EFFECTS OF PATIENT FACTORS ON IMPLANTATION RATES IN IN VITRO FERTILIZATION

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Abstract

The purpose of this report is to evaluate the effects of patient factors and embryo quality on the implantation rate in In Vitro Fertilization (IVF). For this report, statistical methods are used to test the significance of several patient and embryo characteristics on embryo implantation rate. This project is an analysis of data collected from over 36,000 patients.

The analysis concluded several findings. There was enough statistical evidence to conclude that age was significant in regards to implantation rate and FSH levels. As the patient ages, the amount of FSH levels found in their blood increases. A patient with high FSH levels produces less oocytes than a patient with a lower FSH level. A low number of oocytes produced will in return yield a lower implantation rate. When body-mass index and smoking preference were analyzed, the analysis showed that the null hypothesis could not be rejected. When looking at embryo morphology, they played a significant role within percentile groups. The most significance was shown within the embryos that were transferred in day 5. These conclusions support the fact that since embryos are graded as good quality depending on their morphology, any improvements can be seen through the improvement of this selection process.

There are several direct and indirect costs that result from IVF treatments. These treatments are very costly ranging from $16,000 - $800,000, and can represent a significant economic burden for families. Besides having a monetary impact, IVF procedures also have a great psychological impact on patients. Undergoing an IVF treatment can greatly affect a patient's emotional, physical, and relational status.

Introduction

By using various statistical methods, the purpose of this report is to expose and understand the patient factors that affect In Vitro Fertilization (IVF) performance that may potentially contribute to an increased success rate and reduce variation in the process. The human body and this process are so complex that it is impossible to ignore the effects that a patient has on the type of embryo that is produced, or how the clinics method of growing embryos can affect the type of embryo that is produced. This report is going to focus on the patient factor aspect and will have a design analysis around blocking the effect of the clinics and embryo types. The principle patient factors that will be covered in this report are whether the patient was a smoker or not, their age, Follicle Stimulating Hormone (FSH) levels, and their Body-Mass Index (BMI) score. A detailed study of the following objectives will be analyzed in this report:

- Investigate the effect of patient's age on Implantation Rate
- Investigate the effect of patient's smoking preference on Implantation Rate
- Investigate the effect of patient BMI on implantation Rate
- Investigate the effect of patient FSH level on implantation Rate
- Analysis of age effecting FSH level in patient's blood
- Analysis of FSH levels effecting number of oocytes produced
- Analysis of implantation rate by percentile clinics on embryo types
- Analysis of implantation rate effect on number of oocytes retrieved

A master's thesis is currently being conducted encompassing the two factors this report will not cover (Clinic Percentile and Embryo Morphology). Based on the results of this study,

the statistically significant patient factors will help create a better process to improve success rates in the lower percentile clinics.

The proposal for this experiment stemmed from Dr. Alex J. Steinleitner's clinic located in San Luis Obispo, which specializes in In-Vitro Fertilization. Currently, some clinics around the US, including his, have a success rate of over 70 percent while others are closer to 30 percent. Dr. Steinleitner wants to find assignable causes for variation between clinics and implement process controls to increase the success rate of the underperforming clinics. In order to achieve any conclusions, a wide range of data is necessary to begin the analysis phase. IVF clinics throughout the United States have accumulated data for 3 years, in order to make this experiment possible. The data includes but is not limited to the woman's age, embryo score, donor egg/non donor egg.

Literature Review

History of IVF

The search for a cure to infertility has long been researched since 1855 when Dr. J. Marion Sims believed that infertility could be cured through gynecological surgery or artificial insemination. Nearly 30 years later the first child was born through artificial insemination by physician William Pancoast. After this achievement, success in the field of assisted reproductive technology progressed slowly. It wasn't until 1968 that British scientist Robert Edwards teamed with Patrick Steptoe and fertilized the first human eggs in vitro. Over the next nine years, the public analyzed the ethics behind this discovery. In December of 1977 the British team successfully grew an egg fertilized in vitro in a human uterus and on July 25 of 1978 their work was official with the delivery of baby Louise Joy Brown. Since then, in vitro fertilization has become a part of medical vernacular and over 450 IVF clinics have opened across the United States (pbs.org).

The IVF Process

Normally, in order to conceive a baby, an egg and sperm are fertilized inside a woman's body. If the fertilized egg attaches to the lining of the womb and continues to grow, a baby is born about 9 months later. This process is called natural or unassisted conception (Storcke). Unfortunately, about 10% of women ages 15-44 in the United States have difficulty getting pregnant or staying pregnant (Infertility). For this precise reason, Sir Robert Edwards was the first pioneer of in-vitro fertilization (IVF) in 1978 (Johnson). IVF is a form of reproductive assisted technology that helps a woman become pregnant and is usually chosen when other less-expensive fertility techniques have proven to be unsuccessful because IVF itself is so expensive.

There are five basic steps to IVF. The first one is the stimulation step, also known as superovulation. In this step, the patient is given drugs to stimulate her ovaries and develop multiple eggs, since most women usually produce only one egg per month. Step two is called Egg Retrieval. Once the eggs are mature, a minor surgery called follicular aspiration is performed to remove the eggs from the ovaries. During this procedure, a needle is placed through the vaginal opening and into the ovaries containing the eggs. The needle is then connected to a suction device that pulls the eggs out of each follicle, one at a time. The eggs then get transferred to an embryology lab where they are evaluated for maturity. More on how to evaluate the eggs will be discussed further in the literature review. Step three which is called fertilization in the Lab is what comes next. At this stage, a fresh sample of sperm is collected in which the best sperm is chosen for insemination. The sperm is placed together with the best quality eggs and stored in a controlled chamber. If the doctor believes the chance of fertilization is low, the sperm is then injected directly into the egg using a process called intra-cytoplasmic sperm injection (ICSI). If the fertilization is successful, the oocytes and embryos will stay in the lab for about 2-5 days (Keefe).

The next two steps are very important because they dictate which embryo will be the most qualified to be transferred. Step four is called embryo culture/quality. During this step, the laboratory staff will regularly check the embryo to ensure that it is growing properly. Once a good embryo is identified, they are transferred during day 3 or day 5. Day 3 embryos are called cleavage stage embryos and have about 4-8 cells. Day 5 embryos are called blastocyst embryos and look like a ball of cells with liquid inside. The last step is called Embryo Transfer. This is the stage in which the embryos are placed inside the woman's uterus through her vagina. It is a simple procedure that does not require anesthesia. During the process, the embryos are loaded

into a thin tube called a catheter, and then inserted into the woman's vagina. Pregnancy results if an embryo sticks inside the lining of the womb and grows (Keefe).

It is possible to insert more than one embryo into the womb to have twins, triplets, or more. This however is a complex issue that depends on several factors including a woman's age. The embryos that are not used may be frozen and implanted or donated on a later date (Storcke).

Cleavage Stage (Day 3) vs. Blastocyst Stage (Day 5) Embryos

Embryos from assisted reproductive technologies in vitro fertilization (IVF), are usually transferred into the woman's uterus at either the early cleavage stage (Day 2 to 3 after egg collection) or blastocyst stage (Day 5 to 6 after egg collection). Currently, doctors' majority opinion is that transferring embryos at the blastocyst stage is the most biologically correct stage for embryos to be in the uterus since earlier stages are naturally in the fallopian tube (Wang). They also benefit from the embryo staying in the laboratory longer, since it may give doctors more time to select the best quality embryo(s). The objective of this experiment was to evaluate whether the live birth rate and other pregnancy outcomes can be improved by Day 5 to 6 transfer compared with Day 2 to 3 embryo transfer (Wang).

The experiment consisted of seven trials (n=1446 cases). The results confirmed that blastocyst transfer was statistically significantly associated with an increase in clinical pregnancy rate with an odds ratio (OR) of 1.43% and confidence interval (CI), 1.15-1.78], implantation rate (OR 1.38; 95% CI, 1.09-1.74) and ongoing pregnancy rate (OR 2.15; 95% CI, 1.57-2.94), and also a reduction in the probability of first trimester miscarriage rate (OR 0.51; 95% CI, 0.30-0.87). The improvement in the live birth rate was also observed to have (OR 1.77; 95% CI, 1.32-2.37) (Wang).

The above experiment results suggest that indeed, live birth rate and other pregnancy outcomes are significantly improved if the embryo is transferred during the blastocyst stage compared to the cleavage stage.

Quality: Defined

Currently, most labs use embryo morphology as the main quality indicator. It is a noninvasive embryo selection technique that has been utilized since the early days of IVF. Of course, this method has its limitations and room for error. Morphological assessments are not always performed at constant time intervals, thus making variations in the score. For example, an embryo scored early on day 2 can appear significantly different than the same embryo late on day 2. The cell could morph from a 2-cell to a 4-cell during that time period, making this method fairly crude and subjective (Montag, Toth, Strowitzki).

The main points that are analyzed in this widely used method include: fragmentation percentage, the number of cells, shape of the cells at all days, and the appearance of the zona pellucida, inner cell mass and trophectoderm of the blastocyst at day 5 (SART). Another study, performed in 1999, found the characteristics that led to the highest pregnancy success. The "characteristics of these top quality embryos were absence of multinucleated blastomeres, four or five blastomeres on day 2, seven or more cells on day 3, and ≤20% anucleated fragments" (van Royen).

Presently, it is known that the quality of the embryo can only be as good as the woman carrying it. There are many factors that influence the success of IVF. Still, isolating the quality of the embryo is a significant factor used to predict the success of the procedure.

S.A.R.T. Embryo Scoring System

There have been numerous grading systems created to rank embryos in the processes utilized in Assisted Reproductive Technology (ART) labs. Some systems use the overall appearance of the cleavage stage embryo, others at the blastocyst stage, and some are more complex that involve formulas to predict the possibility of pregnancy. Embryo quality has been correlated with pregnancy success, and without having data of each embryo score, high variability in success rates occurred. Because of this diverse grading system and lack of a convention, comparisons between clinics and overall quality control could not be performed. In 2004, "embryo morphology fields" were included in the Society of Assisted Reproductive Technologies (SART) database (Racowsky). After this incorporation, SART developed their own grading/scoring system which was made mandatory to include by March 2010. This system had 3 goals: to be "1) simple, 2) comprised of fields that have a basis in scientific inquiry with some proven predictive value, and 3) easily adopted in laboratories not routinely capturing these parameters" (Racowsky). The following three-point system (Table 1) was developed:

Growth phase	Overall grade	Stage
Cleavage	Good, fair, poor	Cell no.: 1 through >8
		Fragmentation: 0, <10%, 11%–25%, >25%
		Symmetry: perfect, moderately asymmetric, severely asymmetric
Morula	Good, fair, poor	Compaction: complete, incomplete
		Fragmentation: 0, <10%, 11–25%, >25%
Blastocyst	Good, fair, poor	Expansion: early, expanding, expanded, hatched
		Inner cell mass: good, fair, poor
		Trophectoderm: good, fair, poor

Table 1: SART Grading System (Source: Racowsky)

Since SART has mandated that embryo score be included in all datasets, much more accurate predictions have been made in predicting pregnancy.

A study was conducted to evaluate the correlation between the SART scoring method and ART single blastocyst embryo transfers. In the study, each blastocyst was given a grade based on the SART scoring system and then statistical methods such as multiple logistic regression and chi square analysis were used to correlate the scoring system to live birth rate. The study used a sample of 717 single blastocyst transfer cycles that were fresh and autologous (Heitmann).

"The live birth rate was 52 % and included both elective and non-elective [single blastocyst cycles] SBT. Chi square analysis showed higher live birth in good grade embryos as compared to fair ($p = 0.03$) and poor ($p = 0.02$). Univariate binary logistic regression analysis demonstrated SART embryo grading to be significantly correlated with both implantation and live birth ($p < 0.01$). This significance persisted when patient age, BMI, and the stage of the blastocyst were controlled for with multiple logistic regression. In five patients with a poor blastocyst score, there were no live births" (Heitmann).

From this study it can be seen that the SART grading method is a strong indicator in live birth success and should be used when analyzing any ART data.

To test if SART's grading method accounted for all factors influencing success in an embryos morphology, Sophia Kamran, ET. Al. devised a study to see whether or not the symmetry of the day 3 embryo was a significant predictor to pregnancy in addition to the 3 main areas that the SART method uses: cell number, fragmentation and blastomere symmetry. In the study, Kamran used MATLAB to measure the "roundness" of a day 3 embryo. Using statistical methods, she found that this characteristic was not useful as an additional marker for embryo selection. This finding helped to show the appropriateness of the SART grading system in correlation with pregnancy success.

The NYU Langone Medical Center states that there is no standard classification system for all fertility centers. Although most centers use the Gardner grading system for blastocysts,

each center created their own system for grading day-2 and 3 embryos as the technology developed and, at this point, it would be too difficult for all centers to try and use a standard grading method (NYU Langone Medical Center). For this reason, all data that is used in this research is collected in the same database using the same methodology. Only clinics using the SART grading method have contributed data.

Factors Influencing IVF Success:

Several external factors have been identified in reducing the success of IVF pregnancies; ethnicity, smoking, age, patient Body Mass Index (BMI), and paternal BMI.

Ethnicity

Since IVF is a fairly new concept, there are many factors that could be contributing to unsuccessful pregnancies. Most scientists have researched and experimented with the age of the patients to see if this plays a major role in whether the process is successful. It is important however, to take into account all of the possible variables that can influence the outcome of IVF such as ethnicity. Can ethnicity be an influence on IVF? This is the question that a team of researchers at Nottingham University in the UK set out to answer.

Ethnic minorities form a significant portion of couples undergoing (IVF) throughout the world. It is important to let these patients know what their probabilities of success may be based on their genetic makeup and variables that might affect their outcome. There have already been a number of reports published on the relationship between ethnicity and IVF success. Asian infertile women in the United States were reported to have a lower IVF success rate compared with white woman (Jayaprakasan).

In other studies in the US, authors have reported that white woman also have more biochemical pregnancies and live births compared to women from ethnic minorities. Including

but not limited to Hispanic and Asian. In the UK, studies that were published in the late 1990's reported that in the USA, South Asian Indian women had lower live birth rates when compared to white woman despite their younger age and lower basal follicle stimulating hormone (FSH) (Jayaprakasan).A follicle stimulating hormone is an acidic glycoprotein found in women that stimulates the development of ovarian follicles (eggs) and stimulates the release of estrogen. In men, this hormone stimulates the production of sperm (Follicle).

In this particular study, the team aimed to investigate the relationship between ethnicity and IVF outcome in a large population that received treatment over a period of five years between 2006 and 2011. The IVF outcome between minorities and subpopulation groups such as South-East Asian, African-Caribbean, and Middle Eastern were compared to white European groups. For this particular study, all women who participated were undergoing their first cycle of IVF treatment to try and reduce variability. The study was performed in the UK at the Nottingham University Research and Treatment Unit in Reproduction. All of the patients underwent a standard long agonist or antagonist protocol, depending on their ovarian reserve tests. The difference between these two protocols is the time in which the patient received the gonadotrophin-releasing hormone. For the long agonist protocol, the gonadotrophin is started in the midluteal phase of the menstrual cycle while during the antagonist protocol, it is commenced on day two. The starting doses of gonadotropin were dependent on the woman's age and ranged from 150-450 iu. The women were then monitored for follicle development with a serious of transvaginal ultrasound beginning on the fifth or sixth day of simulation. As soon as the doctors noticed three follicles measuring more 18mm or more in diameter, oocyte retrieval was performed 36 hours later. The oocytes are then fertilized in the lab and depending on the number of embryos that developed, a maximum of two embryos was transferred into the uterus at days 2,

3, or 5 after insemination. All of the pregnant women were followed up to record accurately the final outcome of their pregnancies (Jayaprakasan).

The data was then recorded in a Microsoft Excel spreadsheet and analyzed using various statistical methods. Their first step was to test for normality to choose the appropriate statistical test. Continuous data was analyzed by the Student T-test or by the U-test, depending on the data distribution. The Chi Square test and Fisher were performed to analyze the relationship between two categorical variables. Out of the 1517 women who began the treatments, 23 did not reach the egg-retrieval stage, 11 developed an excessive response, 5 had no eggs to be collected, 9 had no mature eggs, 39 failed fertilization, and 1395 had embryo transfer. Their results on a univariate logistic regression analysis, was that ethnicity was an independent predictor of live birth rate with a p-value of less than or equal to .02. On a regression analysis, ethnicity proved to not be a predictor of successful IVF outcome only when the South-East Asian population was included in the population with a p-value of .06 (Jayaprakasan).

Based on the experiment, the data indicate that live birth rates, clinical pregnancy rates, and implantation rates followed by IVF are significantly lower in ethnic groups when compared to European women. It proves that ethnicity may be a major determinant of live birth following IVF treatment. When the subgroups were analyzed, success rates were lower in the South-East Asian, African-Caribbean, and Middle-Eastern groups but not statistically significant possibly because of the small group sizes. It is important to tell patients realistically, what their probabilities are of having a positive outcome. Although this research indicated that ethnicity affects the probabilities of a successful outcome, further research is needed to know the degree of variation in success caused by different ethnic backgrounds. There may be modifications

available to the clinical strategies of IVF to ensure equivalent success rates among all ethnic groups when the relationship between ethnicity and IVF outcomes are thoroughly understood.

Age

JunHao Yan, et al., conducted a retrospective, observational study of 11,830 IVF-ET (in vitro fertilization embryo transfer) cycles. The women were aggregated into four age groups, 21-30, 31-35, 36-40, and 40+. Many factors were analyzed in the study, including; the dosage of Gonadatropin, mean number of oocytes received, 2PN zygote rates, good quality embryo rates, clinical pregnancy rates, miscarriage rates, and birth defect rates. The following graphic shows the significant findings:

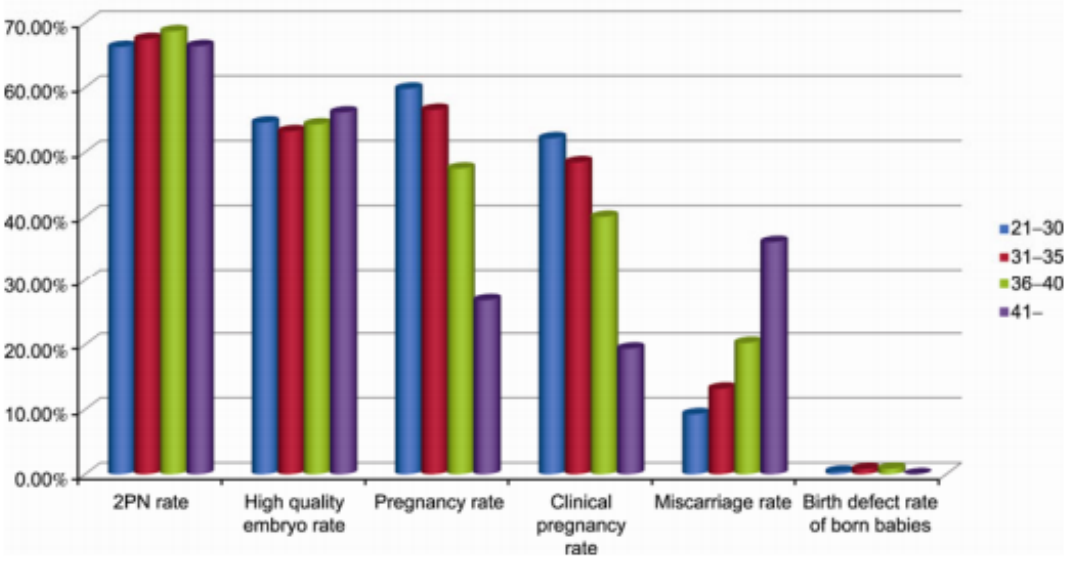


Figure 1: IVF Outcomes of Different Maternal Age Groups

Source: Yan, JunHao, KeLiang Wu, Rong Tang, LingLing Ding, and Zi-Jiang Chen. "Effect of Maternal Age on the Outcomes of in Vitro Fertilization and Embryo Transfer (IVF-ET)." Science China Life Sciences, 55.8 (2012): 694-698.

The findings were interesting in that age does not seem to play a factor in the quality of day 3 embryos and 2PN zygote rates. However, the tests showed significant findings of lower pregnancy rates in those of increasing age, as well as miscarriage rate. The miscarriage rates match those of natural pregnancy though, and thus other factors such as uterine anatomical defects, environmental factors obesity, endometriosis, etc can be in effect. The study concluded that age significantly plays a role in IVF success, and pregnancy in general. The authors state that women 21 through 30 years of age will have the best IVF outcomes of the women of fertile age (Yan).

A similar study conducted at the Reproductive Medicine Center of the Third Affiliated Hospital of Guangzhou Medical College by Hong-zi Du, et. al, looked at the effects of patient age, and number and quality of transferred embryos. The study grouped the infertile women into three age groups, <30, 31-34, and >=35. The results found that it is desirable to select a single good-quality embryo for patients <30 and two good-quality embryos for women >30. As women age, poor-quality embryos should not be transferred at all, and more than two good-quality embryos may need to be transferred (Du).

Body Mass Index

Obesity is also considered to be a factor influencing the success of IVF treatment. Not only does the patient's Body Mass Index (BMI) affect the dosage of gonadotropins, but it can also have effects on the reproductive tissues and organs, leading to increases in maternal and neonatal complications (Bellver). A retrospective analysis was performed by D.K. Shah, et al., on the effect of BMI on IVF outcomes. The results were presented as odds ratios (OR) and confidence intervals. Eight hundred ninety-three women were studied with varying BMIs. Those with Class II and III obesity (BMI of 35.0-39.9 and >=40.0, respectively) had much lower peak

estradiol levels (2123 pg/mL vs. 1664 and 1366, $p<0.05$), fewer oocytes retrieved (15 vs. 11 and 12, $p<0.0001$), and fewer total embryos (14 vs. 11 and 12, $p<0.0001$). Women with Class III obesity also had lower live birth rates (OR=0.4, CI = 0.18-0.88) and a 29% greater incidence of immature oocytes (CI = 1.11-1.81) compared to subjects with normal BMI (18.5-24.9) (Shah).

Another study that accounts for obesity and BMI includes Zaher O. Merhl et al.'s article on male adiposity and how it affects clinical pregnancy rates but not the day 3 embryo quality score. Merhl and company retrospectively studied 344 infertile couples, focusing on several areas: the number of oocytes retrieved, zygote PN-score, total number of embryos available on day 3, number of embryos transferred, composite day 3 grade for transferred embryos, composite day 3 grade per cycle, and CPR. The results found one hundred twenty-one cycles resulted in clinical pregnancy (35.2%). The normal BMI category was associated with a much higher CPR (46.7%) than that of the overweight and obese BMI categories (32.0% averaged CPR). There were no significant differences in any of the other factors analyzed. The study concluded that, "embryo grading based on conventional morphologic criteria does not explain the poorer clinical pregnancy outcomes seen in couples with overweight or obese male partners" (Merhl).

Tobacco Smoking

Cigarette smoking is widely believed to be associated with decreased fecundity outcomes in naturally conceiving populations. The effect of female smoking on pregnancy outcomes in patients undergoing IVF however is still unclear and over the last few decades, smoking among women of reproductive age has increased. Lifestyle habits such as smoking may indeed play a crucial role in the success rates of IVF. Tobacco smoke contains hundreds of substances including but not limited to nicotine, carbon monoxide, and mutagens. There have been 16 studies all divided into retrospective studies, prospective studies, and meta-analysis that have

investigated the effect of smoking on IVF (Kettel). In almost all of these studies, smoking did not uniformly affect the same endpoints. The studies showed that maternal smoking decreased fertilization rates, number of oocytes, pregnancy rates, and increased miscarriage rates. In other studies there was no effect of smoking on fertilization and pregnancy rates. Other studies simply just did not have the adequate power to assess significant differences in pregnancy outcomes. The biggest mistake in some of these was not defining the smoking history of each patient with sufficient details. Smoking was classified when patients first entered the study, but not throughout the procedure where their habits may have changed. Women who stopped smoking cigarettes after commencing the treatment were classified as current smokers given that their habits had changed (Seibel).

The study conducted by Hilary Klonoff-Cohen, Loki Natarajan, Richard Marrs, and Bill Yee focused on evaluating all biological and reproductive endpoints. It involved both females and males and also performed multivariate analyses and adjusted for potential confounders and interaction terms. The quantity, frequency, and duration of smoking was also carefully taken into account. The study included 221 couples undergoing IVF treatment in Los Angeles, Orange and San Diego counties. All subjects were between the ages of 20-40 years old and the subjects over 35 years old were analyzed separately because of an increase incidence of miscarriage. The smoking habits were categorized by the following time periods: lifetime, 1 year, 1 month, 1 week, and one day prior to the procedure. In addition, all of the patients smoking habits were monitored throughout the entire procedure (Kettel).

When conducting multivariate analyses, the RR for not achieving pregnancy was 2.41 with a p-value of .03 for smoking compared with non-smoking couples. There was a 40% decrease in the number of oocytes aspirated from smoking couples during the IVF treatment. Of

the 41 couples who had successful live birth deliveries, 11 had multiple births. The smoking effects on the multiple births were assessed using a logistic regression. For multiple deliveries, there was a 9% higher RR for each additional year that the person smoked before being treated with IVF. The data also concluded that if a woman ever smoked during her lifetime, her risk of not having a successful pregnancy increased by 9% per year that the individual smoked. In conclusion, the above study provides compelling evidence that smoking negatively affects the probabilities of a successful pregnancy outcome (Kettle).

There have been a lot of studies showing how smoking can affect a successful live birth when undergoing an IVF treatment, but can second hand smoking also affect a successful outcome? Secondhand tobacco smoke (STS) is a mixture of over 4000 chemicals, where more than 60 of them are known or suspected carcinogens or reproductive toxins (Lindbohm). In a previous study, self-reported female STS exposure was associated with decreased implantation and pregnancy rates among the 225 women tested undergoing IVF (Neal). The increasing use of assisted reproductive technology, in particular IVF, has helped improve doctor's abilities to study contributing factors that lead to infertility and early pregnancy loss. Follicle Fluid also stated as FF, is the fluid that surrounds the preovulatory oocyte. It is collected during IVF treatment but very seldom used although it has the potential to serve as a matrix to measure markers of exposure to STS or other environmental agents. The amount of cotinine levels in FF are an indicator of a developing oocytes direct exposure to tobacco (Fabro).

The following study was designed to examine the relationship between female STS exposure and failed implantations using the cotinine dosages in FF in women undergoing IVF. The study used women who had an IVF treatment from 1994-2003 at one of three Boston clinics. The total number of participants was 1909 couples with a total of 3270 treatment cycles. The

physicians and technicians retained the FF from the participants during egg retrieval for each cycle. The FF was aspirated from the follicles using a 16 G needle and suction from a Rocket pump apparatus and then transferred to a petri dish. The fluid, which would normally be discarded at this point, was placed into a 15 ml tube and centrifuged for 15 minutes. After, it was transferred to the Brigham and Women's hospital laboratory for analysis (Benedict, Stacey).

The data analysis was performed using SAS software where cotinine concentrations were recorded. The study established that the cycles with STS exposed would yield FF cotinine concentrations of <10 and >1.1 ng/ml and unexposed cycles were < 1.11 ng/ml. The potential confounding variables were female age, BMI, ethnicity, primary infertility diagnosis, site of treatment, months spent trying to get pregnant, etc. (Homer). The results showed a significant increase in the risk of failed implantation among women exposed to STS in comparison to those unexposed. Based on a 95% CI with a p-value of .004, it was concluded that there is also a relationship between STS exposure and IVF treatment success. In an analysis among 921 women who had urine samples available for cotinine measurement, creatinine-adjusted cotinine levels in urine were associated with a slight decrease in first-cycle implantation rates among non-smoking women (11.1% in the lowest cotinine quintile versus 8.2% in the highest quintile; $P = 0.13$ (Meeker).

In conclusion, this study found a significant increase in the risk of implantation failure among women exposed to STS compared with those who were unexposed based on cotinine concentrations measured in the FF of the women. They also found a significant decrease in the odds of achieving a successful live birth among STS-exposed women.

Breakthroughs and Obstacles

IVF doctors and scientists have been at the top of fertility research and treatment since the first successful IVF birth in Australia. This procedure is nowhere near perfect and there is always room for improvement as well as new side effects surfacing. As of now there is not a standardized procedure to determine the quality of an IVF procedure. There are still many variables that can affect the number and quality of oocytes and embryos in patients.

Recent research done by Dr. Rinchen Zangmo the Departments of Obstetrics and Gynecology in New Delhi, India has evaluated the role of dehydroepiandrosterone (DHEA) on the number and quality of oocytes and embryos in patients who have not been successful with IVF cycles. To better understand the experiment done by Dr. Zangmo, it is crucial to have an understanding of what DHEA really is. DHEA is a hormone that is naturally made by the human body, specifically the adrenal gland and brain (Zangmo). This hormone leads to the production of androgens and estrogens in both males and females. Although the human body produces DHEA, scientists have now also found a way to synthetically produce it with chemicals found in wild yam and soy. It is important to note that the human body cannot produce this hormone by simply eating wild yam and soy.

For this experiment, a total of 50 patients with a record of poor ovarian response in the previous IVF cycles participated. The patients were categorized into two different age groups, where half of them were under 35 and the other half were over 35 years of age. They were treated with 25 mg of DHEA three times a day for a period of four months. The oocyte and embryo number and quality were recorded before and after the four months and then analyzed using a student paired T-test. The results showed an increase of mature oocytes after the 4 month period of DHEA treatment with patients under 35 having a $P<.001$ and patients over 35 years of age yielding a $P = 0.002$. There were significant increases in the total number of oocytes

retrieved, fertilization rates, thus increasing the number of embryos available (Zangmo). In conclusion, DHEA can help improve pregnancy rate in poor responders with history of previous failed IVF cycles.

Since there are various factors that may contribute to the successful outcome of IVF treatment, it is important to have a deep understanding of past experiments conducted in these areas. Furthermore, identifying and understanding the important considerations and analysis that have been made in past research is crucial. The studies on these topics will help the team have a thorough understanding of the topics to provide pertinent analysis and assessment to this research project.

Design and Methods

Set-Up

The data that was used for this project was purchased by Dr. Steinleitner and provided by the Society of Reproductive Technologies (SART) and collected over 3 years. The data included over 36,800 patients and had a description of every single patient's age, smoking status, BMI, FSH level, embryo morphology, day of transfer, number of oocytes retrieved, number implanted, number of beating hearts, and live births. The data was initially organized by percentile clinic and patient ID, with each patient's embryo types being grouped together. To be able to analyze each embryo individually, the data was stacked using Microsoft Excel so that each embryo morphology was a unique entry. See Appendix A Table 2 for an example of the stacking before and after process. Embryo morphology encompasses the data of the embryo that includes the day in which the embryo was transferred, the stage it was in, and many other characteristics depending on whether it was transferred on day 3 or day 5.

After this filtering step, the number of entries increased from approximately 36,000 to 73,651. Once the embryos were stacked accordingly, the next step in having useful data for our analysis was to filter out the cases of embryos that would not be of any value to the study. The only cases that were useful were those in which all embryos were successful or not. For example if two embryos were transferred and two embryos implanted, then it could be implied that both embryos were successful and could trace the characteristics for each one. In the cases where the number of embryos transferred was greater that the number that implanted, it was impossible to determine which one of those embryos were the ones that implanted. After filtering the data for a second time, the final number of embryos that were analyzed were approximately 53,000. The

implantation rate of each embryo was then calculated by dividing the number of implantations by the number of embryos transferred. Figure 2, shows the steps taken to create our useful data.

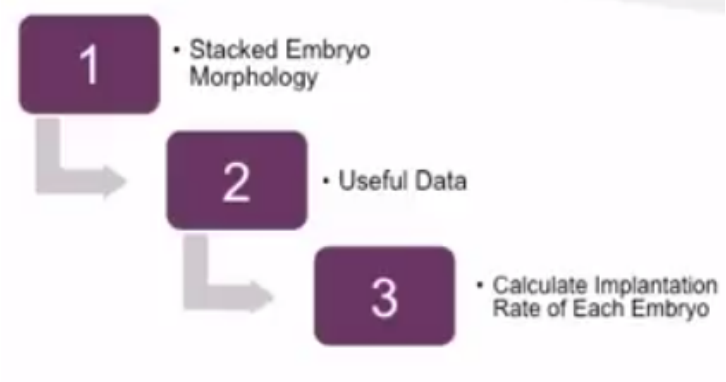


Figure 2: Data Processing Stages

The next phase in this project was to identify the questions that needed to be answered and realize what patient factors could potentially affect each other. Figure 3 below is a diagram that shows the relationships that were analyzed between patient factors and implantation rate.

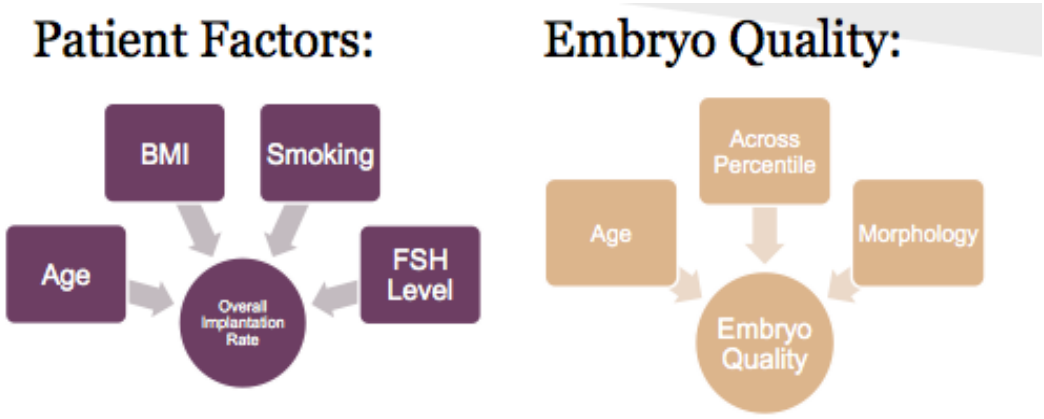


Figure 3: Patient Factor and IR Relationships Analyzed

Once the areas of interest were created, the next step in this process was to group all of the data to make it categorical and analyze it on JMP. Since the age of the patients ranged all the way from 17 to 52 years of age, grouping the data made for the analysis to be much more manageable. According to a previous study made by Dr. Steinleitner, the best groups to separate

age by were, under 36, between 36 and 39, and over 39 years-old. Clinic percentiles were also split into 3 groups composed of percentiles 1-30, 40-60, and 70-90. After all of the data was filtered and grouped, Microsoft Access was used to easily maneuver through the large data set.

Patient Factors

To begin the analysis, several questions were brainstormed to be the basis of the study. The subject matter can be difficult to comprehend initially and these simply stated questions helped to clarify what was being researched. The first question that was sought to be answered was, "Does the patient's age, BMI or smoking preference have a significant effect on implantation rate?" Before running any statistical test, a few key assumptions had to be met. Since the data for implantation rate fell in between 0 and 1, the normality assumption could not be met. In order to normalize the data, a nominal logistic model was used. After reaching normality and testing for constant variance, the model was applied. From Figure 4, the three-factor model is shown to be significant with a chi-squared value of less than 0.001. The Effect Likelihood Ratio Tests table shows that smoking and BMI cannot be concluded to contribute significantly to the model fit. The report shown in this three-factor model supports the conclusion that age is the only factor that has an effect on embryo implantation rate. It is evident based on these results that as a patient gets older, the probabilities of implantation decrease.

Nominal Logistic Fit for Implantation Rate

Converged in Gradient, 18 iterations

Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	67.85244	20	135.7049	<.0001*
Full	427.72832			
Reduced	495.58076			

Effect Likelihood Ratio Tests

Source	Nparm	DF	L-R ChiSquare	Prob>ChiSq
Age Group	10	10	123.045244	<.0001*
BMI_Category	5	5	4.11465217	0.5330
Smoker	5	5	3.13202677	0.6796

Figure 4: Nominal Logistic Model for Implantation Rate

The graph below (Figure 5) shows the relationship between IR and age of the patient. It also shows that based on these conclusions the largest quantity of patients who undergo an IVF treatment are patients over 32 years of age.

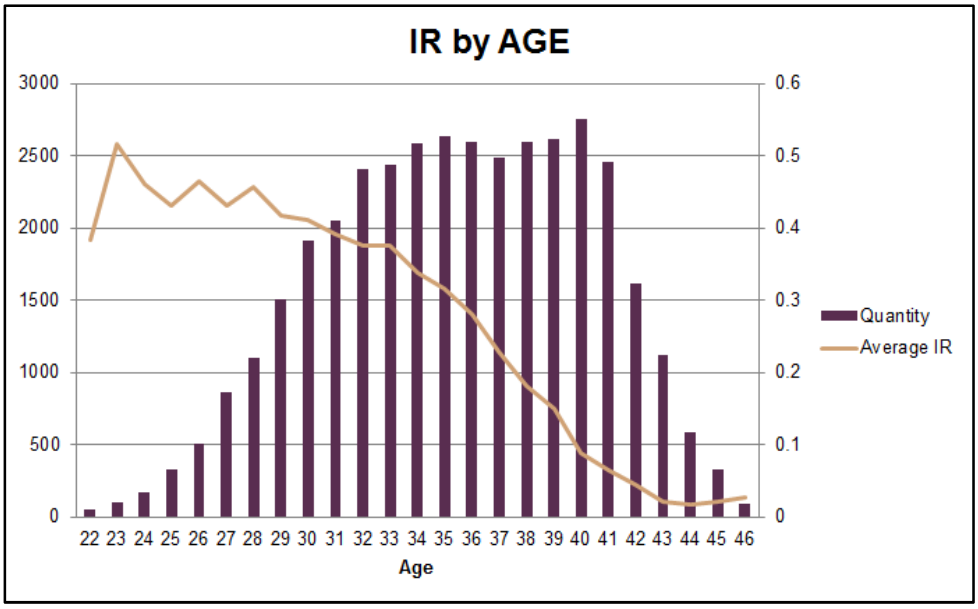


Figure 5: IR by Age

Since smoking preference and BMI could not be proven significant, the effects of only patient's age on other factors possibly influencing implantation rate were studied. This brought about the second question, "Does age play a significant role in the patients FSH blood levels?" FSH is one of the most important hormones involved in the natural menstrual cycle as well as in pharmacological (drug-induced) stimulation of the ovaries. It is the main hormone involved in producing mature eggs in the ovaries.

Figure 6, shows the one way ANOVA that was used to compare the means of the FSH grouping using the F distribution. The data below shows that the constant variance assumption was met.

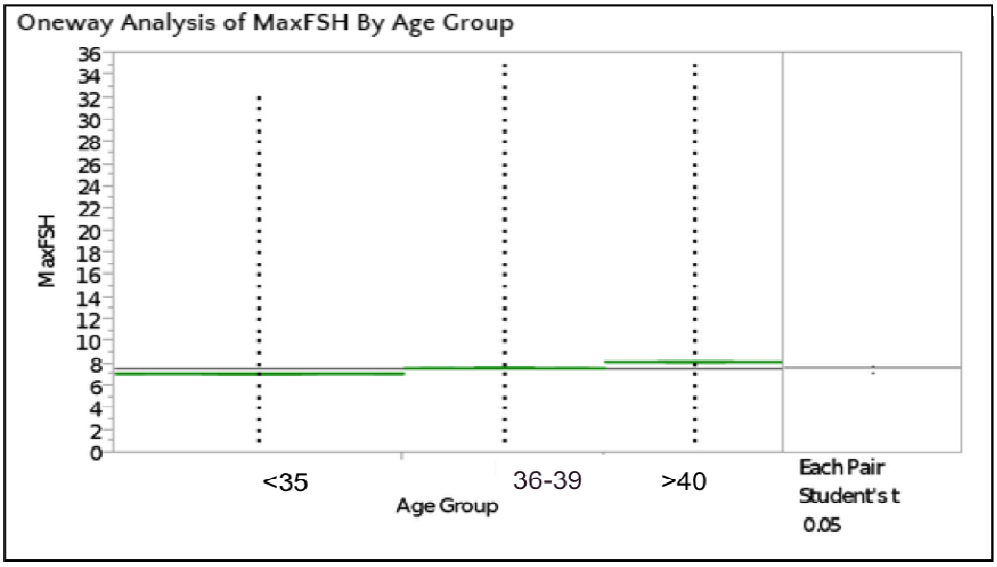


Figure 6: One Way ANOVA of Max FSH by Age Group

The one way ANOVA in Figure 7, concludes that the patient's age was significant with a p-value less than 0.001 in determining the amount of FSH levels in the patient. The connecting letters report shows that all FSH level groups were significant. As the patients get older, they will yield higher levels of FSH than younger patients.

Oneway Anova
Summary of Fit

Rsquare	0.015702
Adj Rsquare	0.015663
Root Mean Square Error	3.583041
Mean of Response	7.620005
Observations (or Sum Wgts)	50277

Connecting Letters Report

Level			Mean
>39	A		8.2566746
36-39		B	7.7180198
<36		C	7.1547383

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Age Group	2	10296.39	5148.19	401.0064	<.0001*
Error	50274	645426.81	12.84		
C. Total	50276	655723.20			

Figure 7: One Way ANOVA with Letters Report of Age Group and FSH

To build upon these results, the correlation between FSH levels and number of oocytes produced were studied. In a regression model, it was found that the maximum FSH level in the patients' blood was significant (with a p-value of < 0.0001) in predicting the number of oocytes to be retrieved. The best-fit line does not fit this data well (R-Square value of only 0.06), but does show the overall trend of the data, indicating some inverse proportionality.

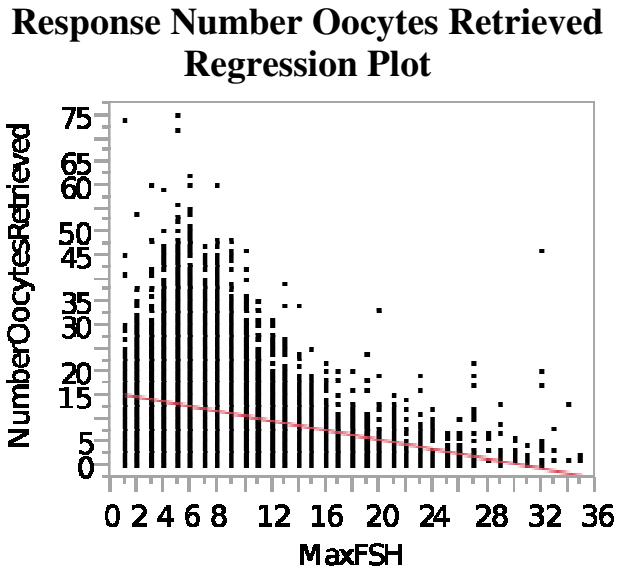


Figure 8: Scatter Plot of Number of Oocytes versus FSH Levels

Summary of Fit

RSquare	0.059971
RSquare Adj	0.059951
Root Mean Square Error	6.970455
Mean of Response	12.12213
Observations (or Sum Wgts)	47563

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	147425.7	147426	3034.246
Error	47561	2310857.9	49	Prob > F
C. Total	47562	2458283.5		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	15.943227	0.076378	208.74	<.0001*
MaxFSH	-0.508195	0.009226	-55.08	<.0001*

Figure 9: Regression Analysis

Finally, the last question to be answered was, “Does the number of oocytes produced affect the embryo implantation rate?” For this question, the numbers of oocytes retrieved were grouped into categories as deemed correct by our professional, clinical reference, Dr. Steinleitner. The categories assigned were, less than 6, between 6 and 35, and greater than 35. As the data shows, the constant variance assumption was met.

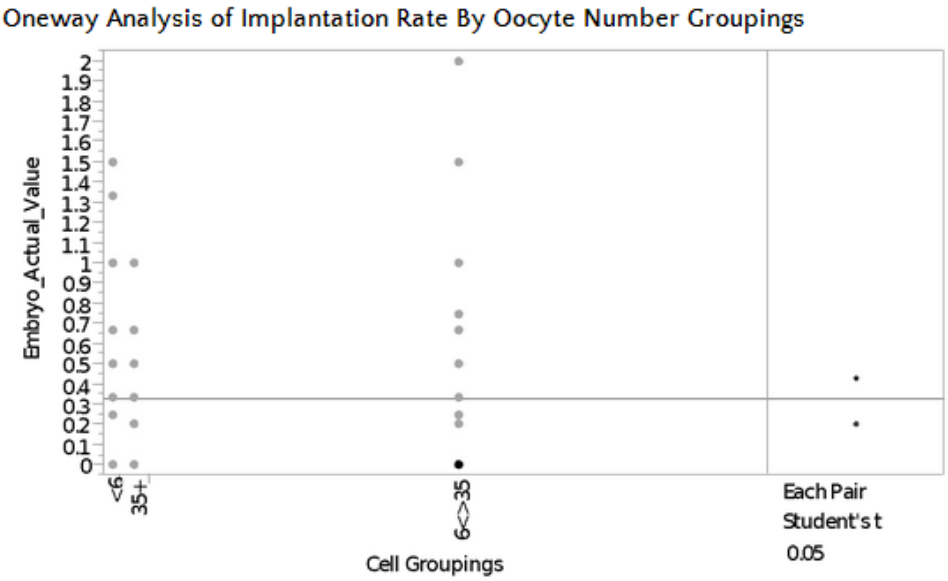
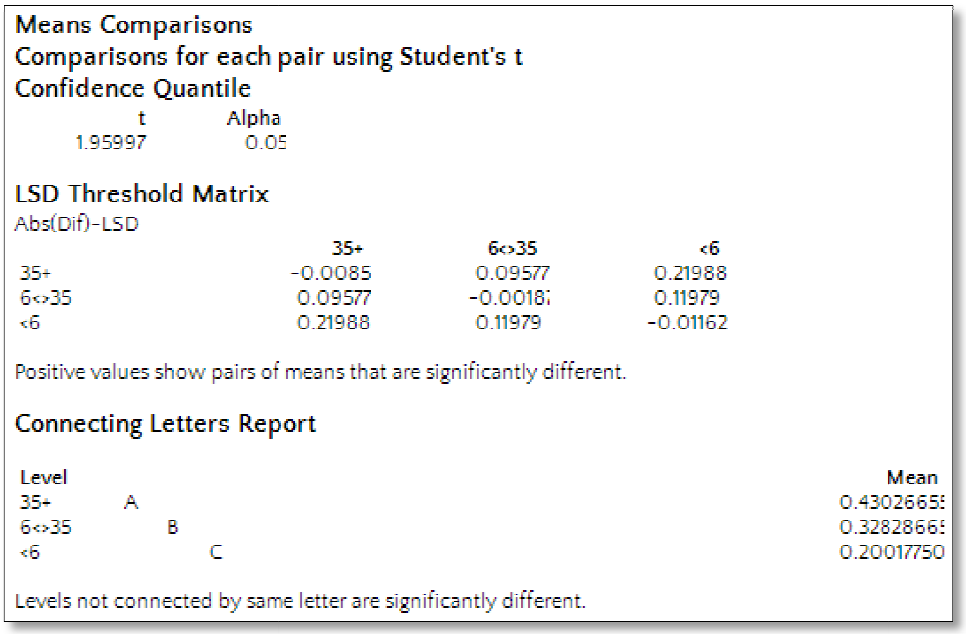


Figure 10: Constant Variance Plot

From the one way ANOVA, the p-value for the model was significant at a level greater than 99%. The connecting letters report shows that each grouping of oocytes retrieved are significant and the mean implantation rate decreases as the number of oocytes decrease.



Means Comparisons
Comparisons for each pair using Student's t
Confidence Quantile

t	Alpha
1.95997	0.05

LSD Threshold Matrix
Abs(Dif)-LSD

	35+	6<>35	<6
35+	-0.0085	0.09577	0.21988
6<>35	0.09577	-0.0018;	0.11979
<6	0.21988	0.11979	-0.01162

Positive values show pairs of means that are significantly different.

Connecting Letters Report

Level			Mean
35+	A		0.4302665
6<>35		B	0.3282866
<6		C	0.20017750

Levels not connected by same letter are significantly different.

Figure 11: One way ANOVA with Letters Report of IR and Oocyte Groupings

Embryo Quality

After producing as many conclusions from the given categories of patient factors data as possible, the focus moved to determining how embryo morphology quality affected implantation rate across percentiles. Percentiles were grouped by categories (for ease of analysis) 1st -30th percentile, 40 – 60th, 70 -90th percentile clinics. To select which embryo groupings were most beneficial to study, a contingency table was created for each morphology type and its unique implantation rate for each patient factor. Those groupings with the highest implantation rates were selected for study. For this analysis, only the youngest age grouping (under 36 years of age) was used, for ease of analysis and because this group has the least repercussions of age's effect on implantation rate. See Appendix B Table 3, for the full contingency table. From the contingency table, the following groups were selected for study: Expanded Blastocyst, Good Inner Cell Mass (ICM), Good Trophectoderm; Hatching Blastocyst, Good ICM, Good

Trophectoderm; 8-cell, 0 Fragmentation, Perfect Symmetry; 8-cell, 1-10% Fragmentation, Perfect Symmetry.

The following analysis shows the implantation rate of Day 5 Expanded Blastocysts with Good Inner Cell Mass and Good Trophectoderm across percentile groupings.

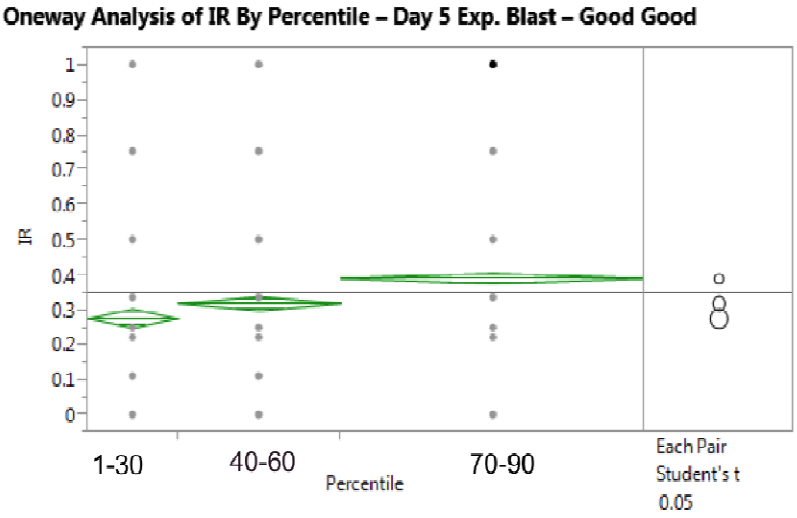


Figure 12: Constant Variance of Percentile Groupings

Oneway Anova
Summary of Fit

Rsquare	0.017394
Adj Rsquare	0.016864
Root Mean Square Error	0.343392
Mean of Response	0.350777
Observations (or Sum Wgts)	3712

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Percentile	2	7.74195	3.87098	32.8277	<.0001*
Error	3709	437.35775	0.11792		
C. Total	3711	445.09970			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
1-30	607	0.276085	0.01394	0.24876	0.30341
40-60	1083	0.318252	0.01043	0.29779	0.33871
70-90	2022	0.390620	0.00764	0.37565	0.40559

Std Error uses a pooled estimate of error variance

Figure 13: ANOVA of IR and Percentile Grouping (Day 5)

Connecting Letters Report

Level			Mean
70-90	A		0.39061985
40-60		B	0.31825177
1-30		C	0.27608457

Levels not connected by same letter are significantly different.

Figure 14 : Letters Report of Day 5 Embryo

The model is found to be significant with a p-value of less than 0.0001, showing higher performing clinics yielding more successful implantation rates. An Expanded Blastocyst in a 70-90th percentile clinic has an average IR of 0.391 (std. error of 0.00764) while the same embryo has an average IR of 0.276 (std. error of 0.01394) in a 1st-30th percentile clinic.

An analysis was also conducted for the day 5 hatching Blastocysts with Good Inner Cell Mass and Good Trophectoderm across percentile groupings. Figure 15 shows that constant variance was met.

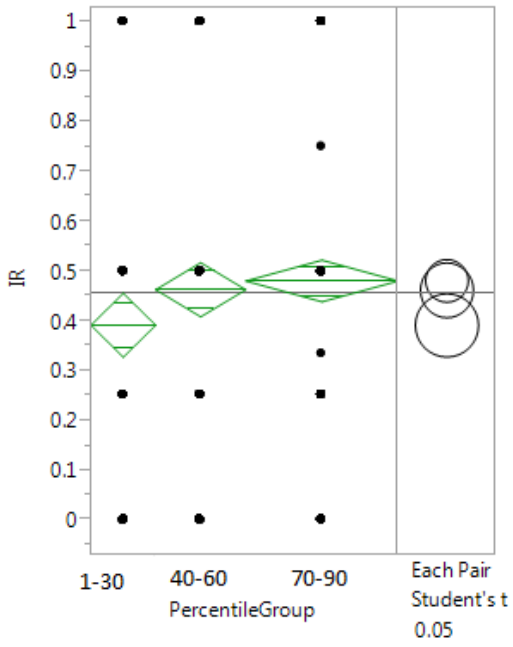


Figure 15: Constant Variance of Day 5 Hatching Blast

Oneway Anova
Summary of Fit

Rsquare	0.008295
Adj Rsquare	0.005136
Root Mean Square Error	0.376894
Mean of Response	0.45589
Observations (or Sum Wgts)	631

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
PercentileGroup	2	0.746117	0.373059	2.6263	0.0731
Error	628	89.206713	0.142049		
C. Total	630	89.952831			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
1-30	133	0.390977	0.03268	0.32680	0.45515
40-60	185	0.462162	0.02771	0.40775	0.51658
70-90	313	0.479766	0.02130	0.43793	0.52160

Std Error uses a pooled estimate of error variance

Connecting Letters Report

Level			Mean
70-90	A		0.47976571
40-60	A	B	0.46216216
1-30		B	0.39097744

Figure 16: ANOVA of Day 5 Hatching Blast with Letters Report

The model is not found to be significant meaning that when all three percentile means are compared, the null hypothesis cannot be rejected. When looking at percentile clinics individually in the connecting letters report from Figure 16, the groupings with 1-30th percentiles and 70-90th percentiles are significant. A Hatching Blast in a 70-90th percentile clinic has an average IR of .427 (std. error of 0.0065) while the same embryo has an average IR of 0.337 (std. error of 0.01245) in a 1st-30th percentile clinic. A full table on each embryo's implantation rate dependent on morphology and percentile grouping can be found in Appendix C. This finding is consistent with clinical knowledge that higher performing clinics tend to have better technology that enables them to grow day 5 embryos, while lower performing clinics lack the technology to support day 5 embryos.

The Day 3 analysis began with 8-Cell, 0 Fragmentation, Perfect Symmetry embryos. Again, an ANOVA test was conducted to detect significance between percentile groupings.

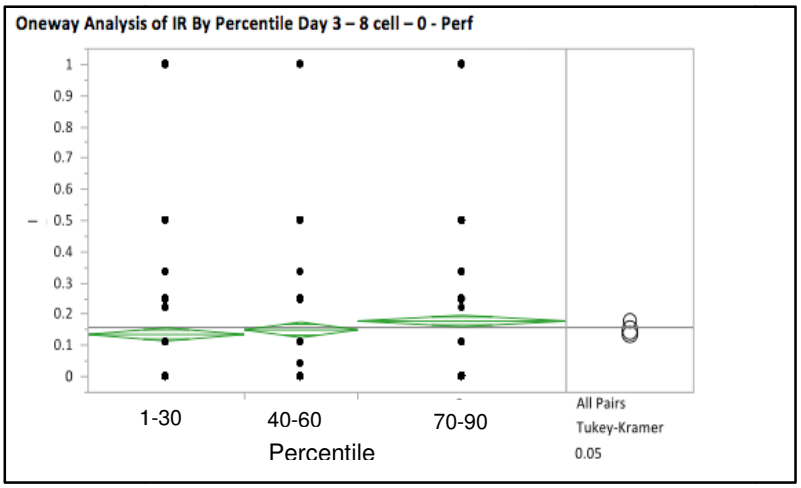


Figure 17: Constant Variance of Day 3 Embryo

Oneway Anova
Summary of Fit

Rsquare	0.006125
Adj Rsquare	0.004838
Root Mean Square Error	0.242918
Mean of Response	0.15778
Observations (or Sum Wgts)	1548

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Percentile	2	0.561819	0.280910	4.7604	0.0087*
Error	1545	91.169458	0.059009		
C. Total	1547	91.731278			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
1-30	505	0.135479	0.01081	0.11428	0.15668
40-60	368	0.150344	0.01266	0.12551	0.17518
70-90	675	0.178519	0.00935	0.16018	0.19686

Figure 18: ANOVA Analysis of Day 3 Embryo

From the results, the model is significant with a p-value of 0.0087. The three percentile groupings are not completely independent.

Connecting Letters Report

Level			Mean
70-90	A		0.17851852
40-60	A	B	0.15034420
1-30		B	0.13547855

Levels not connected by same letter are significantly different.

Figure 19: Letters Report of Day 3 Embryo

Some overlap can be found between the mid-level percentiles. The higher performing clinic group has a mean IR of 0.1785 (std. error of 0.00935) and the lower performing clinics a mean IR of 0.1355 (std. error of 0.01081).

The next day 3 embryo that was analyzed was an 8 cell, 1-10% fragmentation, perfect symmetry. The data was tested for constant variance and passed. The ANOVA test in Figure 20 below showed that he model was significant with a p-value less than .001. In this case it shows that all three percentile groupings are not independent within this particular embryo morphology.

Oneway Anova
Summary of Fit

Rsquare	0.024008
Adj Rsquare	0.022236
Root Mean Square Error	0.24011
Mean of Response	0.140121
Observations (or Sum Wgts)	1105

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Percentile	2	1.562800	0.781400	13.5535	<.0001*
Error	1102	63.533517	0.057653		
C. Total	1104	65.096317			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
1-30	305	0.090164	0.01375	0.06319	0.11714
40-60	503	0.178595	0.01071	0.15759	0.19960
70-90	297	0.126263	0.01393	0.09893	0.15360

Std Error uses a pooled estimate of error variance

Figure 20: Anova of Day 3 Embryo

The higher performing clinic group for this 8 cell embryo has a mean IR of 0.125 (std. error of 0.00468) and the lower performing clinics a mean IR of 0.078665 (std. error of 0.01623). Clinically, the closeness in performance of embryos with day 3 transfers makes sense. All clinics are able to produce day 3 embryos. In practice, the higher performing clinics will only transfer embryos on day 3 if the embryo quality of the patient is low and incubating the embryo any further will most likely not yield a high quality embryo. See Appendix D for an in depth analysis of each embryo morphology analyzed on JMP.

Cost Analysis

The IVF process is extremely intensive, both financially and socially, therefore direct and indirect costs must be taken into account.

Direct Costs

Patients want to spend the least amount of money possible for the best possible results. For IVF, both the upfront price of a procedure, and the number of treatments need to be considered. A high-ranked clinic may charge $10,000 per treatment while a lower-ranked clinic may charge $7,000 per treatment. Even though the upfront costs of the lower percentile clinic is cheaper, in the end, because the success rate is lower in the cheaper clinic, the patient may need several rounds of IVF accumulating two or three times the amount of the upfront costs. The following table illustrates this simple model:

Cost	Cost (Higher % Clinic)	Cost (Lower % Clinic)
Upfront	$10,000.00	$7,000.00
# Trials	1	2
Total Cost	$10,000.00	$14,000.00

Table 2: Cost Comparison

A high-ranking percentile clinic can charge more for their process, because of their higher IR and success rate. These treatments are very costly ranging from $16,000 - $800,000, and often represent a significant economic burden for families. Therefore, making small improvements to their process can increase benefits for the clinic and patient, in increased revenue and less trials, respectively.

Indirect Costs

For women, the pregnancy process is extremely emotional. Hoping for a positive result can be draining, and receiving a negative result, simply earth-shattering. The stress of an unfulfilled wish for a child has been associated with emotional symptoms such as anger, depression, anxiety, and feeling of worthlessness. Some patients have rated the stress of undergoing an IVF cycle as more stressful than or just as stressful as another major life event such as the death of a family member, separation, or divorce. Physically woman are exposed most of the time to various rigorous rounds of medications and injections. Patients have said it is more stressful and uncomfortable to undergo an IVF cycle than a normal menstrual cycle. Despite the relatively low chance of achieving pregnancy in one IVF cycle, many women have unrealistic expectations about the treatment success. Many women report a lack of control during the process and feel like they have little choice but to succumb to the invasive investigations. As a result of this, feelings of depersonalization can occur.

Undergoing infertility treatments can also have an impact on a patient's social life. Social activities are often put on hold because many women are not able or willing to share their experiences. Furthermore the frequent hospital visits can result in missing a lot of work days. It can also put a strain on relationships since partners cope with these situations differently. Overall, IVF treatments put a lot of stress on the patients and their families both psychologically and financially. Any improvements in the success rate of IVF cycles would greatly benefit patients and clinics.

Results and Discussions

In summary, age is significant with regards to implantation rate and FSH level. FSH level played an important role in the number of oocytes retrieved, which in turn plays a large role in determining Implantation success. Also, the older the patient becomes, the higher the maximum FSH blood level will be which in turn is significant in making the implantation rate lower. When analyzing whether smoking and BMI were significant regarding implantation, the null hypothesis could not be rejected. Lastly, Embryo Morphology was significant across percentile groups, especially within day 5. This means that the process used to grow embryos is significant in determining an implantation rate.

As stated in the cost analysis section, increasing the implantation rates of the lower performing clinics could save patients a lot of money and potentially reduce the amount of cycles a woman undergoes. Each cycle has proven to be mentally and physically straining for each patient. They can be a negative impact on relationships and even cost them their jobs.

Future Directions

The amount of information included in this dataset is enormous. There are many areas that can be expanded upon. Several of these areas, such as differences between percentile groups and across embryo morphology are being researched for a Master's thesis. The results of the mentioned thesis are expected to be a breakthrough in IVF. By studying differences between percentile groups and embryo morphology, a standardized grading method/scale are hoped to be produced, along with conclusions about the different processes clinics are using.

Also, using this report as a baseline, expanding implantation rate to live birth rate success would be an interesting step. This could reveal more effects that the patient has on the successful completion of growing the fetus. Perhaps then, a significance can be seen in BMI and smoking preference of the patient.

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Appendix A

Before					
Percentile Group	Patient ID	Age	BMI	Embryo Morphology 1	Embryo Morphology 2
70	11111111	34	24.3	Early Blast, Good	Hatching Blast, Good
50	1232123	37	25.7	8-cell, 0% Frag	8-cell, 1-10% Frag

After					
Percentile	Patient ID	Age	BMI	Embryo Morphology	**MOVED**
70	11111111	34	24.3	Early Blast, Good	
70	11111111	34	24.3	Hatching Blast, Good	
50	1232123	37	25.7	8-cell, 0% Frag	
50	1232123	37	25.7	8-cell, 1-10% Frag	

Table 3: Stacking Process

Appendix B

DAY 5

DayOfTransfer	Stage	InnerCell	Trophoblast	Implantations	Embryos Transfered	IR
5	Expanded Blast	Good	Good	1989	5305	0.374929
5	1 cell	Not Entered	Good	707	2449	0.288689
5	Morula	Not Entered	Good	132	1897	0.069584
5	Hatching Blast	Good	Good	551	1341	0.410887
5	Early Blast	Good	Good	271	1321	0.205148
5	Early Blast	Fair	Fair	166	1077	0.154132
5	Expanded Blast	Good	Fair	301	1055	0.285308
5	Expanded Blast	Fair	Fair	238	1019	0.233562

DAY 3

DayOfTransfer	Stage	Fragmentation	Symmetry	Implantations	EmbryosTransfered	IR
3	1 cell	Not Entered	Not Entered	584	7313	0.079858
3	8 cell	0	Perfect	449	3684	0.121878
3	8 cell	1-10%	Perfect	274	2597	0.105506
3	8 cell	0	Unknown	210	2013	0.104322
3	8 cell	Unknown	Unknown	232	1774	0.130778
3	8 cell	1-10%	Moderate Asym	155	1558	0.099487
3	8 cell	Not Entered	Not Entered	87	958	0.090814
3	8 cell	0	Moderate Asym	123	846	0.14539
3	6 cell	1-10%	Moderate Asym	25	731	0.0342

Table 4: Embryo morphology Contingency Tables

Appendix C

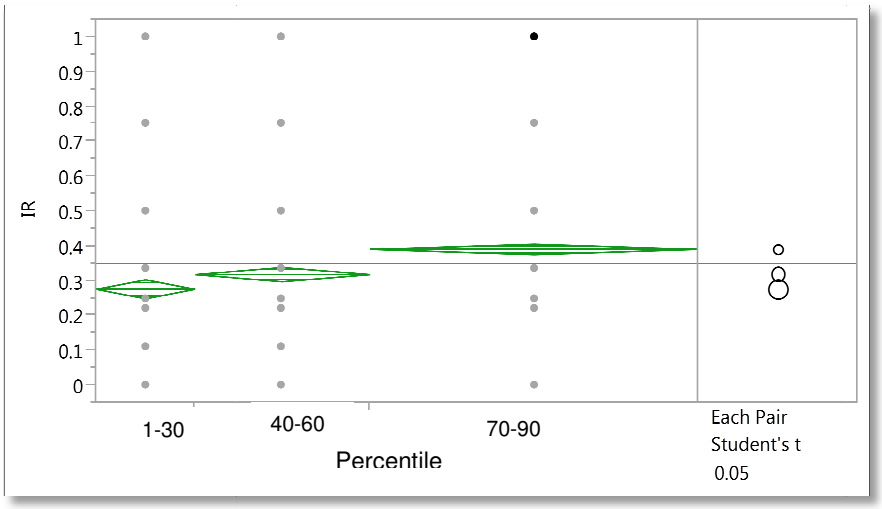
	8 Cell, 0 Frag, Perfect		8 Cell, 1-10 Frag, Perfect		8 Cell, 1-10 Frag, Moc	
	Patient Factor	IR	Patient Fact	IR	Patient Fact	IR
A1-3		0.229		0.166		0.179
A4-6		0.282		0.31		0.179
A7-9		0.331		0.223		0.267
B1-3		0.133		0.047		0.141
B4-6		0.167		0.174		0.07
B7-9		0.201		0.123		0.116
C1-3		0.029		0.023		0.052
C4-6		0.044		0.035		0
C7-9		0.053		0.03		0
		0.163222222		0.125666667		0.111556

	Exp-G-G		Hatch-G-G		Early-G-G	
	Patient Factor	IR	Patient Fact	IR	Patient Fact	IR
A1-3		0.412		0.444		0.271
A4-6		0.513		0.508		0.281
A7-9		0.594		0.571		0.43
B1-3		0.337		0.366		0.151
B4-6		0.368		0.421		0.129
B7-9		0.419		0.43		0.199
C1-3		0.084		0.2		0.071
C4-6		0.348		0.2		0.169
C7-9		0.18		0.28		0.057

Table 5: Percentile Grouping Contingency Table by Embryo Morphology

Appendix D

One way Analysis of IR By Percentile – Day 5 Exp. Blast – Good



Oneway Anova
Summary of Fit

Rsquare	0.017394
Adj Rsquare	0.016864
Root Mean Square Error	0.343392
Mean of Response	0.350777
Observations (or Sum Wgts)	3712

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Percentile	2	7.74195	3.87098	32.8277	<.0001*
Error	3709	437.35775	0.11792		
C. Total	3711	445.09970			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
1	607	0.276085	0.01394	0.24876	0.30341
2	1083	0.318252	0.01043	0.29779	0.33871
3	2022	0.390620	0.00764	0.37565	0.40559

Means Comparisons
Comparisons for each pair using Student's t
Confidence Quantile

t	Alpha
1.96060	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	3	2	1
3	-0.02117	0.04702	0.08338
2	0.04702	-0.02893	0.00803
1	0.08338	0.00803	-0.03865

Positive values show pairs of means that are significantly different.

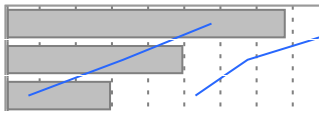
Connecting Letters Report

Level			Mean
3	A		0.39061985
2		B	0.31825177
1		C	0.27608457

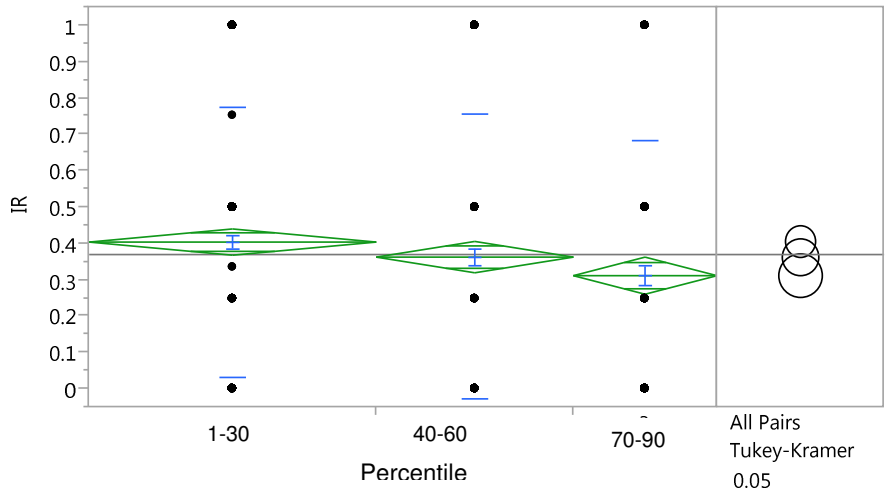
Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
3	1	0.1145353	0.0158928	0.0833758	0.1456948	<.0001*
3	2	0.0723681	0.0129305	0.0470165	0.0977197	<.0001*
2	1	0.0421672	0.0174110	0.0080310	0.0763034	0.0155*



Oneway Analysis of IR By Percentile Day 5 – Hatching- G-G



Oneway Anova
Summary of Fit

Rsquare	0.009245
Adj Rsquare	0.007108
Root Mean Square Error	0.37822
Mean of Response	0.37043

1-30
70-90
Percentile
40-60

Observations (or Sum Wgts) 930

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Percentile	2	1.23743	0.618714	4.3251	0.0135*
Error	927	132.60773	0.143050		
C. Total	929	133.84516			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
1	425	0.404706	0.01835	0.36870	0.44071
2	292	0.363014	0.02213	0.31958	0.40645
3	213	0.312207	0.02592	0.26135	0.36307

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
1	425	0.404706	0.370965	0.01799	0.36934	0.44008
2	292	0.363014	0.392238	0.02295	0.31784	0.40819
3	213	0.312207	0.372955	0.02555	0.26183	0.36258

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.34748	0.05

LSD Threshold Matrix

Abs(Dif)-HSD	1	2	3
1	-0.06091	-0.02579	0.01796
2	-0.02579	-0.07348	-0.02920
3	0.01796	-0.02920	-0.08603

Positive values show pairs of means that are significantly different.

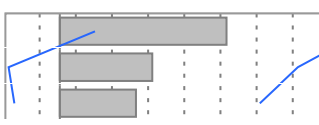
Connecting Letters Report

Level			Mean
1	A		0.40470588
2	A	B	0.36301370
3		B	0.31220657

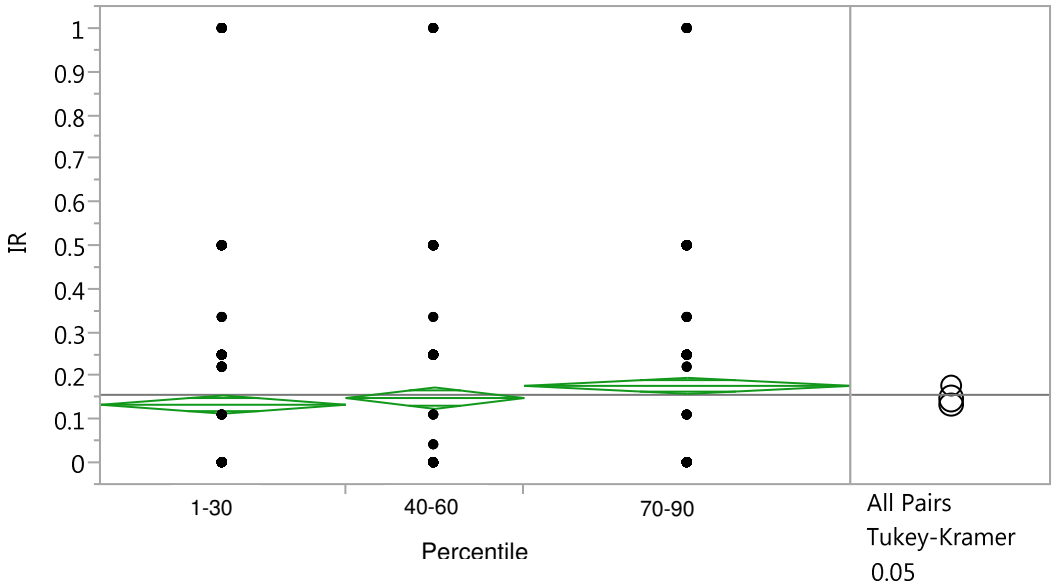
Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
1	3	0.0924993	0.0317520	0.017962	0.1670364	0.0102*
2	3	0.0508071	0.0340807	-0.029197	0.1308109	0.2958
1	2	0.0416922	0.0287487	-0.025795	0.1091791	0.3157



Oneway Analysis of IR By Percentile Day 3 – 8 cell – 0 - Perf



Oneway Anova
Summary of Fit

Rsquare	0.006125
Adj Rsquare	0.004838
Root Mean Square Error	0.242918
Mean of Response	0.15778
Observations (or Sum Wgts)	1548

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Percentile	2	0.561819	0.280910	4.7604	0.0087*
Error	1545	91.169458	0.059009		
C. Total	1547	91.731278			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
1	505	0.135479	0.01081	0.11428	0.15668
2	368	0.150344	0.01266	0.12551	0.17518
3	675	0.178519	0.00935	0.16018	0.19686

Std Error uses a pooled estimate of error variance

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.34597	0.05

LSD Threshold Matrix

Abs(Dif)-HSD	3	2	1
3	-0.03102	-0.00875	0.00951
2	-0.00875	-0.04201	-0.02419
1	0.00951	-0.02419	-0.03586

Positive values show pairs of means that are significantly different.

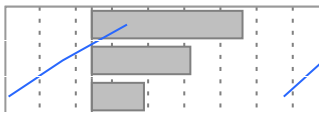
Connecting Letters Report

Level			Mean
3	A		0.17851852
2	A	B	0.15034420
1		B	0.13547855

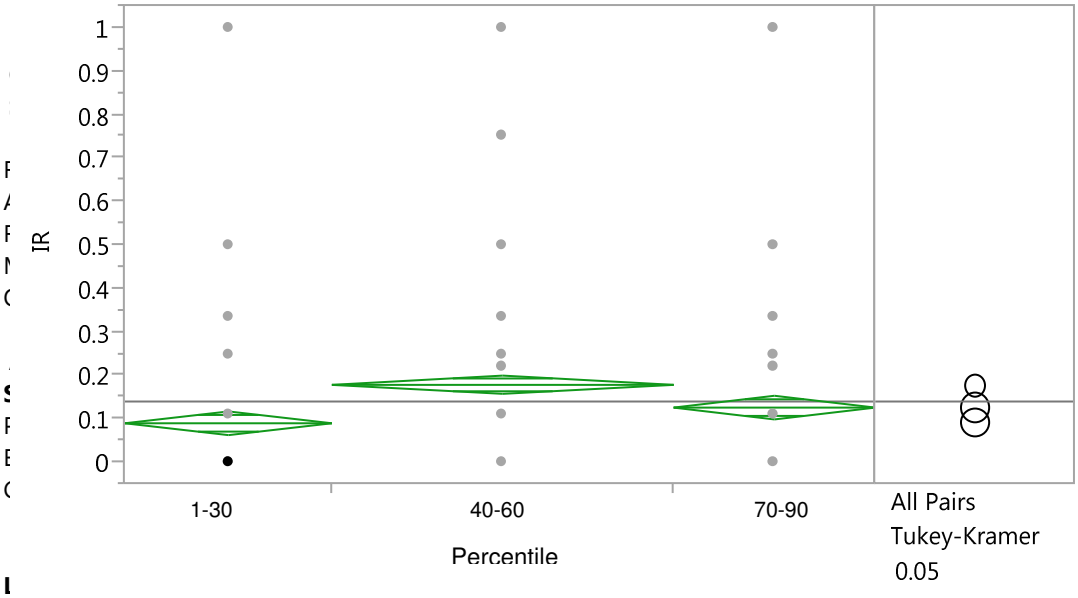
Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
3	1	0.0430400	0.0142924	0.009511	0.0765694	0.0074*
3	2	0.0281743	0.0157408	-0.008753	0.0651017	0.1733
2	1	0.0148657	0.0166494	-0.024193	0.0539245	0.6449



Oneway Analysis of IR By Percentile – 8 Cell – 1-10%- Perfect



1-30	305	0.090164	0.01375	0.06319	0.11714
40-60	503	0.178595	0.01071	0.15759	0.19960
70-90	297	0.126263	0.01393	0.09893	0.15360

Std Error uses a pooled estimate of error variance

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD
Confidence Quantile

q*	Alpha
2.34688	0.05

LSD Threshold Matrix

Abs(Dif)-HSD	2	3	1
2	-0.03553	0.01110	0.04754
3	0.01110	-0.04624	-0.00984
1	0.04754	-0.00984	-0.04563

Positive values show pairs of means that are significantly different.

Connecting Letters Report

Level			Mean
70-90	A		0.17859510
40-60		B	0.12626263
1-30		B	0.09016393

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
2	1	0.0884312	0.0174254	0.047536	0.1293264	<.0001*
2	3	0.0523325	0.0175709	0.011096	0.0935692	0.0083*
3	1	0.0360987	0.0195741	-0.009839	0.0820366	0.1558