation induction or trigger and P4 administration. In the AC, the endometrium is prepared for implantation with estrogen and P4 with or without ovulation suppression. Each method has advantages and disadvantages: the NC and modified-NC methods allow for avoidance of multiple medications and are more cost-effective, whereas the AC allows for better control over ET timing and avoidance of premature ovulation risk. The AC, however, is more labor-intensive and costly. In a prospective RCT comparing a modified-NC vs. an AC with GnRH agonist pituitary suppression for frozen-thawed single euploid blastocyst transfer, no significant differences in the clinical pregnancy, implantation, or miscarriage rates were observed (59).

There are conflicting data on whether there is an advantage for the NC overprogrammed cycles on the implantation and live birth rates of single frozen-thawed euploid transfers. A retrospective study by Wang et al. (60) found that an NC single frozen euploid ET was associated with significantly higher ongoing pregnancy rates than programmed cycles with hormone replacement (60.7%, n = 214, vs. 42.9%, n = 175). Another retrospective cohort study by Melnick et al. (61) also found increased implantation (0.66 ± 0.48 vs. 0.44 ± 0.50, P=.02) and live birth (63.1% vs. 37.5%, P=.007) rates in natural FET cycles in ovulatory women compared with those in programmed FET cycles in anovulatory women after undergoing IVF with PGT-A. This study used programmed cycles in anovulatory women and did not compare the natural and programmed FETs in ovulatory women. Therefore, the results may not be applicable to the ovulatory population.

In patients where ACs are used for endometrial preparation, there is not a consensus on the ideal amount of exposure to estrogen indicated in the proliferative phase. A large retrospective cohort study by Sekhon et al. (62) investigated whether the duration of estrogen administration before euploid ET affected clinical outcomes. This study found that the duration of estrogen administration before frozen euploid ET did not impact the implantation, clinical pregnancy, or live birth rates (62).

One last important consideration is that specific patient populations may have independent risk factors affecting the outcomes of euploid ET. For example, patients with PCOS have a higher risk of miscarriage and decreased chance of live birth after euploid ET (63). In addition, patients with PCOS are more often anovulatory and regularly require the use of an AC before ET. Interestingly, an RCT by Yu et al. (64) of 526 patients with PCOS found no difference in the implantation, clinical pregnancy, and live birth rates in artificial FETs compared with those in stimulated FET cycles with human menopausal gonadotropins. Nonetheless, this study will need to be replicated using euploid embryos only. Overall, more data are needed on the outcomes of FET using an NC vs. AC that leads to differing implantation, pregnancy, and live birth rates in ovulatory women.

Timing of Transfer

Transferring a confirmed euploid embryo should increase the chance of a successful implantation event because it removes a known risk factor for failed implantation and early pregnancy loss. Still, the implantation and live birth rates after the first euploid ET are close to 70% and 65%, respectively. This leaves 30%–40% of cases where the explanation of failed implantation is due to a factor other than ploidy status.

One of the other factors described as a source of implantation failure is endometrial receptivity. The endometrial receptivity assay (ERA) was developed as a means to detect whether a patient with a history of implantation failure has a displaced window of implantation (WOI) as a possible explanation. A study by Tan et al. (65) demonstrated that of patients with at least 1 failed euploid FET, 22.5% were found to have a displaced WOI by the ERA. This study found that patients with personalized FET cycles, on the basis of the ERA results, had higher implantation and ongoing pregnancy rates than those without personalized FET cycles. These differences were not statistically significant based on the small sample size. The results are promising to suggest a benefit to ERA testing in this population, but larger randomized studies are needed to validate this claim (65). Another study by Neves et al. (66) found a nonsignificant lower implantation rate after personalized euploid FET cycles in women with prior euploid implantation failures. It was notable that the ERA group had more prior euploid implantation failures (66). At this time, there are no definitive data to suggest that the timing of the transfer of a euploid embryo can be altered to increase the chance of successful implantation or clinical pregnancy.

P4 Level

It is well known that P4 produced by the corpus luteum in NCs is required for successful embryo implantation and pregnancy maintenance until the luteal placental shift. Progesterone supplementation during the luteal phase of frozen ET cycles has been studied and found to increase live birth rates (67). There is good evidence to support luteal phase P4 in frozen ET cycles, but there are fewer data about the optimal P4 level on the day of frozen ET. Studies have reported unfavorable outcomes when a premature increase in the P4 level is detected after ETs in fresh IVF cycles (68). This premature luteinization is a unique phenomenon in fresh IVF cycles and does not apply to the transfer of euploid embryos during frozen ET cycles. A study by Kofinas et al. (69) investigating the P4 levels on day 19 of frozen euploid ET cycles demonstrated that P4 levels between 10 and 20 ng/mL on day 19 were optimal. When the P4 level was >20 ng/mL, the ongoing pregnancy and live birth rates decreased. In this study, after thawed euploid ET, the ongoing pregnancy and live birth rates were 65% vs. 49% ($P=.02$) in the groups with P4 levels of <20 and >20 ng/mL, respectively. Elevated P4 levels on day 19 may shift the WOI accounting for the decreased success rates in this population. All frozen ETs were performed with euploid embryos. On the other hand, studies have suggested a minimum P4 threshold on the day of ET to optimize the ongoing pregnancy rates (70). While there is no consensus on the most optimal P4 level on the day of transfer, the data suggest that the successful implantation of a euploid embryo is affected by the range of P4 level at the time of transfer (71).

Since the field has transitioned to a segmented freeze-all approach, the P4 level during the retrieval cycle is less likely to play a role in embryo implantation or ongoing pregnancy. The asynchrony between the embryo and endometrium that an elevated P4 level may cause after an egg retrieval is practically eliminated by performing a "freeze-all" cycle. A study assessing the P4 levels on the day of trigger demonstrated that the number of eggs retrieved and the number of euploid embryos available for future ET were not affected by the P4 levels. This study found that elevated P4 levels of >1.5 ng/mL on the day of trigger did not affect the pregnancy and live birth rates in the first subsequent frozen euploid ET cycle (72). Taken together, it appears that the P4 levels before a frozen ET should reach a minimum threshold to optimize the euploid ET success rates.

Being from a Previously Vitrified Egg

Given the more efficient cryopreservation technologies that have been developed, including highly effective vitrification, warming, and later fertilization of cryopreserved oocytes, research has been conducted to determine whether oocyte freezing may cause an increase in aneuploidy and subsequent IVF success outcomes. In addition, the cryopreservation process can cause thickening of the zona pellucida and premature cortical granule reactions, both of which could impact embryonic development and implantation. A study by Forman et al. (73) analyzing the aneuploidy and implantation rates after embryos transferred from vitrified oocytes found that despite the lower efficiency of the IVF process after oocyte vitrification, there was no increased risk of aneuploidy and the implantation rates were equivalent.

Another study by Goldman et al. (74) demonstrated that the long-term cryopreservation of human oocytes did not increase embryonic aneuploidy in their population. In this study, 33 patients with cryopreserved oocytes underwent oocyte thaw, blastocyst culture, trophectoderm biopsy, and PGT-A with aCGH. These embryos were compared with age-matched controls with fresh oocytes whose embryos underwent trophectoderm biopsy and PGT-A during the same time period. The blastocyst formation rate decreased in the group with previously cryopreserved oocytes (54.5% vs. 66.2%), but the number of euploid blastocysts, percentage of euploid blastocysts, and rates of implantation, live birth, and ongoing pregnancy were not statistically different (74).

Fresh vs. Frozen Transfer

Two strategies for the transfer of euploid embryos include the "freeze-all" strategy, where vitrified and then warmed euploid embryos are transferred, and the fresh ET strategy, where a biopsy of an expanded blastocyst is performed on day 5 and the embryo is transferred on day 6 if confirmed euploid. There are benefits and challenges to each approach. Evidence does exist to suggest higher ongoing pregnancy rates with vitrified/warmed embryos in nonstimulated cycles than in fresh transfer in stimulated cycles. In a retrospective study by Zhu et al. (75), the clinical pregnancy rates in fresh vs. vitrified-warmed blastocyst transfer cycles were 36.4% and 55.1%, respectively ($P<.05$).

There is also evidence that the incidence of preterm delivery and low birth weight is lower with vitrified/warmed ETs than with fresh ETs (76). It is important to note that the success of frozen ETs depends on the high survival rate of embryos during the vitrification/warming process, which

can vary from laboratory to laboratory. The advantages of fresh transfer protocols include the ability for a more immediate transfer, limiting the costs and risks of vitrification/warming and storage of embryos, and limiting the cost of additional medications used in frozen ET protocols. One of the disadvantages of the fresh transfer approach is that it requires expanded blastocysts to be available on day 5 and at least 1 of these blastocysts to be euploid for a transfer to take place, thus limiting the chance of a transfer occurring. An RCT by Coates et al. (77) compared these 2 transfer approaches for euploid embryos and found that the ongoing pregnancy and live birth rates were significantly higher in the frozen blastocyst transfer group (80% vs. 61% and 77% vs. 59%, respectively). In addition, Franasiak et al. (78) initiated the discussion that perhaps slow blastulating embryos have lower implantation rates due to embryo/endometrial dyssynchrony and not just reduced embryo quality. In a retrospective review, they found that the implantation rates of fresh day 6 ETs were significantly lower than those of fresh day 5 ETs in individuals aged <35 years (52% vs. 63%) and in those aged ≥ 35 years (32% vs. 48%). However, when day 6 embryos were vitrified and then transferred, they had implantation rates comparable to those of day 5 embryos in both groups (57% vs. 60% in those aged <35 years) and (37% vs. 42% in those aged ≥ 35 years) (78).

There are still advantages to the fresh ET approach that make it a reasonable option, even considering the lower live birth rates determined in this study. In addition to the lower cost, the chance of a more immediate positive outcome compared with that of freeze-all cycles where patients have to wait weeks to months to attempt transfer may be regarded as less stressful. Either approach seems to be a reasonable option, but studies suggest a trend in better outcomes with the freeze-all option when using known euploid embryos.

Another factor to consider is whether there is a benefit to transferring a single euploid embryo compared with that of double ET of untested embryos. Recent practice has shifted to the increasing recommendation to offer eSETs to patients with the primary goal of decreasing multiple gestations and the negative sequelae that can follow. With advances in the field, multiple gestation is almost entirely preventable with the transfer of a single embryo at a time. The original reluctance to accept eSET stemmed from the decreasing chance of live birth compared with that of the transfer of ≥ 2 embryos. With the advent of PGT-A to select out the leading cause of implantation failure and miscarriage, embryonic aneuploidy, the belief is that the success rate of eSET can approach that of the transfer of double ET while continuing to minimize the risk of multiple gestation. A randomized noninferiority trial by Forman et al. (79) investigated whether transferring a single euploid blastocyst can result in an ongoing pregnancy rate equivalent to transferring 2 untested blastocysts. They found that in women aged ≤ 42 years, transferring a single euploid blastocyst resulted in similar ongoing pregnancy rates to transferring 2 untested blastocysts and dramatically reduced the risk of twins. In addition, there was no significant difference in the ongoing pregnancy rates of the PGT-A SET arm that had a fresh or frozen ET. Clinical practices have reflected the results of this study and guidelines for transfer of a known single euploid embryo as the current recommendation in all age groups.

EMBRYOLOGY PROTOCOLS

Laboratory Conditions

It is well known that the conditions of the IVF laboratory can have a significant impact on the success of an IVF cycle. As such, laboratory factors are an interesting topic for research regarding their influence on the implantation of a euploid blastocyst. Currently, studies have investigated the timing of the embryo biopsy, size of the trophectoderm biopsy, type of culture used to grow the embryos, and other factors such as the temperature of the incubator where the embryos are cultured.

Timing of the Embryo Biopsy

Since its introduction in the early 1990s, PGT has been used extensively to assist in the selection of the best embryo. However, there are only a handful of studies examining the impact of biopsy on embryo implantation. Initial studies first examined the timing of the embryo biopsy, comparing blastomere biopsy of cleavage-stage embryos vs. trophectoderm biopsy of blastocysts and its effect on embryo outcomes. Scott et al. (80) demonstrated that cleavage-stage biopsies can provide adequate DNA samples for analysis but can also be detrimental to the embryo. A 2013 RCT by the same group identified that only 30% of cleavage-stage biopsied embryos sustained implantation and led to live-born infants vs. 50% of unbiopsied controls. In contrast, they found that embryos biopsied at the blastocyst stage had equivalent implantation rates (51% vs. 54%) as unbiopsied controls (81). Another study by Mastenbroek et al. (82) of 408 embryos from women aged 35–41 years demonstrated significantly reduced ongoing pregnancy rates in women assigned to cleavage-stage biopsy (25%) compared with unbiopsied controls (37%). Together, these data demonstrate reduced viability and implantation rates with cleavage-stage embryo biopsies, although early studies of PGT-A using blastomere biopsy may be limited.

Size of the Trophectoderm Biopsy

There are also data to suggest that the size of the biopsy obtained during PGT-A has an impact on the euploid embryo implantation rates. This could explain why blastomere biopsies can have a detrimental impact on embryo viability and implantation because a cleavage-stage biopsy requires the removal of 1 or 2 cells from an embryo containing only 6–10 cells. In contrast, embryologists are trained to remove approximately 5–10 trophectoderm cells from a blastocyst, which can contain hundreds of cells.

A 2017 retrospective study by Neal et al. (83) examined the impact of the biopsy size on reproductive outcomes in over 1,100 embryos. They used relative DNA content as a surrogate for characterizing the size of the trophectoderm biopsy because more cells in a sample will have more DNA content, although the exact cellularity could not be determined. Patients were stratified into quartiles based on the highest

relative DNA content. This study found that the trophectoderm biopsies with the relatively highest DNA content were correlated with a lower chance of implantation and ongoing pregnancy. The fourth quartile (corresponding to the highest DNA content) had significantly lower ongoing pregnancy and live birth rates than the other 3 groups (relative risk, 0.84; 99% confidence interval, 0.75–0.95). It is important to note that determining the exact number of biopsied cells is difficult, and purposely trying to obtain a smaller biopsy could lead to uninterpretable results. Therefore, the investigators caution to providing guidelines regarding the optimal size of the trophectoderm biopsy.

Type of Culture Media

The type of culture media used for embryonic growth is another interesting avenue to explore the clinical outcomes of embryo potential. Currently, there are 2 widely used methods for culturing embryos: monophasic media and sequential media. Monophasic or single-step formulations use only 1 type of culture to support the growth and development of the embryo to the blastocyst stage. Sequential media uses a 2-step approach by changing the constituents of the media after the third day of development to mimic the changing metabolic and physiologic processes of the growing embryo. While both approaches demonstrate excellent clinical outcomes, there are little data on the superiority of one over the other. As far as advantages, monophasic medium is more cost-effective and simpler to use as it generally requires less embryo manipulation than sequential medium. A 2015 paired prospective RCT by Werner et al. (84) assessed the blastulation, aneuploidy, and implantation rates among sibling zygotes that were cultured in either monophasic or sequential media. A total of 2,257 fertilized eggs were included in the study, along with 168 patients who completed an ET. A significantly higher blastulation rate was observed with sequential media (55% vs. 47%); however, the implantation rates were equivalent between the 2 groups. The euploidy rates were also similar between the 2 groups. It is important to note that the medium was not "renewed" on day 3 of development in the monophasic group, which is the standard practice in several IVF laboratories. In addition, sequential culture was the default method used at this center, which may introduce the possibility of a bias. Lastly, these findings only apply to the specific medium formulations in this study.

Culture Temperature

Other parameters in embryo culture have also been refined and studied, including temperature, pH, and oxygen tension. In an effort to mimic in vivo conditions, 37 °C has been selected as the default cell culture temperature because this is the standard human core body temperature. This idea has been challenged because of evidence that the reproductive axis may have slightly cooler temperatures. An RCT by Hong et al. (85) sought to evaluate the impact of culture temperature on embryo development in sibling oocytes. After intracytoplasmic sperm injection, over 800 oocytes were randomized to undergo culture at either 36 °C or 37 °C. The mean fertilization rates were similar between the 2 groups; however, the cooler 36 °C group had significantly fewer cells on day 3 and lower blastulation rates (51% vs. 60%) than the standard 37 °C group. The secondary analysis revealed that the implantation and aneuploidy rates were similar between the 2 groups. The results were also consistent in couples undergoing double ET.

Dynamic vs. Static Embryo Culture

An RCT by Juneau et al. (86) in 2020 also examined the impact of using a dynamic culture system on the reproductive potential of embryos. The thought process is that traditional IVF culture techniques are static, forcing the embryo to be "stagnant" throughout its development. In contrast, an in vivo embryo is in constant motion as it is subject to the peristalsis and ciliary actions of the fallopian tube as it makes its way to the uterus. Over 1,200 2-pronuclear zygotes were randomized to be cultured in either dynamic or traditional "static" media. The blastulation rates were similar between both cultures, as were the euploidy rates. To standardize the implantation and pregnancy rates, 72 patients served as their own control by undergoing a double ET (one cultured in a dynamic system and the other cultured in a traditional fashion). Per ET red, there was no difference in the implantation rates between the 2 cultures. However, this study only included women with normal ovarian reserve and only analyzed 1 type of a dynamic culture system. More research is needed to fully elucidate the benefits (if any) of different types of dynamic culture media in a wider range of patients.

Number of Vitrification Cycles

In approximately 2%–5% of biopsies, PGT-A fails to yield a diagnostic result. An inconclusive result is reported to occur in 0.86%–3.8% of cases (87). The option to rebiopsy a no-result blastocyst requires warming, followed by a second round of biopsy and vitrification. There are mixed data on the impact of multiple vitrification and biopsy cycles on clinical outcomes. One retrospective cohort study by De Vos et al. (88) analyzed the impact of 2 rounds of vitrification with ≥ 1 rounds of biopsy. A total of 154 blastocysts were warmed, of which 126 were biopsied and underwent a second round of vitrification. Ninety-two embryos were biopsied for the first time, and 68 underwent a second biopsy, of which a total of 77 were found to be euploid by PGT-A. In this small cohort, 51 blastocysts were subsequently warmed for an FET. The study found comparable clinical pregnancy rates in embryos that underwent double vitrification with a single biopsy (44%) to those in controls that underwent single vitrification and single biopsy (46%). However, there was a trend toward lower clinical pregnancy rates in the double vitrification and double biopsy group (35%), which was not statistically significant. These findings were corroborated by Parriego et al. (89), who found a detrimental effect of double vitrification and double biopsy, as did Neal et al. (90) and Bradley et al. (91). Neal et al. (90) found that embryos that underwent 2 vitrifications and 1 biopsy ($n = 3452$) had ongoing pregnancy and clinical loss rates of 63.2% and 9.8%, respectively, compared

with 50% and 21.7% in embryos that underwent double vitrification and double biopsy (n = 36) (P=.08). Bradley et al. (91) found that double vitrified and double biopsied embryos had a significantly reduced clinical pregnancy rate (31% vs. 54.3%) compared with single vitrification and single biopsy embryos (P=.13). On the other hand, 2 studies found that blastocysts can tolerate a second round of biopsy without compromising clinical pregnancy and live birth rates (87, 92). Although the data are mixed, it appears that at least multiple rounds of vitrification and biopsy may impact the implantation of euploid embryos.

CONCLUSIONS

The goal of PGT-A is to eliminate aneuploid embryos from transfer selection, thus improving the chances of producing a live birth from each transfer. Whether this strategy should be performed in all IVF cycles is beyond the scope of this review. However, by analyzing outcomes from euploid blastocyst transfer, controlling for ploidy, many of the variables involved in IVF success can be more critically examined. This review analyzed the current literature to study the effects of embryonic factors, uterine factors, parental factors, stimulation protocols, and laboratory protocols on euploid embryo success rates. Although the data are limited and mostly retrospective, some conclusions can be made.

Embryonic Factors

In terms of embryonic factors, morphology seems to play the most prominent role in embryo implantation potential. Among euploid blastocysts, preference should be given to the embryo with the best overall morphological grade. If 2 embryos have similar grades, preference may be considered for the blastocyst with the better ICM grade. In addition, preference should also be given to embryos that develop into blastocysts on day 5 or 6 over those that have develop delayed until day 7 and those with an intact zona pellucida.

Uterine Factors

In terms of uterine factors, synchrony between embryo and endometrium as indicated by the P4 level before ET appears to be more predictive of implantation success than the appearance or the thickness of the lining itself. The role of endometrial compaction on outcomes after frozen ET needs further study. Endometrial scratch, on the other hand, has been shown to have no effect on clinical pregnancy rates. Although we were not able to find evidence specific to euploid blastocyst transfers, improved reproductive outcomes after treatment for chronic endometritis suggest that evaluation for this should be considered in patients with a history of implantation failure. The presence of adenomyosis appears to be associated with a higher risk of euploid loss, particularly in women with symptomatic disease. At this time, routine screening for adenomyosis in asymptomatic women is not recommended. There is only limited evidence to suggest that difficult euploid blastocyst transfers may have a negative effect on live birth rates. We recommend trying to anticipate and plan accordingly for such challenges. Lastly, the presence of an arcuate uterus does not appear to impact the success of euploid ET.

Patient Factors

Maternal (BMI) seems to have the most significant impact on the euploid blastocyst transfer success rates of the patient factors analyzed in this review. Overweight and obese patients are significantly more like to experience a spontaneous abortion, even after controlling for euploid status. In addition, it is common practice to recommend maintaining the TSH level below 2.5 mIU/L in early pregnancy because higher levels may be linked with spontaneous abortion, but there does not appear to be a benefit to having lower levels within this range. There may be a maternal age effect with reduced implantation of euploid blastocysts over the age of 40 years, but they still maintain high success rates. There does not appear to be a negative effect on euploid blastocyst implantation from advanced paternal age or vitamin D status. The role of MTHFR gene mutation status, particularly in the embryo, is an area that could be further investigated.

IVF Protocols

The differing types of IVF protocols were also investigated in this review. Recent studies have demonstrated that aggressive ovarian stimulation protocols may increase the number of euploid embryos obtained per cycle due to a proportional increase in oocytes retrieved (93,94). Moreover, there appear to be no toxic effects noted with gonadotropin administration on embryo ploidy status or reproductive potential. Regarding frozen ET protocols, there does not appear to be an appreciable difference in the clinical pregnancy, implantation, and miscarriage rates between an NC FET vs. an AC FET in ovulatory women. The P4 levels have also been investigated extensively. While there is no consensus on the optimal P4 level at the time of transfer, there is evidence that elevated levels above a minimum threshold are deleterious to implantation. Regarding fresh vs. frozen ETs, the sum of the data points toward frozen transfer potentially improving outcomes in select patient populations, primarily through better synchronization of the embryo endometrial relationship.

Laboratory Factors

Finally, the conditions and protocols used in the IVF laboratory can also impact the euploid blastocyst transfer success rates. The current data point to trophectoderm biopsy being superior to cleavage-stage biopsy, as the latter has demonstrated reduced viability and implantation potential with cleavage-stage embryo biopsies. This is likely due to the size of the embryo biopsy relative to the embryo's total size, as larger blastocysts' biopsy samples have also correlated with decreased success rates. At this time, there are 2 types of embryo culture media routinely available (monophasic vs. sequential media). There does not appear to be a significant difference in embryo development, euploidy rate, or implantation between these types of medium formulations. Lastly, there is evidence that double vitrification/double

biopsy of a euploid embryo can negatively impact the implantation and pregnancy rates, whereas double vitrification/single biopsy may not.

In summary, a variety of potential variables impacting IVF success have been reviewed retrospectively and prospectively. Prior literature is clouded by variability in embryo quality, particularly the chromosomal status of the transferred embryos. By analyzing the outcomes of euploid blastocysts exclusively, we believe to clarify the factors that truly have a significant impact on IVF outcomes and use this information to provide recommendations for optimizing the success of euploid blastocyst transfers.

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