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Geeta Bhat Date

A Study of Potential Factors that Impact Implantation Rate
of Assisted Reproductive Technology Procedures

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A Study of Potential Factors that Impact Implantation Rate of Assisted Reproductive Technology Procedures

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2009

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Abstract

A Study of Potential Factors that Impact Implantation Rate
of Assisted Reproductive Technology Procedures
By Geeta Bhat

Purpose: Assisted Reproductive Technology (ART) procedures are becoming increasingly popular as an option to overcome infertility. The expense, time, and effort needed for an ART procedure, however, do not guarantee success. The purpose of the current study was to identify factors that impact implantation rate, determined by dividing the total number of fetal hearts detected on an ultrasound by the total number of embryos transferred per cycle, of ART procedures. ART success can be defined in various ways. Frequently used ART success measures include singleton pregnancy rate, multiple pregnancy rate, singleton live birth rate, and multiple live birth rate. *Methods:* Logistic regression was used to identify individual factors that were significantly associated with implantation rate. A multivariate model was constructed using factors that were significant in bivariate analyses. The factors found to be significant in bivariate analyses were: maternal age, race, body mass index (BMI), nulligravidas, no prior spontaneous abortions, reason for ART, treatment protocol, day of embryo transfer, number of embryos cryopreserved, and use of intracytoplasmic sperm injection (ICSI). *Results:* Increasing age, 'other' race, BMI ≥25, and nulligravida, were significantly associated with decreased odds of obtaining an implantation rate greater than zero. No prior spontaneous abortions, single male factor as reason for ART, day of embryo transfer ≥5, and use of ICSI were significantly associated with increased odds of obtaining an implantation rate greater than zero. Odds of obtaining an implantation rate greater than zero increased as the number of cryopreserved increased. Compared with Agonist Suppression+Folicle Stimulating Hormone (FSH), other treatment protocols had decreased odds of obtaining an implantation rate greater than zero. *Conclusions:* This analysis used implantation rate per ART cycle as a measure of success to confirm several associations noted in previous studies that have used other measures of ART success.

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INTRODUCTION

In 2002, approximately 2% of the 62 million women (~1.2 million women) of reproductive age had an infertility related medical appointment in the previous year (1). Assisted reproductive technology (ART) procedures have been used to overcome infertility since 1978 (2). ART includes all treatments or procedures that involve the handling of human eggs and sperm in a laboratory to establish a pregnancy. United States ART programs must report all ART cycles, even those discontinued before all steps were undertaken, along with outcomes to the Centers for Disease Control and Prevention (CDC) annually as per the Fertility Clinic Success Rate and Certification Act (FCSRCA) passed by Congress in 1992 (2). CDC uses this data to publish an annual ART Success Rates Report.

The CDC National ART Surveillance System (NASS) was implemented to collect and monitor ART use and outcomes as a response to the FCSRCA. The American Society for Reproductive Medicine (ASRM) and the Society of Assisted Reproductive Technology (SART) have partnered with the Division of Reproductive Health at CDC to conduct the ART surveillance system. This study will include analyses of ART patient cycles collected from and reported by clinics providing ART treatment in the United States. This study proposes to use implantation rate per ART cycle as a measure of success. Implantation rate as a success rate was first reported in the 2008 Assisted Reproductive Technology Success Rates National Summary and Fertility Clinic Reports. The primary aim of this study is to identify maternal and treatment factors that impact the implantation rate of an ART procedure in patients undergoing ART for the first time.

LITERATURE REVIEW

ART procedures include infertility treatments in which human eggs and sperm are handled inside a laboratory to establish a pregnancy (2). This includes in vitro fertilization (IVF), gamete intrafallopian transfer (GIFT), and zygote intrafallopian transfer (ZIFT) (1). Depending on the type of treatment, a typical ART procedure begins when a woman starts taking drugs to stimulate egg production or her ovaries are monitored with the intent of transferring embryos to her uterus (2). If eggs are produced, then the eggs are retrieved and combined with sperm in the laboratory (in vitro fertilization). Embryos can then be transferred to the uterus or cryopreserved and thawed for use in a later cycle (2). Embryos can be classified as fresh non-donor, fresh donor, frozen non-donor, and frozen donor. Non-donor eggs are the patient's own eggs. An ART 'cycle' is defined as a process in which 1) a woman has undergone ovarian stimulation or monitoring with the intent of having an ART procedure (even if the cycle was subsequently canceled or no embryos were transferred) or 2) embryos have been thawed with the intent of transferring them to a woman (1).

Success rates can be measured at different phases of an ART cycle. Each measure of success gives slightly different information about the complex ART process. An ART cycle begins when the patient starts taking medication to stimulate development of eggs by the ovaries or when the patient's ovaries begin to be monitored for natural egg production. If and when eggs are produced, the eggs are retrieved and combined with sperm in a laboratory. Successful fertilization yields embryos that can be transferred into a woman's uterus. If one or more of the transferred embryos implant in the uterus, the cycle can progress toward a clinical pregnancy. A pregnancy can then yield a live birth.

Success rates are calculated at various steps of an ART cycle and help provide a better understanding of the chances for success as the cycle progresses. Success rates using different measures are provided in the annual ART Success Rates Report. These measures are: percentage of ART cycles that produced a pregnancy, percentage of ART cycles started that resulted in a live birth, percentage of ART cycles in which eggs were retrieved that resulted in a live birth, percentage of ART cycles in which an embryo or egg and sperm transfer occurred that resulted in a live birth, percentage of ART cycles started that resulted in a singleton live birth, and percentage of ART cycles in which an embryo or egg and sperm transfer occurred that resulted in a singleton live birth (1).

A patient's chance of having a successful pregnancy and live birth through ART is influenced by many factors. The number of embryos transferred in an ART procedure is an important factor when considering the association between ART and success of pregnancy (3). Approximately 1 percent of United States infants born in 2006 were conceived using ART. The United States has no federal regulation on the practice of assisted reproduction. The American Society for Reproductive Medicine (ASRM) in conjunction with the Society for Assisted Reproductive Technology (SART) published guidelines recommending maximal numbers of embryos for transfer according to the woman's age, quality of embryos, and opportunity for embryo cryopreservation (4). There is an inverse relationship between age and the success of an ART procedure (2). Increasing maternal age is significantly associated with reduced odds of conception and live birth (5). Embryo transfer guidelines allow a higher limit for older women compared to younger women (4).

Independent of age, the following factors have been noted as “favorable” prognosis characteristics: first cycle of IVF, good quality embryo, and excess embryos of sufficient quality to warrant cryopreservation. ASRM/SART recommendations based on data available in the year 2007 limit the number of cleavage stage embryos (embryos after 2 or 3 days of fertilization) to transfer to 2 for women under 35 years. For women ages 35-37, no more than 3 embryos should be transferred. For women ages 38-40, the embryo transfer limit increases to 4 embryos. The transfer limit for women ages 41-42 is 5 embryos. For favorable prognosis cleavage stage embryos, the transfer limit per age group decreases by one embryo, except for those women ages 41-42. Recommendations for the transfer of blastocysts (embryos 5 or 6 days after fertilization) are lower. For women under 35, either 1 favorable prognosis blastocyst stage embryo can be transferred or 2 blastocyst stage embryos total can be transferred. For women under ages 35-37, the recommended limit is 2 blastocyst stage embryos. Women between ages 38-40 should limit transfers after 2 favorable blastocyst stage embryos or 3 blastocyst stage embryos total. The recommended limit for women ages 41-42 is 3 blastocyst stage embryos. The recommendations for blastocyst stage embryos are lower because they have higher implantation rates compared to cleavage stage embryos (4). A review of guidelines for the number of embryos to transfer following in vitro fertilization yielded recommendations similar to those of ASRM and SART (6).

Cycles that use fresh non-donor embryos are the most common procedure type, accounting for 70% of cycles. In SART member clinics, there has been a marked reduction in the percentage of fresh non-donor cycles in which four or more embryos were transferred in women under 35 years of age, between the years of 1996 and 2003

(7). Declines in the number of embryo transfers for women ages 38-41 were not significant. (8).

Success of an ART procedure may be influenced by various factors, independent of age and the number of embryos transferred. Previous studies have researched various maternal and treatment variables that could potentially impact ART success. There is an increased risk of spontaneous abortion for women with one or more prior spontaneous abortions (9). Use of intracytoplasmic sperm injection (ICSI) has been associated with increased odds of pregnancy (10). In ICSI, a single sperm is injected into the woman's egg. ICSI is a specialized technique used in some IVF procedures. ICSI and assisted hatching have also been found to have positive effects on conception and continuation of pregnancy through the first trimester (5). A variety of assisted hatching techniques have been used to assist embryo hatching. Assisted hatching involves artificial disruption of the zona pellucida. It has been proposed as a method to improve the implantation capacity of an embryo. Randomized controlled trials studying effectiveness of assisted hatching have revealed that there is no difference in implantation or pregnancy rates between treatment and control groups (5, 11-13). Factors such as the embryologist used for transfer, embryo transfer duration, and type of ART procedure have also been noted to influence pregnancy rates (3, 10).

Demographic factors can also influence pregnancy outcome of an ART procedure. Several studies agree that there is a lower chance of pregnancy for African Americans, Hispanics, and Asians compared with Caucasians (5, 14, 15). Although Asian ART patients have similar baseline characteristics as Caucasian ART patients, they have a decreased pregnancy rate and live birth rate (14). An increased preterm delivery rate

has been noted amongst African American and Hispanic women compared to Caucasian women as well (15). Even within Body Mass Index (BMI) categories, there exist significant disparities in pregnancy and live birth rates by race and ethnicity (16). The results of these studies may have been influenced by a multitude of cultural, social, nutritional, and environmental factors.

Higher BMI in females receiving ART procedures is associated with an increased failure to achieve a clinical intrauterine gestation. This risk is reduced with the use of donor oocytes. The adverse effects of increasing BMI amongst patients who used non-donor oocytes were found to be greater women under 35 compared with older women (17). In a study limited to obese women using donor oocytes, reduced implantation and pregnancy rates were reported, along with higher miscarriage rates (18).

Several studies have explored various aspects of existing stimulation protocols to optimize treatment. Treatment protocols have been compared to determine whether clinical pregnancy and live birth rates differ based on protocol used. A retrospective study which reviewed charts of first time IVF cycles concluded that clinical pregnancy and live birth rates are similar using either a gonadatropin releasing hormone (GnRH) agonist of antagonist (19). Most IVF programs use long GnRH agonist protocols for ovarian stimulation. More recently, GnRH antagonists that induce a rapid suppression of gonadotrophin secretion have become available. GnRH antagonists can be administered at mid-cycle, and this is useful for patients with decreased ovarian reserve (20). A randomized controlled trial of poor responders to ovarian stimulation found that a multi-dose GnRH antagonist protocol appears to be at least as effective as a long agonist protocol. Clinical pregnancy rates in this study were higher for the antagonist group, but

the difference was not statistically significant (20). A previous randomized controlled trial that studied ART treatment protocol in poor responders found a non-significant trend for improvement in clinical pregnancy and implantation rate in the antagonist group (21). A systematic literature search conducted by the European Society of Human Reproduction and Embryology has found however, that most comparative studies suggest a non-significant reduction in the probability of pregnancy after IVF using GnRH antagonist versus GNRH agonist. The ESHRE acknowledges that the role of GnRH antagonists in ovarian stimulation for IVF appears to be promising, but doses and effects must be studied further (22).

Transferring an embryo on day five of culture versus day three of culture has been suggested to improve implantation rate. Studying embryo quality at different stages such as day of embryo transfer may be an option when selecting an embryo with a high chance of implantation. In a comparative study of embryo culture regimes in a private practice, implantation rates for day five transfers were found to be twice that of embryos transferred on day three (23). Other prospective studies have found, however, that day three and day five transfers have similar pregnancy and implantation rates (24, 25). These studies contend that implantation rates can be better assessed after an extended culture period. Five days of culture allows the transfer of a reduced number of embryos without decreasing the overall pregnancy rate (25).

Measuring the success of an ART procedure is a much debated question. Outcome measures can include implantation rate, singleton and multiple pregnancy rates, and singleton and multiple live birth rates. The adverse effects of multiple pregnancy and multiple birth have drawn focus to the promise of elective single embryo transfer.

Redefining ART success as a singleton live birth is a proposal that is starting to be given consideration (26).

METHODS

ART cycles conducted in 2008 and reported to the NASS were used for this retrospective cohort study. This study was submitted to and approved by Emory Institutional Review Board. The goal of this study was to determine maternal and treatment factors associated with implantation rate for a cycle for patients undergoing ART for the first time. Only patients with no prior ART cycles were selected to avoid clustering of observations on patient characteristics and to avoid random effects modeling. Additionally, first cycle of IVF has been previously noted as a favorable prognosis characteristic (4).

Implantation rate for any given cycle is calculated by dividing the total number of fetal hearts detected by the total number of embryos transferred in that cycle and multiplying by 100. The number of fetal hearts detected is defined as the maximum number of fetal hearts detected on ultrasound, prior to any reduction in the number of embryos.

The study population was limited to ART cycles that were initiated in 2008. This is the most recent ART surveillance data available. From the 104,673 cycles initiated in 2008, those that used fresh, non-donor embryos (60,487 cycles) were selected. From these cycles, cycles were selected for which it was the patient's first visit (57,587 cycles). Patients were excluded if they had undergone prior ART procedures. From this subset of cycles, only cycles that were not cancelled or cycles that progressed to the transfer of one or more embryos were selected. The resulting data set consisted of 47,316 cycles for analysis.

Descriptive statistics were performed on all predictor variables [maternal age, maternal race, maternal ethnicity, maternal BMI, gravidity, prior spontaneous abortions, reason for ART, reason for ART grouped by male and/or female factors, ART treatment protocol, day of embryo transfer, number of embryos cryopreserved, use of ICSI]. Average implantation rate per cycle was calculated for each stratum of all predictor variables. Average implantation rate per cycle was obtained by dividing the sum of the implantation rate per cycle for each stratum by the total number of cycles in the stratum. In addition to the demographic factors of age, race, ethnicity, and BMI, the maternal factors studied included gravidity, number of spontaneous abortions, and reason for ART. Patient age at cycle initiation was measured in years and categorized as <35 [reference group], 35-37, 38-40, 41-42, and >42 years. Race was categorized as White [reference group] and Other. Patients that were reported as Black or African American, Asian, Native Hawaiian or other Pacific Islander, American Indian or Alaska Native were categorized as Other due to sample size limitations. Patient ethnicity was classified in the data set as Hispanic or Latino [reference], Not Hispanic or Latino, and Unknown. BMI was calculated from the patient's height and weight that were recorded in the data set. BMI was categorized as underweight (<18.5), normal weight (18.5-24.9) [reference group], overweight (25-29.9), and obesity (>30). Gravidity and number of prior spontaneous abortions were categorized as 1, 2, 3, and ≥4 due to sample size limitations. Reasons for ART documented were: Tubal Factor, Ovulation Disorders, Diminished Ovarian Reserve, Endometriosis, Uterine Factor, Male Factor, Other Factor, Unknown Factor, Multiple Female Factors, and Multiple Factors: Male Factors and Female Factors. Treatment factors studied included treatment protocol, day of embryo transfer, number of

embryos cryopreserved, and use of ICSI. Treatment protocol was categorized as Agonist Suppression + Follicle Stimulating Hormone (FSH), Antagonist Suppression + FSH, Agonist Flare + FSH, FSH Only, and Others. The four specific treatment protocols account for greater than 90% of procedures.

Bivariate analyses were conducted for each predictor to assess significance of the variable. A logistic regression model was run for each predictor versus the dependant binary variable implantation rate zero or greater than zero (at least one fetal heart detected on ultrasound). Gravidity and number of previous spontaneous abortions were made into dichotomous variable. Reason for ART categories were aggregated as follows: Tubal Factor, Ovulation Disorders, Diminished Ovarian Reserve, Endometriosis, and Uterine Factor were classified as single female factor. Male factor was classified as single male factor. Multiple female factors and multiple factors: male factors and female factors remained the same in the new classification. The resulting reason for ART categories grouped by male and/or female factors were: single female factor, single male factor, multiple female factors, multiple factors: male factors and female factors [reference group], and unknown/other factors. Day of transfer was analyzed as ≥5 versus day 0-4.

Variables that were not significant (p-value <.05) in bivariate analysis were not included in the multiple regression model. All other exposure variables were included in the multivariable model. Chunk tests were performed for each variable significant in bivariate analyses to assess significance of the variable as a whole. Multiple logistic regression was used to model the probability of obtaining an implantation rate greater than zero (at least one fetal heart detected on ultrasound). Data were analyzed using Statistical Analysis Software, version 9.2 (SAS, Inc., Cary, NC).

RESULTS

The percentage distribution implantation rate (equal to 0 versus greater than 0) transferred per stratum of each predictor variable is presented in Table 1A-1C. These tables also show the average implantation rate per cycle for each stratum of all predictor variables. Demographic predictor variables are located in Table 1A. Maternal predictor variables are located in Table 1B, and treatment predictor variables are located in Table 1C.

Implantation rate per cycle ranged from 0% to 300%. Implantation rate may be expected to range from 0% to 100%, but this is not always the case. In this study population, 0.6% of cycles were calculated to have an implantation rate greater than 100% because of possible splitting of embryos after transfer. An implantation rate of 0% was calculated for 54.25% of cycles in this study population.

A comparison of these descriptive statistics suggests that average implantation rate per cycle differs based on patient characteristics. Patients under the age of 35 account for 51.53% of the study population. Average implantation rate per cycle decreases as maternal age increases. Race was unknown for 40.13% of the study population, and 47.62% of the study population consisted of White women. The remaining 12.41% were Black or African American, Asian, Native Hawaiian or other Pacific Islander, or American Indian or Alaska Native. Of patients included in this study, 45.07% had a BMI classified as ‘normal,’ or between 18.5 and 24.9. Average implantation rate per cycle was approximately 32% for those patients with BMI categorized as underweight or normal and 27.57% for those with a BMI ≥30. Average implantation rate per cycle increases as the number of embryos cryopreserved increases, but 58.49% of patients did not

cryopreserve any embryos. Increasing gravidity also corresponds with increasing average implantation rate. Patients who had no prior abortions had the highest average implantation rate and accounted for 73.85% of the cycles. ICSI was performed on 71.35% of patients. Use of ICSI did not appear to change the average implantation rate by more than 3%. Male Factor and Multiple Factors: Male Factors and Female Factors were the most frequent reasons for ART. The two most frequent protocols were Agonist Suppression+FSH and Antagonist Suppression+FSH, with Agonist Suppression+FSH accounting for 57.41% of all patient cycles in the study population. Agonist Suppression+FSH also yielded the highest average implantation rate per cycle, 34.85%, when compared with other treatment protocols. Day 3 embryo transfer accounted for 52.08% of the study population. Embryos transferred on day 5 had the highest average implantation rate, 41.48%, when compared with embryos transferred on other days.

The results of the bivariate logistic regression model for the probability of an implantation rate greater than zero are shown in Table 2. An implantation rate of 0% was considered as a failure, and any implantation rate greater than 0% was considered a success. All categorical levels of age, race, number of embryos cryopreserved, and treatment protocol were significant (p-value <.05) predictors of implantation rate in bivariate analyses. Nulligravida, no prior spontaneous abortions, and embryo transfer day ≥5 were also significant predictors of implantation rate in the bivariate analyses. Patient's ethnicity was not significant at any categorical level. Patient BMI was significant for BMI levels ≥18.5. All reasons for ART categories except single female factor were significant predictors of implantation rate in the bivariate analyses. All variables except ethnicity were significant at the overall level as well.

Table 2 also presents the results of the multivariable model containing all exposure variables except ethnicity, which was not significant in a bivariate model. P-values for the chunk tests for each variable are displayed in Table 2 as well. Increasing maternal age was associated with significantly decreased odds of implantation rate. When compared with White women, women of other races had a decreased probability of obtaining an implantation rate greater than zero. Women of other races had 0.77 times the odds of obtaining an implantation rate greater than zero when compared with White women. Women with a BMI ≥25, categorized as overweight or obese, had a decreased probability of obtaining an implantation rate greater than zero compared with women with a normal BMI between 18.5-24.9. Obese women had 0.82 times the odds of obtaining an implantation rate greater than zero than women with normal BMI, and overweight women had 0.94 times the odds of obtaining an implantation rate greater than zero compared with women with normal BMI. Compared with patients who cryopreserved ≥4 embryos, those who cryopreserved either 0, 1, 2 or 3 embryos had significantly decreased odds of obtaining an implantation rate greater than zero. The odds of obtaining an implantation rate greater than zero increased as the number of embryos cryopreserved increased. Nulligravidas had significantly decreased probability of obtaining an implantation rate greater than zero. They had 0.88 times the odds of obtaining an implantation rate greater than zero compared with women who had previously been pregnant. No prior spontaneous abortions, procedures using ICSI, and embryo transferred ≥5 days were all associated with increased odds for an implantation rate greater than zero. Single Male Factor was the only Reason for Art which was significantly associated with implantation rate. The odds of obtaining an implantation rate

greater than zero was 1.09 for those patients with single male factor compared to those patients with Multiple Factors: Male and Female. All categories of treatment protocol were significantly associated with implantation rate. Patients who received Antagonist Suppression+FSH had 0.84 times the odds of obtaining an implantation rate greater than zero compared to patients who received Agonist Suppression+FSH, as did patients who received FSH Only. Agonist Flare+FSH had decreased odds of obtaining an implantation rate greater than zero compared to those who received Agonist Suppression+FSH. The adjusted odds ratio for Agonist Flare+FSH was 0.78. Those with a treatment protocol of 'other' had 0.72 the odds of obtaining an implantation rate greater than zero compared to those that received Agonist Suppression+FSH.

DISCUSSION

These analyses examined some well established predictors of ART outcome using implantation rate per cycle as an ART success measure. The findings of these analyses confirm expected predictors of ART outcome and also present unique associations for certain variables that warrant further analysis in future studies. The average implantation rate per cycle for different strata of the predictor variables ranged from a low of 4.45% for women ages >42 to a high of 45.78% for women who had been pregnant previously or cryopreserved ≥4 embryos. Average implantation rate values are low overall because 54.25% of cycles had an implantation rate of 0, meaning that zero fetal hearts were detected on ultrasound for over half of ART cycles in this study population.

The finding from these analyses that increasing age is significantly associated with decreased ART success coincides with other studies (2, 3, 5).The finding that there is a lower odds of obtaining an implantation rate greater than zero for non-Whites compared with Whites is also supported by other studies (5, 14, 15). Results from this analysis pertaining to the effects of ICSI, nulligravida, and no prior spontaneous abortions are all in agreement with previous analyses that used a different measure of ART success, clinical intrauterine gestation, as the outcome variable (5). Increased odds of implantation rate greater than zero for embryos transferred ≥day 5 supports findings from previous studies (23). The decreased odds of implantation rate greater than zero for obese women agrees with findings from previous studies that have noted adverse effects of female obesity on ART pregnancy and live birth rates (17). Since previous literature has shown a reduction in the failure to achieve a clinical intrauterine gestation in women using donor oocytes, the possibility that embryo quality may be impaired among women

with higher BMI should be explored (17). The finding that Antagonist Suppression+FSH has decreased odds of implantation rate greater than zero compared to Agonist Suppression+FSH echoes previous literature which found similar results studying pregnancy rates (22).

Reason for ART is a variable that has the potential to be researched more extensively. This study found that single male factor increased the probability of obtaining a positive implantation rate and multiple female factors decreased this probability. The aggregation of reasons for ART is unique to this analysis. Further steps can be taken in future analyses to determine specific combinations of female factors that impact implantation rate. Number of embryos cryopreserved is another variable to possibly study further. Examining the relationship between embryo quality and embryo cryopreservation would be worthwhile. Embryo cryopreservation could be an indicator of higher embryo quality.

Strengths and Weaknesses

This study has several limitations. Due to the retrospective nature of this study, analysis was limited to the variables that already existed in the database. Only a limited number of predictor variables were available for analysis because the study population was obtained from a surveillance database that collects data on select patient and treatment characteristics. The data collected include information pertaining to the patient's medical history, clinical information for the ART procedure, and information on resulting pregnancies and birth. Co-morbid conditions such as diabetes mellitus, hypertension, and heart are not captured in this data set.

Another study limitation is that several variables were recategorized due to sample size limitations. In this study population, the race and ethnicity was classified as unknown for a large number of cycles. This causes the results for these variables to be biased. The race variable was recategorized as White and Other due to sample size limitations. Thus, differentiations between races termed as 'other' could not be made. Given the significant difference in odds of implantation rate between the two categories in this analysis, more detailed collection of race information may aid in better understanding the impact of the race variable on ART outcome.

The inability to confirm if embryo splitting after transfer contributed to implantation rates greater than 100% is another study weakness. This was the assumed explanation for obtaining implantation rates greater than 100%. In this study, any implantation rate greater than 0 was categorized as a success. Thus, including implantation rates greater than 100% could lead to misclassification bias if cycles where this occurred were in reality not due to an embryo's splitting after transfer. The results of this study may be reporting an increased number of implantation rate successes due to this bias. Since implantation rate is calculated by dividing the number of fetal hearts detected on an ultrasound by the number of embryos transferred, implantation rate is influenced by the number of embryos transferred in an ART cycle.

The lack of independence between implantation rate and the number of embryos transferred is another study limitation. This analysis assumes that given other characteristics, the implantation rate is independent of the number of embryos transferred. To the extent that this assumption does not hold, the results may be invalid and confounded by the number of embryos transferred and other risk factors for

implantation. Lastly, because this analysis only used data for patients undergoing their first cycle of ART, the generalizability of these results is limited.

A strength of this study is its use of a different measure of ART success as the outcome variable. Implantation rate in this study was calculated per ART cycle. It was not calculated as a percentage from the total number of ART cycles in the study population. Almost all of the exposure variables considered in this analysis were found to be significant predictors of implantation rate, and these findings correspond with other studies that assessed factors influencing ART success. Additionally, since the FCSRCA mandates that all ART procedures conducted in the United States be reported to the CDC, the dataset used for analysis is estimated to capture more than 92% of ART clinics (1). The large study population size allows greater power to assess relationships between predictor variables and implantation rate.

Continued data collection and research is necessary to better understand and draw conclusions concerning favorable predictors of ART success. However, from the current analyses, it is apparent that there are several factors that are associated with better rates of ART success. Implantation rate is a good measure of ART success that offers information about the likelihood of a successful implantation at one phase of the ART and pregnancy process.

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Table 1A. Percentage distribution of demographic factors by number of embryos transferred and the average implantation rate for U.S. women receiving ART, 2008

	Total Number	Number of Embryos Transferred (%)			Average Implantation Rate (per cycle)
DEMOGRAPHIC FACTORS	n	1	2	≥3	
Age (years)					
<35	24383	11.61	72.62	15.77	39.09%
35-37	10286	11.66	56.68	31.66	29.14%
38-40	8144	11.74	35.79	52.47	18.81%
41-42	2989	14.55	24.69	60.76	10.29%
>42	1514	19.68	22.79	57.53	4.45%
Race					
White	22531	11.27	60.18	28.55	31.86%
Other		13.34	55.54	31.12	25.97%
Unknown	18988	12.67	56.66	30.67	30.32%
Ethnicity					
Hispanic	2864	9.74	54.71	35.54	29.22%
Not Hispanic	29685	11.84	58.52	29.64	30.38%
Unknown	14767	13.03	58.22	28.75	31.03%

Table 1A. (continued) Percentage distribution of demographic factors by number of embryos transferred and the average implantation rate for U.S. women receiving ART, 2008

	Total Number	Number of Embryos Transferred (%)			Average Implantation Rate (per cycle)
DEMOGRAPHIC FACTORS	n	1	2	≥3	
Patient Body Mass Index					
Missing	9010	11.99	53.01	35.01	29.17%
<18.5 underweight	1178	16.21	59.34	24.45	32.62%
18.5-24.9 normal	21326	12.73	59.77	27.50	32.08%
25-29.9 overweight	8968	11.32	58.74	29.94	30.10%
≥30 obese	6834	10.52	59.19	30.29	27.57%

Table 1B. Percentage distribution of maternal factors by number of embryos transferred and the average implantation rate for U.S. women receiving ART, 2008

	Total Number	Number of Embryos Transferred (%)			Average Implantation Rate (per cycle)
MATERNAL FACTORS	n	1	2	≥3	
Gravidity					
Missing	141	9.93	41.84	48.23	20.44%
0	26107	11.35	61.69	26.96	32.08%
1	10238	12.22	55.32	32.46	35.78%
2	5383	13.82	53.72	32.45	39.23%
3	3020	14.37	52.58	33.05	40.53%
≥4	2427	12.86	50.56	36.59	45.78%
Prior Spontaneous Abortions					
Missing	339	15.04	35.40	49.56	18.80%
0	3491	12.03	59.93	28.04	31.77%
1	7666	11.51	55.01	33.49	27.94%
2	2759	12.79	51.90	35.30	25.83%
3	1026	13.26	51.56	35.19	26.35%
≥4	585	15.73	50.94	33.33	25.38%

Table 1B. (continued)Percentage distribution of maternal factors by number of embryos transferred and the average implantation rate for U.S. women receiving ART, 2008

	Total Number	Number of Embryos Transferred (%)			Average Implantation Rate (per cycle)
MATERNAL FACTORS	n	1	2	≥3	
Reason for ART					
Tubal Factor	4463	9.97	62.40	27.63	30.69%
Ovulation Disorders	3725	11.62	68.56	19.81	38.34%
Diminished Ovarian Reserve	3644	16.99	36.86	46.16	16.95%
Endometriosis	2388	9.80	62.27	27.93	33.71%
Uterine Factor	627	14.35	55.18	30.46	28.88%
Male Factor	9933	10.08	64.66	25.26	34.12%
Other Factor	3879	16.60	51.22	32.17	27.51%
Unknown Factor	6558	11.54	60.26	28.19	32.63%
Multiple Female Factors	4345	13.72	52.01	34.27	26.56%
Multiple Factors: Male and Female	7754	11.61	56.72	31.67	29.48%
Reason for ART by male and/or female factors					
Unknown/Other	10437	13.42	56.90	29.67	30.73%
Single Female Factor	14847	12.27	57.35	30.38	29.64%
Single Male Factor	9933	10.08	64.66	25.26	34.12%
Multiple Female Factors	4345	13.72	52.01	34.27	26.56%
Multiple Factors: Male and Female	7754	11.61	56.72	31.67	29.48%

Table 1C. Percentage distribution of treatment factors by number of embryos transferred and the average implantation rate for U.S. women receiving ART, 2008

	Total Number	Number of Embryos Transferred (%)			Average Implantation Rate (per cycle)
TREATMENT FACTORS	n	1	2	≥3	
Protocol					
Agonist Suppression+ FSH	27163	11.15	64.16	24.68	34.85%
Antagonist Supression+FSH	12968	12.20	51.20	36.61	25.46%
Agonist Flare + FSH	4445	13.72	45.80	40.47	20.62%
FSH Only	1304	9.66	54.29	36.04	29.52%
Others	1436	25.84	50.42	23.75	25.57%
Day of Transfer					
0-2	2077	24.75	45.31	29.95	18.05%
3	24644	9.53	48.40	42.08	23.66%
4	1222	12.36	42.96	44.68	24.20%
5	18321	13.72	73.15	13.13	41.48%
6	1052	18.35	70.34	11.31	31.85%

*FSH=Follicle Stimulating Hormone

Table 1C. (continued) Percentage distribution of treatment factors by number of embryos transferred and the average implantation rate for U.S. women receiving ART, 2008

	Total Number	Number of Embryos Transferred (%)			Average Implantation Rate (per cycle)
TREATMENT FACTORS	n	1	2	≥3	
Number of Embryos Cryopreserved					
Missing	435	14.02	51.49	34.48	26.82%
0	27673	13.28	47.58	39.14	22.54%
1	2898	8.70	68.98	22.33	35.78%
2	3960	8.28	74.60	17.12	39.23%
3	3067	9.46	73.82	16.73	40.53%
≥4	9283	12.00	74.62	13.38	45.78%
Intracytoplasmic sperm injection					
Missing	17	5.88	41.18	52.94	32.84%
Yes	33758	12.00	57.74	30.26	29.72%
No	13541	12.31	59.35	18.34	32.48%

**Number of embryos cryopreserved refers to number of fresh embryos cryopreserved

Table 2. Potential predictors for Implantation Rate Greater than Zero among US women receiving ART in 2008 (Odds ratio, adjusted odds ratio (AOR), 95% confidence interval (CI) and P-value)

Variable	Odds Ratio	95% CI	Overall P-value	AOR	95% CI	Overall P-value
MATERNAL FACTORS						
Age (years)			*<.0001*			*<.0001*
<35	1.00	Reference		1.00	Reference	
35-37	0.70	0.67-0.73		0.80	0.76-0.84	
38-40	0.46	0.44-0.49		0.62	0.58-0.66	
41-42	0.27	0.25-0.29		0.40	0.36-0.44	
>42	0.10	0.08-0.12		0.17	0.14-0.20	
Race			*<.0001*			*<.0001*
White	1.00	Reference		1.00	Reference	
Other	0.74	0.69-0.78		0.77	0.72-0.83	
Unknown	0.94	0.90-0.98		0.97	0.93-1.02	
Ethnicity			*0.1541*			--------
Hispanic	1.00	Reference		---------	----------	
Not Hispanic	1.01	0.94-1.09		---------	----------	
Unknown	1.05	0.97-1.14		---------	----------	
Patient Body Mass Index			*<.0001*			*<.0001*
<18.5 (underweight)	0.95	0.84-1.07		0.90	0.80-1.02	
18.5-24.9 (normal)	1.00	Reference		1.00	Reference	
25-29.9 (overweight)	0.91	0.87-0.96		0.94	0.89-0.99	
≥30 (obese)	0.80	0.76-0.85		0.82	0.78-0.87	
Gravidity			*<.0001*			*<.0001*
0	1.14	1.10-1.18		0.88	0.84-0.94	
≥1	1.00	Reference		1.00	Reference	
Prior Spontaneous Abortions			*<.0001*			*<.0001*
Yes	1.00	Reference		1.00	Reference	
No	1.21	1.16-1.27		1.17	1.10-1.25	
Reason for ART			*<.0001*			*0.0034*
Unknown/Other	1.06	1.00-1.13		1.01	0.94-1.08	
Single Female Factor	1.00	0.94-1.05		0.98	0.92-1.05	
Single Male Factor	1.25	1.17-1.32		1.09	1.02-1.17	
Multiple Female Factors	0.87	0.80-0.93		0.93	0.86-1.02	
Multiple Factors: Male and Female	1.00	Reference		1.00	Reference	

Table 2. (continued) Potential predictors for Implantation Rate Greater than Zero among US women receiving ART in 2008 (Odds ratio, adjusted odds ratio (AOR), 95% confidence interval (CI) and P-value)

Variable	Odds Ratio	95% CI	Overall P-value	AOR	95% CI	Overall P-value
TREATMENT FACTORS						
Protocol			*<.0001*			*<.0001*
Agonist Suppression + FSH	1.00	Reference		1.00	Reference	
Antagonist Suppression + FSH	0.65	0.62-0.68		0.84	0.79-0.88	
Agonist Flare + FSH	0.52	0.49-0.55		0.78	0.72-0.84	
FSH Only	0.81	0.72-0.90		0.84	0.73-0.98	
Others	0.59	0.53-0.66		0.72	0.63-0.84	
Day of Transfer			*<.0001*			*<.0001*
0-4	1.00	Reference		1.00	Reference	
≥5	1.88	1.81-1.95		1.43	1.37-1.50	
Number of Embryos Cryopreserved			*<.0001*			*<.0001*
0	0.38	0.36-0.40		0.53	0.50-0.56	
1	0.71	0.66-0.78		0.78	0.71-0.86	
2	0.78	0.72-0.84		0.81	0.74-0.88	
3	0.82	0.75-0.89		0.85	0.77-0.93	
≥4	1.00	Reference		1.00	Reference	
Intracytoplasmic sperm injection			*<.0001*			*<.0001*
Yes	1.09	1.05-1.14		1.14	1.08-1.20	
No	1.00	Reference		1.00	Reference	

*FSH=Follicle Stimulating Hormone

**Number of embryos cryopreserved refers to number of fresh embryos cryopreserved

***OR=Unadjusted Odds Ratio

****AOR=Adjusted Odds Ratio. Model adjusts for all variables listed except ethnicity

APPENDIX A – IRB APPROVAL

EMORY UNIVERSITY

Institutional Review Board

TO: Geeta Bhat
Principal Investigator

CC:

Drews-Botsch Carolyn Epidemiology

DATE: January 21, 2011

RE: **Notification of Expedited Approval**
IRB00048059
A Study of Trend and Potential Factors which Influence Implantation Rate of Assisted Reproductive Technology Procedures from 2000-2008

This is your notification that your above referenced study was reviewed and APPROVED under the Expedited review process per 45 CFR 46.110, category 5. The approval is valid from 1/20/2011 until 1/19/2012 . Thereafter, continued approval is contingent upon the submission of a continuing review request that must be reviewed and approved by the IRB prior to the expiration date of this study.

Any reportable events (serious adverse events, breaches of confidentiality, protocol deviation or protocol violations) or issues resulting from this study should be reported immediately to the IRB and to the sponsoring agency (if any). Any amendments (changes to any portion of this research study including but not limited to protocol or informed consent changes) must have IRB approval before being implemented.

In all correspondence and inquiries concerning this research study please include the IRB ID, the name of the Principal Investigator and the Study Title.

Sincerely,

Andrea Goosen, MPH
Research Protocol Analyst
This letter has been digitally signed