The Effects of a History of Eating Disorders on Women's Fertility and Pregnancy

Ashna Aggarwal • Francis Parker School

Objective

The purpose of this poster is to examine the effects of a history of eating disorders, as compared to a history of other psychiatric disorders or no psychiatric disorders, on women's fertility and pregnancy. Specifically, this poster will demonstrate how a history of eating disorders affects women's rates of undergoing fertility treatment, rates of twin births, and rates of complications during pregnancy. Additionally, this poster will examine how a history of eating disorders affects women's rates of unplanned pregnancies and their feelings regarding unplanned pregnancies.

Abstract

In the last few years, eating disorders, including Anorexia nervosa (AN) and Bulimia nervosa (BN), have had increased prevalence in societal discussions. This increase in attention can be attributed to the rising percentage of Americans, especially women, suffering from eating disorders–currently, 0.5% of women suffer from AN, and 2-3% of women suffer from BN. AN is characterized by an abnormally low body weight, a disturbance in body image, and an intense fear of gaining weight; BN is characterized by both phases of binge eating and compensatory behaviors as well as a high value placed on weight and image[5]. Both AN and BN are associated with medical complications which have a relevant impact on fertility and pregnancy. Past studies have demonstrated that complications can arise from eating disorder behaviors *during* pregnancy, including preterm delivery, low birthweight and increased odds of Caesarean birth[2]. Often overlooked–yet vital to women in these circumstances–are the effects of a *history* of eating disorders on the fertility and pregnancy of women. Both a lifetime history of eating disorders (AN, BN, or both) or a past history in women can affect their rates of seeking fertility treatment and rates of twin births. Additionally, this history affects women's rates of unplanned pregnancies and their feelings regarding this pregnancy. Lastly, a history of eating disorders can impact the chances of complications arising during pregnancy.

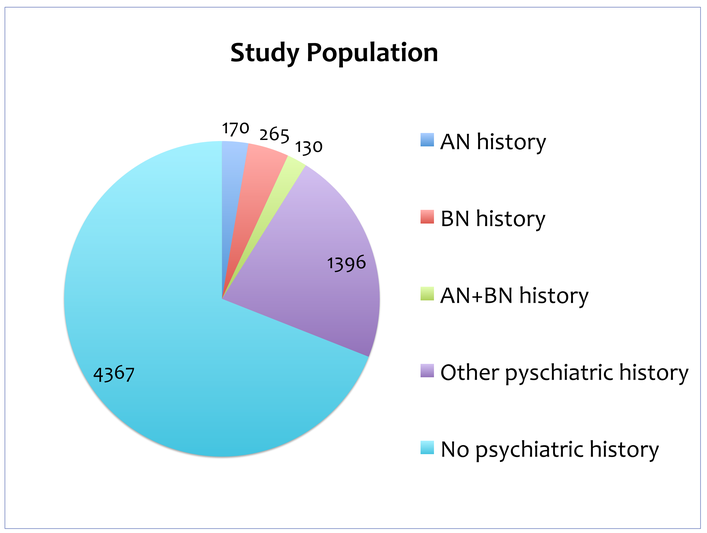

Figure 1. Study population of first study based in Rotterdam, the Netherlands. Micali, N., et al. (2014). Fertility treatment, twin births, and unplanned pregnancies in women with eating disorders: findings from a population-based birth cohort. BJOG, 121(4). Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24206173

Methods and Materials

In one study, a group of women from Generation R (a prospective general population cohort study based in Rotterdam, the Netherlands at the Erasmus Medical Centre) was divided into those with AN history, those with BN history, those with AN+BN history, those with other psychiatric history, and those with no psychiatric history (see Figure 1). The AN, BN, and AN+BN groups were broken down further into those with lifetime history, those with past history, and those with history in the last year before pregnancy. Because of the small percentage of women that fell into the category of history within the last year, sufficient data wasn't obtained and thus this group will not be the focus of this poster. The women were eligible for enrollment if they had a delivery date between April 2002 and January 2006; of the 8,880 recruited, 6,328 were selected based on the women's completion of a questionnaire used to determine exposure for the study. Data regarding patients' psychiatric history was obtained from patients' self-evaluation based on a provided medical vignette, data regarding fertility treatments and twin births was obtained from obstetric records, and data regarding unplanned pregnancies and women's feelings about unplanned pregnancies was obtained from a questionnaire given to women upon enrollment.

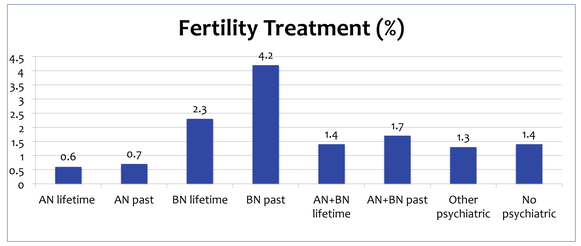

Table 1. Fertility Treatment (%). Micali, N., et al. (2014). Fertility treatment, twin births, and unplanned pregnancies in women with eating disorders: findings from a population-based birth cohort. BJOG, 121(4). Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24206173

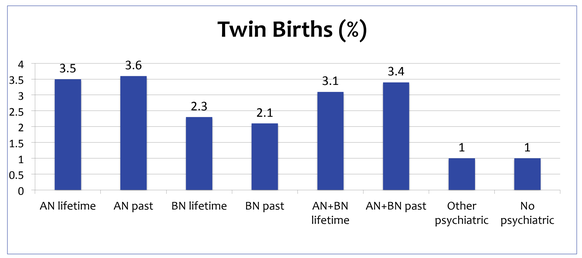

Table 2. Twin Births (%). Micali, N., et al. (2014). Fertility treatment, twin births, and unplanned pregnancies in women with eating disorders: findings from a population-based birth cohort. BJOG, 121(4). Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24206173

Results

According to the study, women with a history of BN, either lifetime or past, had an increased rate of seeking fertility treatment as compared to women with other or no psychiatric history, whereas women with AN history, either lifetime or past, had a decrease in this rate[4] (see Table 1). Additionally, a history of eating disorders, whether lifetime or past, is associated with increased rates of twin births[4] (see Table 2). It was also found that AN (lifetime or past) and AN+BN (lifetime or past) history is associated with increased rates of unplanned pregnancies as compared to women with other or no psychiatric history; additionally, women with these histories were more likely to have continually mixed feelings regarding this pregnancy[4]. This was corroborated by a second study in the Avon area, UK, which found that women with lifetime AN or lifetime AN+BN were more likely to view motherhood as a personal sacrifice[1]. A third study, conducted in Rotterdam by the original group of scientists, found that lifetime AN+BN history is associated with approximately doubled rates of hospitalization during pregnancy and lifetime AN history is associated with an 80% increased rate of fetal distress as compared to women with no psychiatric history[3].

Conclusions

In conclusion, a history of eating disorders has a proven impact on women's fertility and pregnancy. A history of eating disorders is associated with increased rates of twin births. A lifetime or past history of BN is associated with increased rates of fertility treatment. A lifetime or past history of AN or AN+BN is associated with increased rates of unplanned pregnancies and continued mixed feelings regarding this pregnancy. Lastly, lifetime histories of AN and AN+BN are associated with complications during pregnancy. Fertility treatment experts should keep in mind that a history of eating disorders may underlie fertility problems and pregnancy complications.

Applications to Biotechnology

Advances in biotechnology allowed fertility options to be available to those women (primarily those with a history of BN) who sought fertility treatment. Of the women with a history of lifetime bulimia who sought fertility treatment, approximately 60% were treated with induced ovulation and approximately 40% were treated with IVF[4].

Acknowledgements

I would like to extend my sincerest thanks to Dr. Ericka, for her enthusiasm, dedication, knowledge, and insight. I would like to extend thanks to Sheriden Smith, for her humor and help, to Dr. Chang, for his knowledge and wisdom, and to Mrs. Winter, for her patience and commitment. I would also like to thank all of the doctors and researchers that shared their insight and made this program possible. I'd like to thank my parents and AP science teachers, for always encouraging me and supporting me in my pursuit of science. And, last but most definitely not least, I would like to thank my OSA sisters of 2015 for their help, encouragement, and friendship.

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Comparison of Live Birth Rates Resulting From Embryo and/or Egg Cryopreservation Via Vitrification

Simran Budhwani

Mount Carmel High School

Objective

This research will explain the comparison of two forms of fertility preservation and live birth rates resulting from embryo and egg cryopreservation via vitrification. Additionally, data will illustrate how maternal age at the time of implantation plays a crucial role in live birth rates.

Abstract

Cryopreservation, or the process in which reproductive factors such as eggs, tissue, ovaries, and embryos are frozen to be used at a later time, has become increasingly popular with women who have fertility issues, either from genetic or external factors such as cancer treatment. Within the presented research is a comparison between the two most prevalent forms of fertility preservation--egg and embryo freezing--and how effective each treatment is as indicated by successful live births. One of the major problems of cryopreserving eggs and embryos is the formation of ice crystals. A new method of freezing, vitrification, is a more effective method of cryopreservation because of its high amount of cryoprotectants. A university based hospital in East Asia conducted a study of live birth rates of embryos by vitrification versus slow freezing. The study contained 8,824 cryopreserved human cleavage stage embryos of which 7,482 were vitrified while 1,342 were frozen by slow freezing. The survival rate of the vitrified embryos was far greater compared to slow freezing, with a 15:1 ratio. A second study done, citing meta analysis data from different reproductive centers using both vitrification and slow freezing of oocytes demonstrates that vitrification still has higher survival rates. Out of the 13,079 total oocytes that were thawed, there was an 85% survival rate of those oocytes that underwent vitrification. One of the major problems of cryopreserving eggs and embryos is the formation of ice crystals. This research presents further information of how using vitrification as a freezing method, with an embryo or egg, results in higher live birth rates. Along with this, it will inform patients about the comparison between freezing an embryo versus an egg, and how the maternal age during implantation can determine the chances of a live birth.

Comparison Of Embryo Preservation

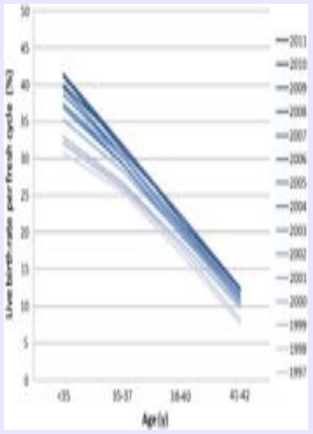

Figure 1. ***Percentage of all initiated fresh cycles resulting in live births per year from 1997 to 2011 in the United States***

Mastenbroek, S., & Repping, S. (2014). Cryopreservation of human embryos and its contribution to in vitro fertilization success rates. Fertility and Sterility, 19-26.

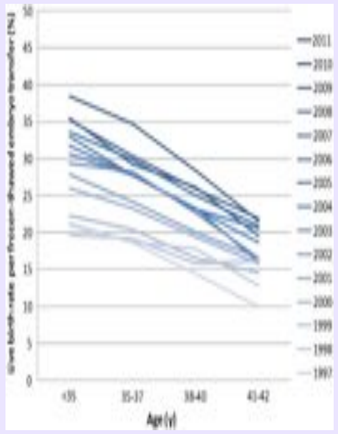

Figure 2. ***Percentage off frozen-thawed embryo transfers resulting in live births per year from 1997 to 2011 in the United States***

Mastenbroek, S., & Repping, S. (2014). Cryopreservation of human embryos and its contribution to in vitro fertilization success rates. Fertility and Sterility, 19-26.

Methods and Materials

A University based Hospital in East Asia, conducted a study of live birth rates of embryos by vitrification versus slow freezing. The study contained 8,824 cryopreserved human cleavage stage embryos (blastocysts) of which 7,482 were vitrified while 1,342 were frozen by slow freezing. Four individual investigations studies were conducted using a protocol of ovarian stimulation either by Long agonist/rFSH or CC/hMG. A second study done, citing meta analysis data from different reproductive centers used both vitrification and slow freezing of oocytes to test survival rates. There were 1,805 infertile patients undergoing nondonor oocyte cryopreservation with a total of 13,079 oocytes and 2,265 thaw cycles. A third study done by the Midland Fertility Services cites data from Human Fertilization and Embryology Authority from 2006 shows the live birth rates of patients undergoing IVF and ICSI between the ages of 38 and 45.

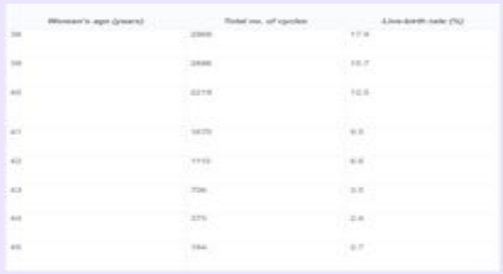

Figure 3 ***UK live-birth rates for cycles completed in 2006 of frozen embryo transfers***

Lockwood, G. (n.d.). Social egg freezing: The prospect of reproductive 'immortality' or a dangerous delusion? Reproductive BioMedicine Online,23(3), 334-340.

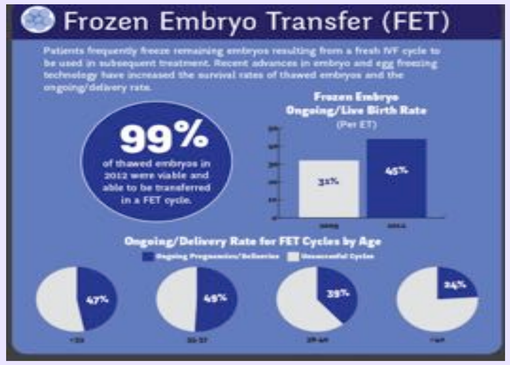

Figure 4. ***Frozen embryo transfers done at Shady Grove Fertility Center in 2012***

Frozen Embryo Transfer Success Rates - HRFnd. (2014, March 19). Retrieved August 10, 2015.

Results

The first study contained 8,824 cryopreserved human cleavage stage embryos of which 7,482 were vitrified while 1,342 were frozen by slow freezing. The survival rate was significantly higher after vitrification as compared with slow freezing. The odds ratio was a 15.57, 95% confidence level as compared to slow freezing the odds ratio had 2.20, 95% confidence level, essentially a 15:1 ratio. The results of the second study which used both vitrification and slow freezing of oocytes demonstrated that vitrification still has higher survival rates. Out of 13,079 total oocytes that were thawed, there was an 85% survival rate of those oocytes that underwent vitrification. While reviewing both studies, it became apparent, that the factor of maternal age played a critical role in the improvement of live birth rates. The third study further demonstrates this fact by focusing mainly on the relationship between age and live birth rates. The study showed that a woman who would undergo an assisted reproductive treatment with her own fresh eggs at 42 would have a live-birth rate of 6.6% per cycle. The rate, had she used cryopreserved eggs harvested at age 30, would be 40% greater per transfer when implanted at 42 years of age.

Conclusions

Vitrification, the process of freezing an embryo or egg to a glass -like state, was proven to have a higher live birth rate in both cryopreserving an embryo and egg. It has a higher level of cryoprotectant agents and ultrafast cooling and warming rates which eliminates the chance of intracellular and extracellular ice formation within and around the cell. The effects of the stage of development in which the method implemented cryopreservation as well as embryo transfer plays a critical role in the live birth rates. This fact is highlighted by data showing pregnancy rates above 50% for young patients undergoing embryo vitrification. Egg freezing, however, is a young practice in medicine, making it controversial in whether or not it is a plausible practice because it is investigational and experimental. Due to lack of technology and ice crystals puncturing the membrane of the egg, an average of 30% of the cryopreserved eggs are not even viable for implantation. It was discovered that maternal age played a significant role in the rate of successful live birth rates regardless of whether the embryo or the egg was cryopreserved. This is important to note for both female patients and physicians that the age at which they decide to implant their embryo or egg for a pregnancy, along with the type of freezing method-vitrification or slow freezing, is vital when wanting to pursue the most effective fertility preservation method.

Relation to Biotechnology

Due to the rise of biotechnological methods such as vitrification and slow freezing, freezing eggs alone is becoming a more hopeful option for women who are currently not in a relationship, do not want a sperm donor, or who want to have children after establishing a career. Although it is a known fact that fertility rapidly declines at age 37-- having less viable eggs for embryos as a woman ages-- this technology could possibly change the rates of successful pregnancies, even in women who are premenopausal.

Acknowledgments

I would like to thank my family, especially my mother, and Dr. Ericka Senegar-Mitchell for always having faith in me and for supporting me throughout this journey. They have encouraged me to have more confidence in myself and for being a female scientist and I could not be more grateful. I would also like to thank Ms. Winter and Sheriden for their incredible dedication and time to the program along with, Dr. Chang, and all the incredible scientists and doctors we had the privilege of meeting this summer. I feel so blessed to have been a part of a program and have learned so much alongside my amazing OSA sisters.

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The Role of Lysophosphatidic Acid as a Biomarker for Early Stage Epithelial Ovarian Cancer Compared to CA-125

Nayeli Diez de Bonilla

High Tech High Chula Vista

Objective

Ovarian cancer ranks fifth in cancer deaths among women, yet if this disease is caught in an early stage, more than 90% of patients live longer than 5 years. If there was a method of detecting ovarian cancer in an early stage, doctors could potentially lower the amount of deaths caused by this disease. The purpose of this poster is to address the urgent need for a biomarker for ovarian cancer that can be used to screen the general population of women. This poster will also compare the efficiency of using CA-125 and Lysophosphatidic acid (LPA) as potential biomarkers for early stage ovarian cancer.

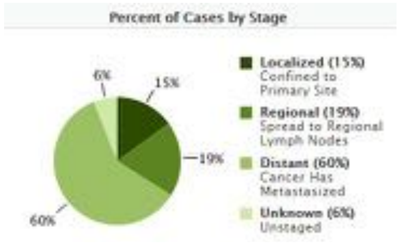

Figure 1 This pie graph represents all of the women who are diagnosed with ovarian cancer. It shows what stage the women's disease was at the time of diagnosis.[6]

Abstract

Although ovarian cancer is in the top 20 of cancer diagnoses worldwide, over 70% of ovarian cancer cases are diagnosed in a late stage, due to lack of early detection methods. Because of inefficient detection methods, only women who are identified as high risk are screened; however, data suggests that there are alternative methods that could be used to screen all women, rather than exclusively high risk women. Finding a biomarker for ovarian cancer that is present in early stages would provide doctors with the ability to detect ovarian cancer earlier. Therefore, the amount of deaths attributed to this disease could possibly be reduced. CA-125 is a cancer antigen that is primarily used to detect and monitor the response of ovarian cancer to treatment. Many studies have been published in which researchers compared the levels of CA-125 and LPA in the bloodstream of three categories of women: healthy women, women with benign ovarian tumors, and women with epithelial ovarian carcinoma(EOC) at different stages. Using the cutoff level of 35 U/L for CA-125 and 1.3 µmol/L for LPA, researchers were able to compare the specificity and sensitivity of these potential biomarkers to early stage ovarian cancer. Researchers found that initial levels of LPA were significantly higher in patients with EOC than in patients with benign ovarian tumors and healthy women. Researchers determined that measuring LPA levels is a better method of diagnosing ovarian cancer, but that tracking CA-125 levels after the detection of cancer is the more effective way to monitor the disease's response to treatment. Overall, LPA has proven to be a promising biomarker for EOC, but further studies should be conducted to confirm the role of LPA in EOC and to determine the potential role of LPA in other gynecological cancers. If measuring LPA levels could become the widespread method of screening for ovarian cancer, countless lives could be spared through ea

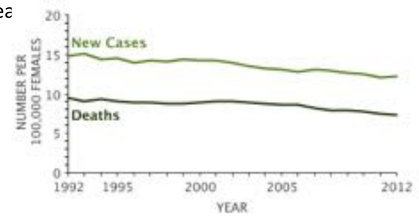

Figure 2 This graph compares the amount of new cases of ovarian cancer per year to the amount of deaths caused by ovarian cancer per year.[6]

Methods and Materials

In a study performed at Istanbul University in Turkey, 50 healthy women, 74 patients with benign ovarian tumors, and 87 patients with epithelial ovarian carcinoma(EOC) were tested to measure the amount of LPA and CA-125 in their system. Of the 87 EOC patients, 10 patients had stage I, 63 patients had stage III, and 14 patients had stage IV. Women who were diagnosed with germ cell tumors and sex cord stromal tumors were not included. The participating women had 2 samples of 5 mL's of blood drawn. One sample was then analyzed for LPA using a biochemical method that other studies had used. First, the lipids were extracted and then the LPA was separated from other lipids via thin layer chromatography. Then, after hydrolysis and deprivation, an analysis was performed using gas chromatography and mass spectrophotometry (GC-MS). The cutoff value for LPA was set at 1.3 µmol/L, based on previous studies. The second sample was then analyzed for CA-125 using a CA-125 II kit that utilizes the Radioimmunoassay (RIA) technique. The cutoff value for CA-125 was set at 35 U/mL, based on other studies that have been conducted. Researchers compared the levels of LPA and CA-125 within all experimental groups. If a woman's levels of LPA were not detected, their levels were assumed to be 0.1 µmol/L for statistical analysis.

Results and Interpretations

The mean total plasma LPA level for women with EOC (n=87) was 4.29±4.52 µmol/L. Compared to women with benign ovarian tumors (n=74) who had a mean total plasma LPA level of 1.57±0.92 µmol/L and healthy women (n=50) who had a mean total plasma LPA level of 0.6±0.42 µmol/L, mean total plasma LPA levels for patients with EOC were significantly different. Using the cutoff value of 1.3 µmol/L, 83 of 87 (95%) EOC patients had LPA levels greater than or equal to that value. In comparison, 46 of 50 (92%) healthy women had total plasma LPA levels lower than 1.3 µmol/L. The mean CA-125 levels of women with EOC was 764.63±1183.44. For women with benign ovarian tumors, the mean CA-125 level was 38.14±69.40 and for healthy women the mean CA-125 level was 23.90±16.73. Mean CA-125 levels are significantly higher when compared to patients with benign ovarian tumors and healthy women. There is no significant difference between the mean CA-125 level in women with benign ovarian tumors and healthy women.

Table 1. LPA and CA-125 levels in EOC, benign ovarian tumors and healthy controls

	No.	Mean LPA* (µmol/L)	Mean CA-125† (U/mL)
EOC	87	4.29±4.52	764.63±1183.44
Benign ovarian tumor	74	1.57±0.92	38.14±69.40
Healthy women	50	0.6±0.42	23.90±16.73

EOC: epithelial ovarian cancer, LPA: lysophosphatidic acid.
*Mean LPA levels were significantly different between the groups ($p_s < 0.001$).
†Mean CA-125 level in EOC patients was significantly higher than the level in healthy women or in patients with benign ovarian tumor ($p_s < 0.001$). However, the levels were not significantly different between the patients with benign ovarian tumor and healthy women ($p_s = 0.69$).

Figure 3 This table displays the results of a study and it compares the number of participants, mean LPA, and mean CA-125 between healthy women, women with benign ovarian tumors, and women with EOC. [1]

Conclusion

Total plasma LPA levels are significantly different between women with EOC, women with benign ovarian tumors, and healthy women. This suggests that monitoring LPA levels could help differentiate between women with EOC, benign tumors, and no cancer which could be a major contribution to screening the general population. Both mean total plasma LPA levels and CA-125 levels were elevated in women with EOC compared to women with benign ovarian tumors and healthy women. These results align with evidence from other studies that have been conducted comparing LPA levels of healthy women and women with EOC. While mean LPA levels are significantly different between women with benign ovarian tumors and healthy women, we saw that mean CA-125 levels for women with benign ovarian tumors and healthy women was not significantly different. This shows us that LPA is a more sensitive option than CA-125. Further research should be conducted to compare LPA levels and CA-125 levels by stage of EOC.

Relevant Applications to Biotechnology

In a year, around 22,000 women are diagnosed with ovarian cancer and there are around 14,000 fatalities. Early diagnosis can potentially help lower the fatality rate for this disease. However, one of the many challenges to early diagnosis is that the ovaries aren't as accessible as other organs and most of the methods used for screening today don't achieve the necessary sensitivity or specificity required. Another challenge of this disease is that 90% of ovarian cancer cases occur in women who aren't in an identifiable high risk group. If researchers were able to identify a biomarker for ovarian cancer, they could develop a new method of screening the general population. This new method, which could potentially involve LPA, would have to be very sensitive and have a high specificity rate if it were to be applied to the general population. LPA appears to be playing a promising role in this area, but further tests are required to confirm this.

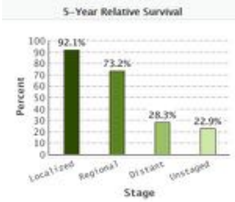

Figure 4 This graph shows the 5 year relative survival rate of women who are diagnosed with ovarian cancer depending on what stage it is detected in.[6]

Acknowledgements

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Effects of Autologous and Allogeneic Bone Marrow Transplants on Female Fertility

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Southwest High School

Introduction

The objective of this poster focuses on patients who suffer from leukemia have 3 possible treatments they can receive: chemotherapy, autologous bone marrow transplant, and allogeneic bone marrow transplant. However, one of the possible long term effects is the loss of fertility. Infertility occurs when radiation affects oogenesis and decreases the production of oestrogen and progesterone. Doctors conducted several studies to determine which treatment resulted in the most infertile patients. They also studied the possible effects preparation for these treatments could have on infertility such as hormone therapy and radiation. In addition, doctors analyzed different outcomes between different age groups.

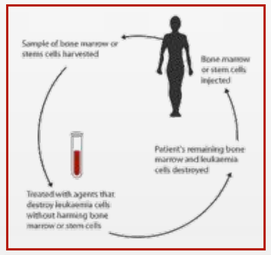

Figure 1. Process of Autologous BMT (ABMT) Source: http://www.abc.net.au/health/library/stories/2004/10/18/1830091.htm

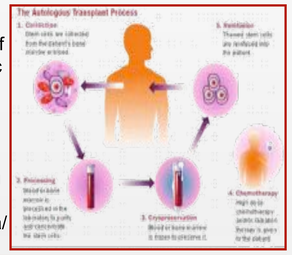

Figure 2. Process of Allogeneic BMT (Allo BMT) Source: http://cumc.columbia.edu/lymphoma/file/147

Abstract

Several post leukemia patients have reported a loss or hindrance of infertility after treatment. Three major treatments (chemotherapy, Allogeneic BMT, and Autologous BMT) are possibly associated with infertility. Chemotherapy is radiation that kills off cancer cells, allogeneic BMT (bone marrow transplant) requires a donor, and autologous BMT is a bone marrow transplant from the patient's own body. Doctors conducted multiple studies to discover any possible effects that could be observed in the fertility of post-treatment leukemia patients. In a particular study, doctors admitted 576 women into the study whose ages ranged between 10 to 60 years old. However, 25 women were removed from the study. The participants received one of three treatments: chemotherapy, analogous BMT, or allogeneic BMT. By the end of the study, only 98 patients completed the follow-up questionnaire, which provided a summary of the quality of their lives after treatment. In particular, the sexual disorder questionnaire revealed that infertility occurred in 32 (one third) of all patients, especially in the Allogeneic BMT-treated patients. Thirty-four patients who either underwent ABMT or Allogeneic BMT procedures also reported painful sexual intercourse. Doctors concluded that Allogeneic BMT resulted in more infertile patients than the other two leukemia treatments. Other doctors who have conducted similar studies also show similar results and agree that allogeneic BMT causes 83% of women to lose their fertility due to gonad damage. Gonadal damage occurs when oogenesis is affected and the production of oestrogen and progesterone is decreased. Preparation for Allogeneic BMT, such as TBI, also increases the patient's chances of infertility from 80% to 89%. Doctors should further examine if any combination of these three treatments against one, for example, chemotherapy and autologous BMT against just autologous BMT, will increase the chance of preserving fertility. Doctors should recommend chemotherapy or autologous BMT to patients who preferably want to save their fertility. In sum, patients will understand the effects different bone marrow treatments have on fertility and will be provided with reassurance by being aware of the potential risks before they are treated.

Methods and Materials

In a particular study, doctors used 576 women whose ages ranged between 10 to 60 years old in order to investigate the long-term effects of treatment for post-leukemia patients. They were divided into three test groups based on one of three types of treatments: chemotherapy, analogous BMT, or allogeneic BMT. Only 98 patients completed the entire follow-up study, which occurred a year after receiving treatment. Patients completed multiple QLQs (Quality of Life Questionnaire) to evaluate their QOL (Quality of Life). The demographic QLQ contained questions regarding marital and employment status after fertility. The EORT (European Organization for Research and Treatment of Cancer) asked about physical, cognitive, emotional and social functioning. The leukemia BMT model QLQ explored the somatic symptoms patients experienced after treatment. The sexual functioning and infertility questionnaire surveyed patients about sexual drive, pain, and infertility. The final questionnaire asked for the patients' perception on changes in their family, professional, social and leisure life. Furthermore, doctors continued to investigate the effects of allogeneic BMT on both genders. They admitted patients who were treated between 2000-2005, and whose ages ranged from 4-28 at the time of treatment. They also participated in a long term follow-up study 3 to 12 years after treatment. Clinicians evaluated data obtained from spermiograms, testicular volume measurements, menstrual cycles, hormone analysis, and evaluated the health of any children patients have had after treatment. In addition, doctors analyzed differences in fertility rates between patients who were treated before 13 years of age and patients who were treated after 13 years of age. These patients also answered survey questions that assessed their quality of life after treatment.

Figure 3. Fertility Classification in Allogeneic BMT-Treated Patients[3]

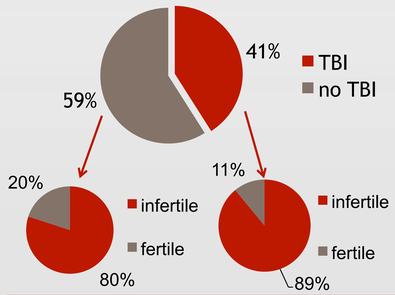

Table 1. Fertility in Patients after Treatment.[5]

Treatment	Fertility Rates
Chemotherapy	78%
ABMT	70%
Allo BMT	32%

Results

Of the 98 patients who completed the entire study, 32 reported infertility in the QLQs, most of whom received allogeneic BMT. Chemotherapy-treated patients had a 22% chance of becoming infertile, ABMT-treated patients had a 30% chance of becoming infertile, and Allo BMT treated patients had a 68% chance of becoming infertile. Patients who underwent AMBT or allo BMT experienced pain during sexual intercourse. The study that focused solely on allogeneic BMT classified eighty-three percent of the women in the study as infertile. Of those classified, 80% of women who did not receive Total Body Irridation (TBI) were classified as infertile, and 89% of women treated with TBI became infertile. Furthermore, females were more like to loose their fertility after age 13 (91%) than before age 13 (72%). Although Doctors have not been able to pinpoint the exact time in which infertility occurs, they have learned that infertility due to treatment is progressive, not immediate.

Conclusions

Doctors have concluded that Allogeneic BMT results in the most cases of infertility. The preparation radiation for this treatment, TBI, also increases the chances of infertility by 9%. Allogeneic BMT and ABMT treated patients suffer similar side effects, however, cancer reoccurrence is more likely to occur in an ABMT-treated patient since the bone marrow is from the patient's own body. Patients treated with chemotherapy experience the same side effects, however, they are less likely to lose their fertility. If possible, patients who want to preserve their fertility should asks clinicians if they can receive chemotherapy. However, some patients are in need of an allogeneic BMT. In this case, patients should ask their doctors if TBI is necessary since it raises a patient's chances of infertility from 80% to 89%. The results from these studies enable doctors to thoroughly inform their patients of all the risks each treatment has, and if the patients have any doubts, they are fully aware of the consequences of treatment before hand.

Relevance to Biotechnology

In the past, leukemia patients only had chemotherapy as an option for treatment. Biotechnology has enabled doctors to treat patients who need a bone marrow transplant. New technology has empowered doctors with the ability to collect data after treatment such as analyzing menstrual cycle patterns as well as measuring and comparing levels of hormones. Biotechnology has opened several doors for doctors who wish to help leukemia patients preserve fertility.

Acknowledgements

Thank you Mrs. Winter, Dr. Ericka Senegar-Mitchell, Sheriden Smith, and OSA sisters for guiding me throughout this academy and making it an enjoyable experience. A special thank you to all of the guest speakers who educated us and gave us a new perspective on oncofertility. I would like to thank my parents for all their support, and a special thanks to Emma Creek, who has been here with me through everything.

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Oncofertility Potential in Fertility Preservation Among Transgender Youth

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Objective

This poster investigates current Oncofertility practices that may be adapted for use in the transgender community. Specific attention is given to ethics and quality of life for transgender youth. The word transgender is an umbrella term that is used to describe a very diverse group of individuals. This project will focus on transmasculine ("assigned a female sex at birth who identify somewhere along the masculine gender spectrum") and transfeminine ("assigned a male sex at birth and identify somewhere along the feminine gender spectrum") youth between the ages of 12 and 24.

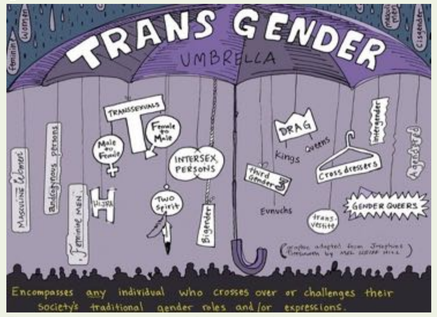

Figure 1: A visual representation of the various distinctions found on the transgender spectrum. (Hill, M. R., & Mays, J. (n.d.). Transgender Umbrella [The Gender Book]. Retrieved August 5, 2015.)

Figure 2: LGBTQ pride flag. Retrieved August 10, 2015, newlifefertility.com

Figure 3: The logo for a film that intimately documented transgender parenthood. (Huberdeau, R. (n.d.). Transgender Parents [Digital image]. Retrieved August 10, 2015, from transgenderparentsdoc.com)

Abstract

The ideas and processes surrounding fertility preservation have been long debated topics in the medical world. The development and success of IVF (in vitro fertilization) in the field of Oncofertility has opened doors to many men and women. It has been used with those that are infertile and those with cancer who are at a high risk of damaging their fertility. In these contexts, many fertility procedures have become routine. These procedures can be applied to transgender youth as well. However, they face many of the biases that once surrounded these now standard procedures. Hormonal regimens employed by young people during their transitions can cause fertility to be compromised. There is a critical window of development if a young person wishes to suppress puberty. In the past, loss of fertility was viewed as "the cost of transitioning" however this is no longer a scientific inevitability. Fertility preservation processes are becoming more accepted in the medical community when applied to minors with cancer. On the other hand, personal beliefs may challenge the acceptance of the application of these methods with individuals that identify as transgender. It is important to consider that when a youth is preparing to begin cross sex hormones or undergo sexual reassignment surgery, they may not be considering a desire for a future family or child. By giving them the opportunity to preserve their fertility, scientists can keep the option of a family open. Viable options for post pubescent youth include egg freezing and sperm collection. A study done on transgender demographics used multiple surveys, mental health screening, and risk behavior assessment to conduct a statistical analysis of the participants. A study done in Sweden focused on nine transmasculine people. It analyzed clinical characteristics, fertility preservation procedures (and outcomes), and assessed patients' psychological perceptions. There are now guidelines in place for the use of puberty suppressing drugs and hormonal regimens for youth. However, these guidelines do not include recommendations for oncofertility treatments. A few studies have begun to collect information on whether or not transgender people wish to have biological families. While results vary, many people would have pursued fertility preservation if presented the option. Still, there are cases where this technology has been employed in the transgender community. There are a host of ethical concerns that are only now beginning to be addressed. In time, these cases may be accepted in the manner of earlier procedures. These technologies provide a world of opportunity, and should not exclude any one group of people. Thus, utilizing fertility preservation in transgender youth needs to remain an open dialogue.

Materials and Methods

A review of relevant literature was conducted to explore the composition of the transgender community, the challenges that they face, and the possibilities that oncofertility can provide for the community. Effort was also spent to understand and appreciate the evolving terminology needed to report in a sensitive manner. The review led to an understanding that existing Oncofertility practices must be offered to young transgender individuals. Currently, egg banking and sperm banking is available for youth who have undergone puberty. Scientists are developing different methods to help to preserve the fertility of prepubescent cancer patients. These new treatments could also be applied to pre pubescent transgender youth. This conclusion led to the debate over medical treatments for transgender youth and their effects on fertility post-treatment. Although these treatments are still surrounded in controversy, this study examined cases where transgender youth have undergone oncofertility procedures.

Relevant Applications to Biotechnology

There are a myriad of oncofertility treatments, both standard and experimental that have the potential to enhance the life of transgender youth. Transmasculine youth have one current fertility-saving option - they can remove and freeze their eggs. At this point, the eggs can only be removed and saved for potential embryo creation after puberty has begun. Menses is required to bring the eggs through follicular development. However, one of the up and coming developments in the field of oncofertility is the ability to take an egg and bring it (almost all the way) through follicular development. Scientists predict that this will soon become an experimental fertility preservation method, such as the aforementioned egg freezing method. On the other end of the spectrum, transfeminine youth have two possible options; one standard and one experimental. Sperm banking through self stimulation is the standard method. It involves obtaining the sperm through self stimulation and then freezing it. Sperm banking through an alternate collection method is the experimental method. Sperm is obtained through testicular sperm extraction or aspiration. To date, both transmasculine methods are only possible after puberty. However, much like the research that is being done to bring eggs through follicular development, scientists are trying to develop a way for sperm to be brought to maturity before puberty.

Figure 4: Cole Carman, 18, is the first transgender teen that has undergone a procedure to preserve his fertility. (Carman, C. (2015, July 30). [A photo of transgender youth, Cole Carman]. Retrieved August 5, 2015.)

Results and Interpretation

Transgender medicine is evolving. Transgender youth are faced with many tough decisions. They can now choose to suppress their puberty until they decide to use cross sex hormones or sexual reassignment surgery to complete their transition. An issue that is not commonly addressed is transgender fertility. There is a lack of consensus concerning the ethical repercussions of treating transgender youth. These unresolved issues lead to uninformed patients and lack of fertility preservation treatment. A Swedish study done amongst 9 transmasculine people concluded that "standards of care for [fertility preservation] in [the transgender] clinical setting are lacking." When asked, fertility preservation is not always a major concern of the transgender community. A study found that amongst transfeminine people, "...[m]ore than 90 [percent] of the respondents stated that loss of fertility was not an important reason to delay their transition." In a separate study of transmasculine people, "... researchers found that 77 percent had not considered freezing their eggs at the time of HRT..." Within the same study, "54 percent of respondents still expressed a desire to have children, which may indicate a lack of action on fertility preservation prior to HRT and certain gender-confirming surgeries did not align with underlying reproductive desire." A study done on transmasculine men in Belgium "found that 54 percent wished to have children, and 38 percent would have considered freezing their eggs if the procedure had been available." Other Belgian research done with transfeminine people "found that 40 percent wanted to have children, and 77 percent felt they should have the option to preserve their sperm before hormone treatment." Despite having very little data on the reproductive desires of transgender youth, these initial studies clearly warrant more research on the topic and show that fertility preservation must be an option for this community.

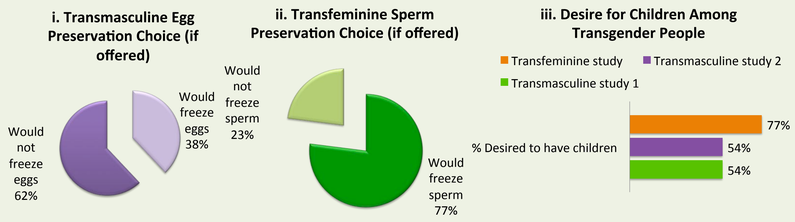

Figure 5: Graphs i and ii represent a transgender person's decision if provided the option to preserve their fertility.[2,6] Graph iii represents the desire for children among transgender people from three different studies. [2,6,7]

Acknowledgments

A special thank you to Dr. Ericka Senegar-Mitchell, Dr. Jeffrey Chang, and Sheriden Smith for their guidance. I would like to thank Hiral Dave, M. D. for her insightful presentation on the world of Bioethics. Most importantly, thank you to my parents, Lauren and Michael Gabby for their continuous love and support.

Conclusions

Precise transgender demographics do not exist. Data is especially rare on transgender people ages 12 to 24. One study, done at a clinic in Los Angeles, included 101 transgender patients. Participants were given a variety of psychological and physiological tests. One of the important results of the study demonstrated that "...baseline physiologic data [is] in line with the normal ranges of the same assigned sex non-transgender youth population..." There are many biases and misunderstandings among health professionals that create barriers for transgender individuals. Gender dysphoria is a real psychological condition that doctors often misconstrue as an issue that is brought on by "hormone imbalances." This study clearly shows hormonal imbalances are not a factor.

All people should have the right to biological children if the scientific possibility exists. The field of Oncofertility utilizes fertility preservation techniques to save the fertility of cancer patients. Often, transgender patients face similar loss of fertility. However, using these proven oncofertility techniques on transgender patients is sometimes seen as ethically wrong and is not accepted on a large scale. Laura Nixon, a sexual and reproductive health activist, states, "the objection that a gender dysphoria diagnosis is not equivalent to a cancer diagnosis is grounded in general belief that a mental health diagnosis is less legitimate than a physical health diagnosis, and a more specific belief that gender dysphoria is not existent or simply decisional." The existing treatment for transmasculine youth seeking to retain reproduction options after hormone use, is (experimental) egg freezing. On the other end of the spectrum, existing treatments for transfeminine youth with the same desires for biological offspring are (standard) sperm retrieval by self-stimulation and (experimental) sperm retrieval by testicular extraction or aspiration. Transgender youth have a critical pubescent time window to consider transitioning. When a patient wishes to block puberty and transition into the other sex, they must consider the consequences of that action. Fertility is akin to a one-way street, once it is compromised by cross sex hormone treatments there is no going back. According to Dr. Clark, a firm advocate of fertility preservation in transgender youth, there have been "viable sperm collection[s] from adolescents as young as 11 years of age." The immature sperm go through a procedure dubbed ROSNI. ROSNI takes the round spermatids (precursors of mature sperm) and injects them directly into oocytes. For now, scientists can only use this experimental procedure to preserve the fertility of pubescent or post pubescent transgender people. This is less than ideal for transgender youth as it can mean beginning (or fully experiencing) unwanted secondary sex characteristics and becoming even more uncomfortable in the bodies with which they were born. Waiting until a certain age can also mean delaying their transition, or going through painful and emotionally difficult hormone regimens (transmasculine youth for egg retrieval purposes). Some practitioners are hesitant to provide medical options for transgender youth citing studies that report incidents of transgender youth who have reconsidered their transition. Puberty suppressants do not have long-term results, but once a young person starts cross sex therapy, their fertility and ability to reverse the change is compromised. Despite the controversy, there are clinics and doctors willing to treat transgender youth. In some cases, physicians such as Dr. Olson, Dr. Eyvazzadeh, Dr. Pang, Dr. Clark, and Dr. Eyler have begun using oncofertility techniques in their practices. Further study in this area is needed as transgender medicine becomes more accepted and this marginalized group demands equality of reproductive rights.

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Treating Cervical Cancer with Monoclonal Antibody Bevacizumab

Isabel Gandarilla
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Objective

This poster intends to demonstrate how the use of the monoclonal antibody, bevacizumab increases survival rate of patients while decreasing late effects. Advanced cervical cancer is commonly treated with chemotherapy. Often resulting in tissue damage and and other toxic effects that severely impair quality of life. In order to decrease these traumatic symptoms an alternative to chemotherapy is being evaluated. Bevacizumab is used to stimulate the destruction of cancerous cells by attaching to them and inhibiting angiogenesis. A combined chemotherapy and antibody regiment will be compared to the usual chemotherapy only regiment on patients with stage four cervical cancer.

Abstract

In many developing regions in Latin America, Asia, and India, cervical cancer continues to be a leading cause of death in women. Many survivors continue to have long-term effects due to treatment options such as chemotherapy and radiation. In the case of cervical cancer, an alternative that is being tested is the monoclonal antibody bevacizumab, which imitates the immune system and degrades the cancerous cells. The treatment provides not only an effective cure for cervical cancer but less prevalence of late effects after the treatment. The effect of the monoclonal antibody was tested on 452 patients with metastatic (stage 4) cervical cancer who were then divided randomly into two overarching groups. The first group received two standard doses of chemotherapy drugs: 50 mg of cisplatin per square meter of body area as well as a 135/175 mg dose per square meter of paclitaxel. The second group, in addition to the chemotherapy, received a 15mg/kg dose of the antibody. The group with a combined regimen of chemotherapy and bevacizumab resulted in an extended survival rate of about 4 months (13.3 vs. 17.0 months). In addition, patients who received the antibody as part of their treatment had a higher response rate to the treatment (36% vs. 48%). Finally, those who were treated with the antibody also had fewer neurotoxic symptoms than the patients treated only with chemotherapy. Overall, the use of the monoclonal antibody bevacizumab in addition to chemotherapy was most effective at treating cervical cancer. The combined treatment extended the survival rate of patients while decreasing traumatic symptoms. In comparison, those patients treated with chemotherapy alone experienced a higher number of late effects and a shorter survival rate. Therefore, the effective use of the monoclonal antibody bevacizumab to treat cervical cancer indicates the development of a promising alternative to cancer treatment.

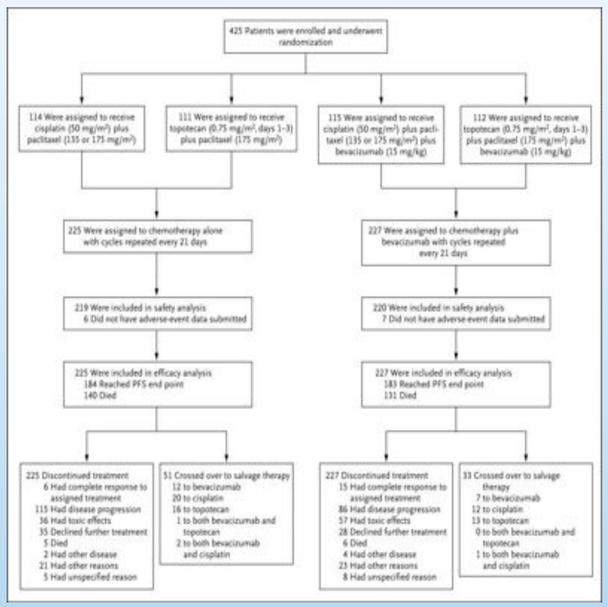

Figure 1: Flow chart demonstrating the randomization of patients into four treatment groups. *Tewari KS, Sill MW, Long HJ 3rd, Penson RT, Huang H, Ramondetta LM, Landrum LM, Oaknin A, Reid TJ, Leitao MM, Michael HE, Monk BJ. Improved survival with bevacizumab in advanced cervical cancer. N Engl J Med. 2014 Feb 20;370(8):734-43.*

Materials & Methods

A study sponsored by the National Cancer Institute was conducted in the United States and Spain to observe the effects of the monoclonal antibody bevacizumab on patients with metastatic cervical cancer. Bevacizumab is a monoclonal antibody that slows down angiogenesis by inhibiting vascular endothelial growth factor (VEGF). VEGF functions in blood vessel growth. A total of 452 patients were included in the study. All patients were female with metastatic cervical cancer and healthy bone marrow, hepatic, renal, and thromboembolism function. All of the patients were given a physical examination and a radiography prior to the studies initiation. The patients were divided into two overarching categories. The control group consisted of patients treated with a standard chemotherapy regiment. This regiment consisted of 50mg of cisplatin, which was administered per square meter of body surface area as well as 135-175mg of paclitaxel, administered also per square meter of body surface area. This study also measured the effect of the chemotherapy drug topotecan and the results in combination with bevacizumab. Cisplatin, paclitaxel, and topotecan are chemotherapy drugs that trigger cell apoptosis. After the treatment was terminated, the effects of bevacizumab in combination with the chemotherapies were evaluated by measuring tumor size every three months for two years. Three surveys were used to measure the quality of life for patients after the treatment. The FACT-Cervix survey (0 to 4 scale) was utilized to measure overall well being and the FACT/GOG survey (0 to 4 scale) was used to measure neurotoxicity. The BPI survey (0 to 10 scale) was used to measure pain after treatment.

Results & Interpretation

The implementation of the antibody bevacizumab to cervical cancer treatment increased the survival time of patients by about 4 months. Patients treated with the chemotherapy ONLY regiment survived for about 13.3 months as opposed to those patients who were treated with the antibody as well; they survived about 17.0 months. In addition, patients responded best to the combined regiment of the antibody and chemotherapy. Out of the 452 patients, 48% responded to the combined treatment whereas only 36% responded to the standard chemotherapy treatment. In addition, patients treated with the combined therapy answered about 1.2 points lower on the FACT/GOG-NTX survey which demonstrated quality of life on a scale of 1 to 4, 4 being the worse state of health. Patients treated with the combined regiment also had a 3% increase of gastrointestinal fistulas and thromboembolic

Conclusions

Cervical cancer can be more effectively treated if the monoclonal antibody bevacizumab is used in combination with chemotherapy. Patients treated with the antibody survive for a longer period of time and experience a smaller amount of late effects from the treatment. Late effects such as neurotoxicity and pain are reduced with the use of the antibody. Improvement in overall quality of life is also an effect of the antibody. Patients treated with the antibody reported in a survey to have had an overall improvement in the quality of life. In addition, neither topotecan or cisplatin is a more effective chemotherapy drug. Both drugs were equally effective at treating cervical cancer.

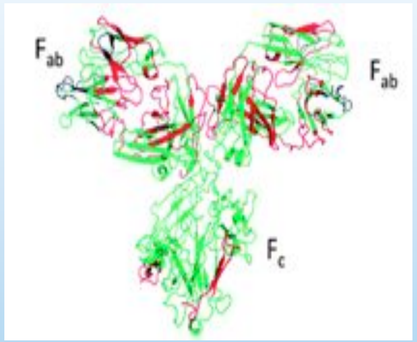

Figure 2: Representative protein model of monoclonal antibody bevacizumab. *Thompson, N., Rosati, S., Rose, R., & Heck, A. (n.d.). The impact of mass spectrometry on the study of intact antibodies: From post-translational modifications to structural analysis. Chem. Commun., 538-548.*

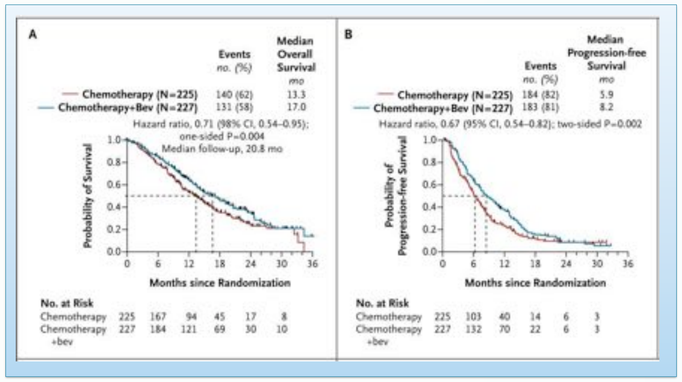

Figure 3: Graph A demonstrates the improved survival rate of patients treated with a combined chemotherapy and bevacizumab regiment. Graph B demonstrates the higher probability of progression free survival of those patients who received the combined treatment. *Tewari KS, Sill MW, Long HJ 3rd, Penson RT, Huang H, Ramondetta LM, Landrum LM, Oaknin A, Reid TJ, Leitao MM, Michael HE, Monk BJ. Improved survival with bevacizumab in advanced cervical cancer. N Engl J Med. 2014 Feb 20;370(8):734-43.*

Relevant Applications to Biotechnology

Immunology is being incorporated with technology in many different ways. Scientists have realized the immense power and influence of our own immune systems ability to cure disease. Research is focusing on detecting antibodies on cancerous cells. Such nanoparticles can be detected by advanced imaging techniques. Lasers are being used to detect these microscopic antibodies on the surface of cells. Due to such advancements in technology, the nano world is no longer an uncharted area of study and is leading to major improvements in medical therapies and treatments.

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The Effects of Preimplantation Genetic Screening and Preimplantation Genetic Diagnosis on Child Development

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La Costa Canyon HS

Objective

The objective of this poster is to present a synthesis of the most up-to-date research on the effects of PGS/PGD on child development to help assess the risks of embryo biopsy on the embryo's growth and development years later. The research indicates that PGS/PGD has no correlation to major differences in physical and neurological development among PGS/PGD children, IVF children, and children from natural pregnancies.

Abstract

Preimplantation genetic screening (PGS) and preimplantation genetic diagnosis (PGD) are new technologies that are used in vitro fertilization (IVF) treatment to help select blastocysts for implantation that will lead to a successful pregnancy and choose embryos with the optimal genes, respectively. PGS is commonly confused with PGD; however, they are different in that PGS only screens for genetic mutations that reduce the likelihood of implantation such as aneuploidy, while PGD screens out embryos with specific genes that the parents deem undesirable, such as the BRCA1/2 mutation. They are similar in that both utilize embryo biopsy to perform genetic tests on one or two cells from each embryo. Concerns about the possible harms of embryo biopsy used in PGS/PGD have led to several studies on their effect on the health and development of children. These studies are necessary to assess the risks of PGS/PGD, two invasive procedures often recommended to advanced maternal age and repetitive failure IVF patients. Research in the area of child development utilized anthropometric data at birth[1], two months[1], two years[3,5], and four years[7] and neurological assessment of general movements, motor skills, mental development, and behavioral development at infancy[2], two years[4], and four years[6] to compare the development of PGS/PGD children with IVF and natural conception children. Neurological examination utilized the Hempel test to classify the mental health of children into categories: neurologically normal, simple motor neurone disease (MND), complex MND, or neurologically abnormal.[2,6] Other methods employed include IQ testing to assess cognitive ability and the child behavior checklist to investigate behavioral development.[4,6] Physical development was measured using weight; height; arm, waist, and head circumference; and counting morphological abnormalities, classifying them into major and minor categories.[3,5] The results of these studies show that PGS/PGD children have no significant morphological or neurological health differences from other IVF children and the general population up to four years of age. Further studies are required to determine the long-term neurological and physical effects past age four before PGS/PGD are recognized as low-risk procedures for IVF/ICSI treatments.

Methods and Materials

To determine the effects of PGS/PGD on child development, research groups used a number of different metrics at different ages. Physical development was tracked using biometrics and anthropometrics. One group collected data on height, weight, and head circumference of two month old infants, comparing 995 PGD children with 1507 ICSI children at the same medical center. The study combined identification of malformations with medical history of neonatal biometrics and 2-month measurements.[1] In another study, 70 PGS/PGD children were compared with 70 ICSI (intra-cytoplasmic sperm injection) children and 70 NC (natural conception) children for biometrics at birth and at 2 years of age, controlling for race (all Caucasian), language (Dutch, French, or English), and number (all singletons). The NC and ICSI groups matched the PGS/PGD group with mother tongue, maternal education level, gender, and birth order. The data collected included the biometrics of the first study in addition to arm and waist circumference and examination of skin and eyes.[5] A third study comparing 50 PGS children, 72 IVF children, and 66 natural conception children examined each at 2 years of age for morphological abnormalities using photography in combination with medical records and anthropometry.[3]

Methods and Materials (continued)

At four years of age, blood pressure and anthropometrics including body fat, heart rate, pulse pressure, height, weight, waist, head circumference, and frequency of hospitalization and paramedical care were measured between 49 PGS children and 64 IVF/ICSI children.[7]

Neurological development was measured using various assessments. One study measured the neurological development of infants, comparing PGS children and IVF children, at 2 weeks, 3, 4, 10, and 18 months of age. Their general movements were examined and classified as normal-optimal, normal-suboptimal, mildly abnormal, and definitely abnormal. At 18 months, five areas of functionality were assessed in the context of play in the Hempel neurologic examination: "fine motor function, gross motor function, posture and muscle tone, reflexes, and visuomotor function."[2] The neurological development at 2 years of age was assessed by another study that calculated the mental development index based on visual/auditory processing, memory, language skills, hand-eye coordination, imitation, and problem-solving ability and the psychomotor development index based on fine and gross motor skills using the Bayley Scales of Infant Development. Parents were also asked to fill out the Child Behavior Check List (CBCL) to collect data on behavior issues. This study compared 54 PGS children to 77 IVF children.[4] Four-year-olds from the anthropometric study above were tested for neurological and behavioral issues using the Hempel test outlined above converted to a neurological optimality score (NOS), CBCL, and IQ tests.[6]

	PGD/PGS	ICSI	NC
Weight (kg)	13.0 ± 1.3	13.4 ± 1.7	13.5 ± 1.5
Height (cm)	90.6 ± 5.2	91.9 ± 3.9	91.4 ± 3.4
Head Circumference (cm)	48.8 ± 1.4	49.0 ± 1.4	48.8 ± 1.6
Left Arm Circumference (cm)	15.7 ± 1.2	15.9 ± 1.3	16.0 ± 1.4
Waist Circumference (cm)	48.7 ± 3.5	48.0 ± 3.1	49.0 ± 3.7

Table 1: Biometric data was collected in PGD/PGS, ICSI, and NC groups at two years.[5]

Results

Birthweight of PGD singletons (3262.8 ± 543.5 g) and multiples (2299.8 ± 581.1 g) were similar to the ICSI control group (3236.5 ± 583.2 g for singletons and 2248.1 ± 582.1 for multiples). Birth head circumference (cm) was also comparable: 34.30 ± 1.64 (PGD) compared with 34.21 ± 1.90 (ICSI) in singletons and 32.35 ± 2.38 (PGD) compared with 32.13 ± 2.36 (ICSI) for multiples. More ICSI multiples exhibited low birthweight (17.8%) compared with PGD multiples (16.2%).[1] See Table 1 for anthropometric data of two-year-olds, Chart 1 for morphological abnormalities of two-year-olds, and Table 2 for the anthropometric measurements of four-year-olds.[5,3,7]

The neurological study on infants indicated that 25% of PGS infants had at least one neurological problem by 18 months compared with 15% of IVF infants. 20% of the PGS group were diagnosed with MND compared with 13% of the control.[2] In the study of PGS children compared with IVF only children at two-years, the mental development index was 103 for both.[4] The neurological outcome results (PGS:control) from the Hempel test showed 87% : 95% normal, 7% : 4% simple MND, 4% : 1% complex MND, 2% : 0% cerebral palsy. The total median CBCL results were 43.0 : 46.0. In the study of four-year-olds, scores of PGS singletons: control singletons were 12.2 (fluency), 49.3 : 48.7 (NOS), 113.4 : 114.4 (total IQ), and 45.7 : 47.7 (CBCL).[6]

	PGS	IVF/ICSI
Weight (kg)	19.5	18.7
Height (cm)	110.7	109.3
Head Circumference (cm)	51.2	51.1
Waist Circumference (cm)	54.5	55.1
SBP/DBP (mm Hg)	102 ± 3/64 ± 3	100 ± 3/64 ± 3

Table 2: Blood pressure and anthropometrics of PGS and IVF/ICSI four-year-olds were measured.[7]

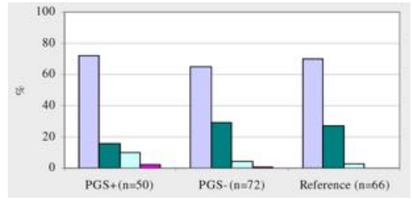

Chart 1: Percentages of major abnormalities per child for the PGS group (PGS+), IVF/ICSI group (PGS-), and natural conception group (reference). Purple = 0, dark green = 1, light green = 2, pink = ≥3.[3]

Conclusions

The results of the studies indicate that PGS/PGD children have similar anthropometric data to their IVF/ICSI and natural conception counterparts. Their neurological development is equally similar, especially as the children aged. There were very few differences: lower birth weights among IVF/ICSI multiples compared with PGD multiples, increased fine motor dysfunction and dysfunctional posture and muscle tone, and 7% greater chance of MND in PGS infants. None of these findings, however, have been replicated, and they are both results observed in small sample populations, where the difference is one or two children. As a result, it appears that PGS/PGD poses no significant risks in the physical and neurological development of PGS/PGD children up to four years of age.

Applications to Biotechnology

Given that PGD and PGS are frequently used to avoid passing on hereditary illnesses and maximize the chances of a successful implantation and pregnancy after IVF/ICSI, it is extremely important that embryo biopsy carries few risks. Tracking the neurological and physical health of PGD/PGS children is essential to determining these risks so that IVF/ICSI patients can make informed decisions and weigh the benefits of PGS/PGD with the risks.

Acknowledgements

I am grateful for this opportunity to learn about oncofertility in a multi-faceted and interactive way through this program. I greatly appreciate the support from my OSA sisters and Ms. Patricia Winter. I am especially thankful for Sheriden Smith's and Dr. Ericka Senegar-Mitchell's help and valuable discussions.

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The Correlation Between Breast Cancer Rates and Fertility Stimulants Clomiphene Citrate and Follicle Stimulating Hormone

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Blessed be God!

Objective

The objective of this poster is to determine if there is a relationship in the intake of fertility drugs and the risk of breast cancer it creates in pre-menopausal women. The research focuses on fertility drugs such as Clomiphene Citrate [CC] and Follicle-Stimulating Hormone [FSH].

Abstract

Fertility drugs, such as Clomiphene Citrate (CC) and Follicle Stimulating Hormone (FSH), stimulate the release of multiple eggs when administered clinically due to the increase of estrogen in a woman's body, which induces ovulation. This large amount of estrogen promotes the growth of hormone receptor positive breast cancer cells in pre-menopausal women. The objective of this research is to examine the relationship between fertility drugs such as CC and FSH, and the risk of breast cancer diagnoses for women under 50 years old. Within the conducted research, the National Cancer Institute recruited 1,422 breast cancer patients who underwent IVF previously, as well as their biological sisters (1,669 women) who were cancer free. Each patient reported the use of CC, FSH, or both and whether or not they were pregnant for 10 or more weeks after fertility treatments. Women who used ovulation-stimulating drugs and did not get pregnant showed a decreased possibility of breast cancer, compared to women who had never used fertility drugs. Participants who underwent assisted reproduction and successfully got pregnant—10 weeks gestation—showed an increased possibility of breast cancer, when compared to the non-pregnant women in the study; however, the possibility did not increase with participants who did not undergo fertility treatment. Conclusively, it can be seen that throughout the research women who received hormone treatment and successfully conceived, had a higher risk of becoming diagnosed with breast cancer. Even though fertility drugs do increase the risk for a pre-menopausal female of getting breast cancer, the risk is not higher than expected for the general public.

Characteristic	Control sisters (n = 1669)	Case sisters (n = 1422)
Race, No. (%)		
Non-Hispanic white	1487 (89.1)	1253 (88.1)
Black	75 (4.5)	71 (5.0)
Hispanic	63 (3.8)	57 (4.0)
Other	44 (2.6)	41 (2.9)
Relative birth order among participating sisters, No. (%)†		
First (oldest)	915 (54.8)	530 (37.3)
Second	610 (36.5)	792 (55.7)
Third or younger	144 (8.6)	100 (7.0)
Education, No. (%)		
High school or less	217 (13.0)	177 (12.4)
Some college but no degree	280 (16.8)	210 (14.8)
Associate or technical degree	253 (15.2)	200 (14.1)
Bachelor degree	525 (31.5)	480 (33.8)

Characteristic	Control sisters (n = 1669)	Case sisters (n = 1422)
Age at first birth among parous women§, No. (%)		
<25 y	501(30.0)	346 (24.3)
25–<30 y	465 (27.9)	423 (29.7)
30–<35 y	242 (14.5)	255 (17.9)
≥35 y	102 (6.1)	94 (6.6)
Total duration of breastfeeding in weeks, mean (SD)	46.3 (70.8)	44.5 (64.4)
Body mass index at ages 30–39 y‡, No. (%)		
<18.5 kg/m²	53 (3.2)	33 (2.3)
18.5–24.9 kg/m²	1157 (69.5)	1002 (70.8)
25.0–29.9 kg/m²	312 (18.7)	283 (20.0)
≥30.0 kg/m²	143 (8.6)	98 (6.9)
Use of hormonal birth control‡, No. (%)		
Nonuser	163 (9.8)	135 (9.5)
<10 y	889 (53.4)	700 (49.3)
≥10 y	590 (35.4)	568 (40.0)
Unknown duration	24 (1.4)	17 (1.2)
Cigarette smoking‡, No. (%)		
Never-smoker	1044 (62.6)	859 (60.4)
<1 pack-year	148 (8.9)	157 (11.0)
1–<10 pack-years	235 (14.1)	217 (15.3)
≥10 pack-years	240 (14.4)	189 (13.3)
Alcohol drinking in the 10 years preceding index age‡, No. (%)		
Nondrinker	143 (8.7)	151 (10.7)
<13 drinks/y	388 (23.7)	313 (22.1)
13–<48 drinks/y	306 (18.7)	260 (18.4)
48–<180 drinks/y	425 (25.9)	373 (26.4)
≥180 drinks/y	378 (23.0)	318 (22.5)
Menopausal status‡, No. (%)†		
Premenopausal	1396 (83.7)	1269 (89.2)
Postmenopausal	147 (8.8)	76 (5.3)
Premenopausal hysterectomy, with retained ovarian tissue	124 (7.4)	77 (5.4)

Figure 1-2. Characteristics of sisters in the Two Sister Study. Fei, C., Deroo, L., Sandler, D., & Weinberg, C. (2012). Fertility Drugs and Young-Onset Breast Cancer: Results From the Two Sister Study. JNCI Journal of the National Cancer Institute, 1021-1027.

Methods and Materials

In order to get the most accurate results, researchers gathered data from participants who had sisters with a diagnosis of breast cancer and who were younger than 50 years old. Both sisters did the same two-part computer assisted telephone interview (CATI) on their health conditions, lifestyle, reproductive factors, and more. In addition the breast cancer diagnosed sisters did CATI on treatments they had undergone, breast cancer diagnosis, and the characteristics of the tumor. Of the 3,283 participants only 3,091 qualified to participate in the study. Before the interview, sisters were shipped memory aids such as a life calendar to mark any type of surgery and births and a list of medications. Participants were also asked in the CATI if they had ever taken medication to help them get pregnant. If they did take medication they had to provide the name of the medication, when they took it, the number of menstrual cycles and if the treatment helped them get 10 or more weeks pregnant. After getting the list of medications, researchers considered two types of ovulation stimulation drugs, CC and FSH. In the first model, scientists gathered the women's data in groups, fertility-drug use was categorized as nonuser, FSH only, CC only and both FSH and CC. In the second model scientists divided the groups into two simpler ones, nonusers and users.

Variable	Control sisters (n = 1669) No. (%)	Case sisters (n = 1422) No. (%)	Adjusted OR (95% CI)†	Adjusted OR (95% CI)‡
Model I				
Nonusers of fertility drugs	1511 (90.5)	1292 (90.9)	1.00 (referent)	—
CC only	107 (6.4)	86 (6.0)	0.80 (0.58 to 1.09)	0.61 (0.41 to 0.90)
FSH only	12 (0.7)	17 (1.2)	1.40 (0.63 to 3.12)	1.03 (0.44 to 2.40)
CC and FSH	39 (2.3)	27 (1.9)	0.73 (0.43 to 1.24)	0.53 (0.29 to 0.96)
Stimulated pregnancy	69 (4.1)	72 (5.1)	—	1.82 (1.10 to 3.02)
Model II				
Nonusers of fertility drugs	1511 (90.5)	1292 (90.9)	1.00 (referent)	—
Use of fertility drug(s)	158 (9.5)	130 (9.1)	0.82 (0.63 to 1.08)	0.62 (0.43 to 0.89)
Stimulated pregnancy	69 (4.1)	72 (5.1)	—	1.82 (1.10 to 3.00)

Figure 3. The relationship of young-onset breast cancer with fertility drugs and stimulated pregnancies. Fei, C., Deroo, L., Sandler, D., & Weinberg, C. (2012). Fertility Drugs and Young-Onset Breast Cancer: Results From the Two Sister Study. JNCI Journal of the National Cancer Institute, 1021-1027.

Variable	Control sisters (n = 1067) No. (%)	Case sisters (n = 907) No. (%)	Adjusted OR (95% CI)†	Adjusted OR (95% CI)‡
Model I				
No fertility-drug use	968 (90.7)	816 (90.0)	1.00 (referent)	—
CC only	67 (6.3)	58 (6.4)	0.88 (0.60 to 1.31)	0.61 (0.37 to 1.01)
FSH only	7 (0.7)	11 (1.2)	1.71 (0.60 to 4.89)	1.20 (0.40 to 3.62)
CC and FSH	25 (2.3)	22 (2.4)	0.99 (0.54 to 1.81)	0.65 (0.32 to 1.32)
Stimulated pregnancy	39 (3.7)	51 (5.6)	—	2.23 (1.19, to 4.16)
Model II				
No fertility-drug use	968 (90.7)	816 (90.0)	1.00 (referent)	—
Use of fertility drug	99 (9.3)	91 (10.0)	0.96 (0.68 to 1.34)	0.66 (0.42 to 1.04)
Stimulated pregnancy	39 (3.7)	51 (5.6)	—	2.23 (1.20 to 4.14)

Figure 2. The relationship of invasive estrogen receptor-positive young-onset breast cancer with stimulated pregnancies. Fei, C., Deroo, L., Sandler, D., & Weinberg, C. (2012). Fertility Drugs and Young-Onset Breast Cancer: Results From the Two Sister Study. JNCI Journal of the National Cancer Institute, 1021-1027.

Results

A significant decrease of young-onset breast cancer appeared on women who only took CC or CC and FSH in comparison with women who did not take these two drugs. Those who unsuccessfully used only FSH weren't found to have any apparent risks, but the number of participants was low. When the first model of treatment of fertility drug use history with the following categories: nonusers, users of CC, users of FSH, and users of both CC & FSH was compared with the second model where fertility drugs were accumulated to create two categories such as exposed or not exposed it became clear that the effects of CC and FSH weren't detectable in the data. Participants with a fertility drug use background appeared to have a significant decrease in the risk of young on-set breast cancer in comparison to females who were nonusers. Women who reached a 10 week or more pregnancy under treatment appeared to have an increased risk of young on-set breast cancer in comparison with other users who had taken fertility drugs but did not have a successful pregnancy. As for participants who did not reach a 10 week or more pregnancy under treatment, they appeared to have decreased chances of getting breast cancer in comparison to nonusers. In conclusion, participants who did or didn't get pregnant under the treatment, did not have a notable increase risk of young on-set breast cancer in comparison with participants that were untreated.

Conclusions

Researchers discovered that there is no significant difference in the rates of developing breast cancer between the women who have used fertility drugs in the past versus the general public. Scientists concluded that fertility drugs do not increase the chances of breast cancer, in fact they lower the risk in females who do not sustain a 10 week or more pregnancy.

Relevant Applications to Biotechnology

Scientists have come a long way since the first IVF treatments. Scientists have figured out a new method of IVF treatment that always observes the growth of embryos. This new technology is called EmbryoScope; it helps fertility professionals control the development of fertilized eggs every twenty minutes. This technology has 54% success rate in comparison with normal IVF which only has 45%. With EmbryoScope, it is much easier for specialists to find important changes or problems.

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The Effectiveness of Targeted Therapy Using Olaparib to Resensitize Resistant Tumor Cells

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Objective

Olaparib is a novel, orally active poly(ADP-ribose) polymerase (PARP) inhibitor that induces synthetic lethality in homozygous *BRCA*-deficient cells[1] .With that background information, this poster will demonstrate about the effectiveness of these group therapies: Combined administration of 400mg Olaparib and platinum-based chemotherapy; monotherapy Olaparib and monotherapy Bevacizumab to see which group makes treatment resistant tumor cells more sensitive.

Abstract

Ovarian cancer is the fifth leading cause of cancer death in women; approximately 10% of all ovarian cancers are hereditary and of these, more than 90% are associated with BRCA1 or BRCA2 germline mutation.[4] Scientist discoverers that targeted therapies are a new type of cancer treatment that uses drugs or other substances to find and attack cancer cells while doing little damage to normal cells. There are 2 types and each works different: Bevacizumad and Olaparib. Bevacizumad works as angiogenesis inhibitor, which helps, block the formation of new blood vessels and as a combination use with standard chemotherapy for cancers.[3] Meanwhile, Olaparib is one new type therapy of PARP inhibitor, which is a repair of DNA single-stranded breaks (SSB) through via homologous- recombination repair pathway.[1] This is why the focus of this study is to see what is the most effective therapy that can revert tumor cell from resistance state to sensitivity state in ovarian cancer. In this study, we will compare three different groups of therapy: Combined administration of 400mg Olaparib and platinum based chemotherapy, monotherapy Bevacizumab; monotherapy Olaparib. The result of this study is the objective response rate (ORR) in group-combined Olaparib and platinum-based therapy is 12% for administration of monotherapy Bevacizumab 15mg is 21%.[3,5] However, the objective response rate for group monotherapy Olaparib 400mg is 33%.[2] In addition, the Progression-free survival (PFS) of Bevacizumab group itself is 4.7 months[3] ,combined administration of 400mg Olaparib and platinum-based therapy is 8.4 months and for Olaparaib group itself is 9 months. [2,5] The conclusion of study prove that monotherapy Olaparib 400 mg twice daily has antitumor activity heavily on ovarian cancer. Monotherapy Olaparib proves that it has most effective in revert treatment resistant tumor cells to a sensitive stage in recurrent ovarian cancer patients.

Materials and Methods

Patients were eligible if they were 18 years of age or older and had recurrent ovarian cancer. All studies start in Phase 2 of clinical trial.[2,3,5] However, the materials and methods for each study will be different. In Ledermann's study (combined Olaparib and platinum-based therapy) includes 68 patients were randomly administrated at a dose of 400mg twice daily.[5] These patients receive two or more platinum-based regimens and had a partial or complete response to their most recent platinum-based chemotherapy .The second study was conducted by Gynecologic Oncology Group, Burger's study (monotherapy Bevacizumab) includes 62 patients were randomized to with Bevacizumab 15mg repeated every three weeks.[3] Audeh's study (monotherapy Olaparib) includes 34 patients were administrated with Olaparib 400mg twice daily.[5] All patients were followed until progression of disease.PFS was assessed with the use of computed tomographic scans obtained every 12 weeks and was calculated on the basis of measurement of tumor size.[1,3,5]

Results

The result of Ledermann's study is the median PFS is 8.4 months for the group combined Olaparib and platinum-based chemotherapy.[5] Based on the RECIST guidelines, the response rate is 12% in the study group.[5] At that time, the data had to cut-off due to few deaths had occurred for a survival analysis to be performed. The toxicity of group combined Olaparib 400mg and platinum-based therapy was majority of adverse events grade 1 or 2 and did not require interruptions of the treatment.[5] In Burger's study, the activity of Bevacizumab was analyzed in 62 patients, the response rate is 21% and the median PFS is 4.7 months.[3] In addition, 40% patients were progression-free for at least 6 months.[3] So far in the study, there are only two patients discontinued the study because of adverse effect and these adverse effects are manageable and mild in majority of cases so the toxicity in Bevacizumab was graded 3.[3] Finally, Audeh's study showed that greater Olaparib activity is seen at high dose of 400mg, the response rate of this monotherapy Olaparib is 33% which higher than other therapy groups.[2] The median PFS is 9 months.[2] However, throughout the process of treatment with monotherapy Olaparib 400mg, there were the most frequently reported adverse events and most events were mild in intensity, toxicity was graded 3 or 4.[2]

Conclusion

In conclusion, monotherapy Olaparib 400mg can make treatment resistant tumor cells most effective. Throughout all 3 studies, monotherapy Olaparib showed that the therapy itself has an antitumor effect that slows growth of ovarian cancer tumors. However, giving patients a high dose of Olaparib 400mg itself can lead to adverse events which may cause relapse in patients because of an overdose in Olapraib. Furthermore, the ORR didn't differ between the group with combined Olaparib and platinum-based therapy and monotherapy Bevacizumab because each study had a different amount of patients that participated in the study, which could have affected results. In the end, results showed that monotherapy Olaparib 400mg is a promising therapy and can be used to revert resistant tumor cells to a sensitive, responsive state.

Relevant Applications to Biotechnology

The advancement of biotechnology have helped us invented many good medication that can cure cancer. Especially in ovarian cancer, targeted therapies, such as PARP inhibitor and anti-angiogenesis inhibitors, these are designed to target the cancer's specific genes, proteins, or the tissue environment that contributes to cancer growth and survival.

Figure 1. Protein structure of Bevacuzinab[3]

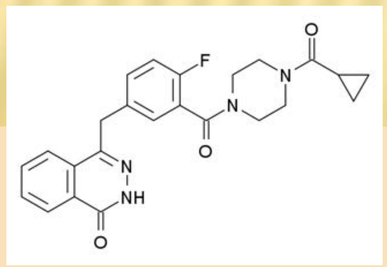

Figure 2. Chemical structure of Olaparib[5]

Therapy	Patients	Dosage	ORR	PFS	Toxicity
Combined Olaparib and Platinum-based therapy	68 BRCA 1/2 patients[5]	400mg Olaparib[5]	12%[5]	8.4 months[5]	1 or 2[5]
Monotherapy Olaparib	34 BRCA 1/2 patients[2]	400mg Olaparib[2]	33%[2]	9 months[2]	3 or 4[2]
Monotherapy Bevacizumab	62 BRCA 1/2 patients[3]	15mg Bevacizumab[3]	21%[3]	4.7 months[3]	3[3]

Acknowledgment

I would like to thank Oncofertility Saturday Academy for giving me this opportunity to learn about Oncofertility, IVF, Cancer and Bioethics in medical. I would like to specifically thank Dr. Ericka Senegar-Mitchell, Ms. Partrica Winter, Dr. R. Jeffrey Chang , Dr. Sender and big sister Sheriden for sharing their knowledge with me. Thank you to my OSA sisters and special sister Hailey for being my guidance and support me throughout the academy.

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The Effects of Mitochondrial DNA on Embryonic Implantation

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Academy of Our Lady of Peace

Objective

What are the effects of Mitochondrial DNA levels on embryonic implantations? The embryos from women ranging of twenty-six to forty-two years old were transferred and observed for any signs of implantation. The amount of mtDNA in the embryos were measured using real-time PCR and then compared to the implanting and non-implanting embryos to observe if there is any relationship. This poster will demonstrate the effects that high and low levels of mitochondrial DNA have on the success rate of embryonic implantations.

Abstract

As maternal age increases, the chance of embryonic chromosomal abnormalities as well as complications with implantation increases. Abnormal mitochondrial activity can damage oocytes by causing augmentation of reactive oxygen super-oxides in the cell, affecting implantation rates. The mitochondrial genome proofreading system is not as strong as the nuclear genome's, so chromosomally normal (euploid) blastocysts have lower levels of mtDNA than chromosomally abnormal (aneuploid) blastocysts. In one study done, 92.9% of euploid embryos developed into blastocysts while 42.1% of aneuploid embryos developed into blastocysts; a possible connection could be the 75% chance of aneuploidy in the oocytes of women over 40 years old. Through real-time Polymerase Chain Reaction (PCR), microarray comparative genomic hybridization (aCGH), and next generation sequencing (NGS), the mtDNA levels in the embryonic genome can be measured. The data will reflect the amount of mtDNA in the embryos and will then be observed for implantation potential. The patients were in IVF clinics in the US and UK; gender and age ranges were kept constant for the duration of the study. The ranges for reproductively younger women were age 26-37 and reproductively older women were age 38-42. Successful implantations were shown where maternal age was low and where mtDNA levels were 0.003 or lower. Where mtDNA levels were higher than 0.003, and came from maternally older women, the embryos had a lower tendency to implant. The data suggests that high levels of mtDNA increase embryonic implantation failures which points to a connection among maternal age, mtDNA, and embryonic implantations for both euploid and aneuploid blastocysts. Knowing the health of blastocysts, pre-implantation, is important so that pregnancies can occur.

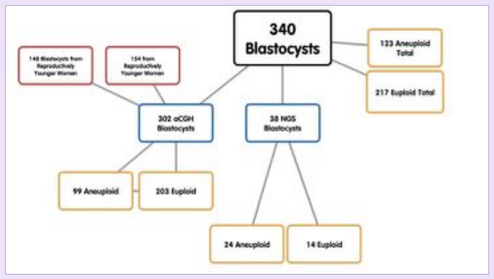

Figure 1: Further clarification of the methods and results of the experimentation process presented in the poster. Fragouli, E., Spath, K., Alfarawati, S., Kaper, F., Craig, A., Michel, C.-E., … Wells, D. (2015). Altered Levels of Mitochondrial DNA Are Associated with Female Age, Aneuploidy, and Provide an Independent Measure of Embryonic Implantation Potential. *PLoS Genetics*, *11*(6), e1005241.

Methods and Materials

In IVF clinics in the US and UK, 340 blastocysts from 161 couples (average maternal age 38 years) were cytogenetically tested for aneuploidies. One of the cytogenetic techniques used was microarray comparative genomic hybridization (aCGH) in which 302 blastocysts were analyzed. Out of the 302 blastocysts from aCGH, 148 blastocysts were from reproductively younger women with a range 26-37 years and an average age of 34.8 years. The remaining 154 blastocysts came from reproductively older women with ages ranging from 38-42 years and an average age of 39.8 years. These 302 blastocysts were studied through real-time Polymerase Chain Reaction (real-time PCR) to determine their chromosomal status and amount of mtDNA; additionally, 38 blastocysts were studied through NGS to search for the same (see figure 1 for reference).

After the relationships between mtDNA, aneuploidy, and maternal age were analyzed, 89 blastocysts from 85 patients (average maternal age 38.3 years) were transferred into uteruses. 81 blastocysts went through single embryo transfers (SETs), and 8 through double embryo transfers (DETs). The mtDNA copy number quantification was found via real-time PCR assessment of the implanting and non-implanting 89 blastocysts. In this study, the factors were maternal age (younger vs. older), embryonic chromosomal status (euploid vs. aneuploid), and embryonic implantation (pregnancy vs. failure to implant).

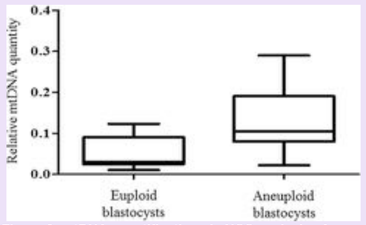

Figure 2: mtDNA quantification via NGS analysis of chromosomally normal and abnormal blastocysts. Fragouli, E., Spath, K., Alfarawati, S., Kaper, F., Craig, A., Michel, C.-E., … Wells, D. (2015). Altered Levels of Mitochondrial DNA Are Associated with Female Age, Aneuploidy, and Provide an Independent Measure of Embryonic Implantation Potential. *PLoS Genetics*, *11*(6), e1005241.

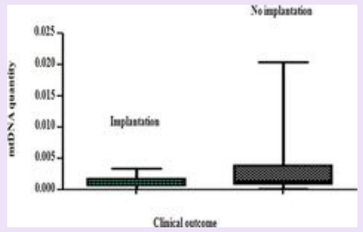

Figure 3: The mtDNA content of chromosomally normal blastocysts in relation to clinical outcome. Fragouli, E., Spath, K., Alfarawati, S., Kaper, F., Craig, A., Michel, C.-E., … Wells, D. (2015). Altered Levels of Mitochondrial DNA Are Associated with Female Age, Aneuploidy, and Provide an Independent Measure of Embryonic Implantation Potential. *PLoS Genetics*, *11*(6), e1005241.

Results

From the 302/340 blastocysts that were analyzed through aCGH, 99 were aneuploid and 203 were euploid. Table 1 depicts the relationship between mtDNA quantity, maternal age, and chromosomal status. Out of the 38/340 blastocysts that were analyzed via NGS, 24 were aneuploid and 14 euploid. Through real-time PCR was used on the 38/340 blastocysts to compare the amount of mtDNA in the aneuploids and 14 euploids. Figure 2 illustrates the low levels of mtDNA in euploid blastocysts and high levels of mtDNA in aneuploid blastocysts. From the 89 blastocysts transferred through SETs and DETs, 42 resulted in ongoing pregnancies while 47 failed to implant. Real-time PCR analysis indicated that the implanting blastocysts had noticeably lower amount of mtDNA than the non-implanting (figure 3). 100% of the 42 blastocysts that implanted had mtDNA quantities lower than 0.003, while 30% of the 47 that failed to implant had mtDNA quantities higher than 0.003. What was found was that the baseline of mtDNA for embryos that implant is 0.003, or lower. The embryos that had an mtDNA quantity of 0.003 or lower, tended to be from the reproductively younger women. These results suggest that maternal age is connected to embryonic mtDNA quantity and that an older maternal age increases the chances of failure to implant.

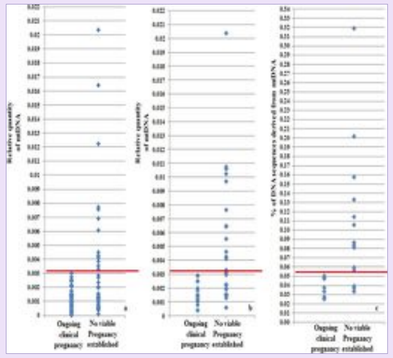

Figure 4: Blastocyst mtDNA quantity threshold in relation to clinical outcome. Fragouli, E., Spath, K., Alfarawati, S., Kaper, F., Craig, A., Michel, C.-E., … Wells, D. (2015). Altered Levels of Mitochondrial DNA Are Associated with Female Age, Aneuploidy, and Provide an Independent Measure of Embryonic Implantation Potential. *PLoS Genetics*, *11*(6), e1005241

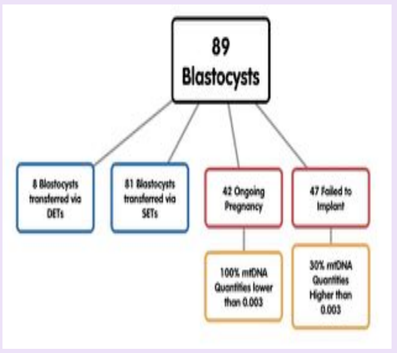

Figure 5: Further clarification of the results of the 89 Blastocysts. Fragouli, E., Spath, K., Alfarawati, S., Kaper, F., Craig, A., Michel, C.-E., … Wells, D. (2015). Altered Levels of Mitochondrial DNA Are Associated with Female Age, Aneuploidy, and Provide an Independent Measure of Embryonic Implantation Potential. *PLoS Genetics*, *11*(6), e1005241.

The average relative quantities of mtDNA observed in association to female age and blastocyst chromosome status.

Female age (years)	Number of embryos assessed	mtDNA quantity range/ euploid blastocysts	Average mtDNA quantity/ euploid blastocysts	mtDNA quantity range/ aneuploid blastocysts	Average mtDNA quantity/ aneuploid blastocysts
26	1 euploid	0.002273	N/A	N/A	N/A
29	1 euploid	0.0029	N/A	N/A	N/A
30	9 euploid 5 aneuploid	0.000065–0.0017	0.000482	0.000095–0.0069	0.00161
31	9 euploid 3 aneuploid	0.000093–0.00231	0.000998	0.00166–0.0044	0.00248
32	7 euploid 3 aneuploid	0.000318–0.00462	0.00137	0.000324–0.00275	0.00145
33	9 euploid 3 aneuploid	0.00013–0.002532	0.00115	0.00048–0.00218	0.00159
34	8 euploid 1 aneuploid	0.001–0.0034	0.0043	0.00244	0.00244
35	10 euploid 2 aneuploid	0.00076–0.00765	0.00265	0.00289–0.0034	0.00315
36	26 euploid 9 aneuploid	0.00032–0.00968	0.002	0.00026–0.0082	0.00212
37	29 euploid 12 aneuploid	0.000038–0.015	0.0023	0.000097–0.00416	0.00142
38	28 euploid 6 aneuploid	0.000294–0.01	0.00283	0.00037–0.007	0.00285
39	18 euploid 18 aneuploid	0.00063–0.0164	0.0025	0.00053–0.00722	0.00393
40	19 euploid 7 aneuploid	0.0004–0.0064	0.002	0.00036–0.0038	0.002
41	16 euploid 15 aneuploid	0.00025–0.006	0.0026	0.00027–0.05	0.0079
42	13 euploid 15 aneuploid	0.00013–0.012	0.0048	0.0001–0.016	0.0053

Table 1: The average relative quantities of mtDNA observed in association to female age and blastocyst chromosome status. Fragouli, E., Spath, K., Alfarawati, S., Kaper, F., Craig, A., Michel, C.-E., … Wells, D. (2015). Altered Levels of Mitochondrial DNA Are Associated with Female Age, Aneuploidy, and Provide an Independent Measure of Embryonic Implantation Potential. *PLoS Genetics*, *11*(6), e1005241.

Conclusion

Maternal age is an influencing factor on chromosomal status. With increasing maternal age, particularly over 37 years old, the cases of embryonic aneuploidies in this study were more prevalent. Moreover as maternal age increased, the amount of mtDNA in blastocysts increased as well, and the instances of embryonic implantations decreased. In women 36 years and younger, the cases for aneuploidy were lower and success rate of embryonic implantation were higher. This is important to note for women experiencing fertility issues, as their age could play a major role.

Relevant Applications to Biotechnology

Through cytogenetic testing methods such as real-time PCR, aCGH, and NGS aneuploidies can be found in blastocysts before transferred into the uterus. Using real-time PCR is important to measure the amounts of mtDNA in blastocysts so that it can be known if implantation of the blastocysts, and ultimately a successful pregnancy, is possible. For the future of biotechnology, perhaps medicine can be developed to lower/steady the mtDNA levels in older women trying to have children. Analysis and future medicine can increase the chances of having an ongoing pregnancy because more healthy blastocysts would be able to be transferred in utero and will implant.

Acknowledgements

I would like to thank Dr. Ericka Senegar-Mitchell for teaching, motivating, and inspiring us in how to be better scientists. I would also like to thank Ms. Winter for helping to run the OSA program. Additional thanks to Dr. Chang for instructing us on the dynamics of Oncofertility and answering all of our questions. My OSA sisters, including Sheriden Smith, have given me guidance and support throughout this program and it is much appreciated. Lastly, to my friends and family that have unknowingly helped me by supporting me in all that I do, especially my sister who watches over me in all that I do.

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CRISPR-Cas9 Mediated Treatment of HPV16-Related Cervical Malignancy

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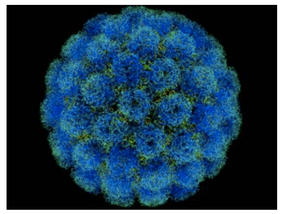
Objectives

With the advancement of technology, it is still often questioned why there is not yet a "cure" to cancer. The objective of this experiment, in which CRISPR-Cas9 systems are used in order to treat cancer cells infected with HPV16, is to serve as a stepping-stone toward solving this age-old question.

Figure 1. HPV16 Virus



Each year, about 12,000 women are diagnosed with cervical cancer. Human Papillomavirus (HPV) is the leading cause, and anyone who is sexually active is at risk. Although preventable with an HPV vaccine and easily detectable with Pap smears, diagnoses still occur.
Rotaru, P. (n.d.). Everything About the Virus HPV (Human papilloma virus). Retrieved August 6, 2015.

Abstract

High-risk Human Papillomavirus (HPV), notably HPV16, is responsible for virtually all cases of cervical cancer. When a host cell is infected, the E6 oncoprotein proliferates and disrupts the p53 tumor-suppressor protein, leading to unregulated cell growth. With the intent to eliminate cervical cancer in women without causing harm to healthy cells, CRISPR-Cas systems can be used to mutate the *E6* oncogene introduced by HPV16, essentially curing cervical malignancy. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) is a new genome-editing tool in which a targeted segment of DNA can be excised with great specificity. Four different cell lines were transfected and studied in vitro, two of which were HPV16-positive cervical cancer cell lines (CaSki and SiHa) and two were HPV16-negative (C33A and HEK293). Each cell line was transfected with a plasmid encoding the Cas9 enzyme and a gRNA sequence. Three different segments of guidance RNA (gRNA) were used in order for the Cas9 (CRISPR associated) enzyme to cut the *E6* gene in the HPV genome at a specific site. When the double stranded breaks (DSB) in the DNA are repaired by the error-prone nonhomologous end joining (NHEJ) repair system, a frameshift mutation occurs, deactivating the gene. An annexin V-FITC (fluorescein isothiocyanate) apoptosis-detection kit demonstrated that apoptosis increased from an average of 5% to 40% in the HPV-positive CaSki and SiHa cells while the HPV-negative C33A and HEK293 cells were unaffected. Western blot analysis indicated a reduction in E6 activity by nearly 50% while p53 activity nearly tripled as compared to the control. The CRISPR-Cas system has great potential as it allows for a less expensive and less harmful treatment for HPV-related cervical cancer. Since the CRISPR-mediated mutations are permanent, they can be passed down from generation to generation. A cure for cervical cancer is only the beginning, as CRISPR has the potential to treat many other genetically based diseases.

Figure 2. CRISPR-genome editing.

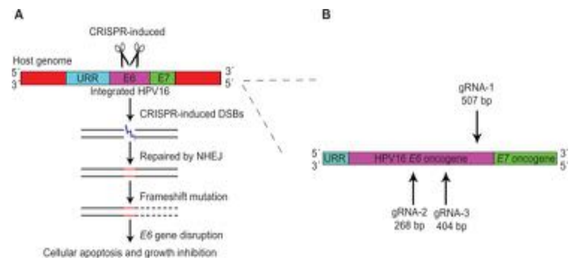
CRISPR is a newgenome-editing tool discovered by Jennifer Doudna, was found to have great potential for targeted treatment. Unlike other chemotherapeutics and genome editing tools, CRISPR operates with great specificity and minimal cytotoxicity. Bridger, H. (2013, January 9). Genome engineering gets CRISPR. Retrieved August 6, 2015.

Methods and Materials

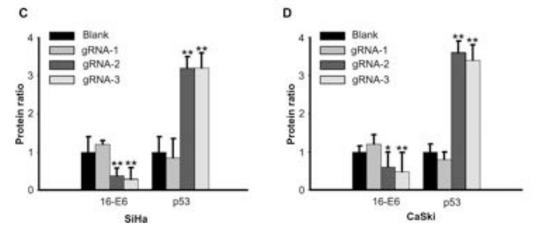
Four types of cells were used in order to test the efficacy and specificity of the CRISPR-Cas9 system: HPV16-positive SiHa and CaSki cells, and HPV16-negative C33A and human embryonic kidney (HEK)-293 cells. The cells were maintained in 5% humidity in Modified Eagle's Medium at 37°C. For the SiHa, C33A, and HEK293 cells, the DNA transfection reagent X-tremeGene HP was used while JetPEI was used for the CaSki cells. Transfected into the cells was a plasmid containing the *Cas9* gene and a short gRNA sequence intended to guide the Cas9 enzyme to the targeted area on the E6 gene. Three gRNA sequences were created (referred to as gRNA-1, gRNA-2, and gRNA-3); each plasmid contained only one of the three sequences.

The CRISPR-Cas9 system is a very powerful genome-editing tool. It consists of the Cas9 endonuclease, guidance RNA sequence, and the CRISPR DNA sequences themselves. The Cas9 endonuclease is an enzyme that acts as a pair of "molecular scissors" that can excise a targeted DNA segment if it matches the gRNA sequence. Using the three gRNA strands, the Cas9 enzyme induces double-stranded breaks (DSB) in the HPV16 E6 gene. These breaks are then "fixed" by the error-prone NHEJ system, causing a frameshift mutation and therefore disrupting the targeted gene. When the E6 gene is disrupted, the p53 tumor-suppressor gene regains function and signals the cancerous cell for apoptosis and growth inhibition. As well, four assays were conducted in order to assess the success of the CRISPR/Cas9 systems.

1. In order to verify that the CRISPR-Cas9 system properly cleaved the E6 gene, a T7 endonuclease I (T7E1) assay was performed. After the initial transfection of the CRISPR-containing plasmid, the targeted E6 gene was amplified via PCR (polymerase chain reaction). Four DNA samples, three containing a unique gRNA sequence and one without a gRNA sequence.
2. Forty-eight hours posttransfection, an annexin V-FITC apoptosis-detection kit was used. Both HPV16-positive and HPV16-negative cells were analyzed with FACSCalibur™ in order to calculate the rate of CRISPR-induced apoptosis.
3. Using the Cell Counting Kit-8 (CCK-8), cell proliferation assays were performed 24 hours posttransfection. In each well of the 96-well plate, 10 μL of CCK-8 solution was added. After 3 hours of incubation, a microplate reader measured absorbance values at 490 nm.
4. Western blot analysis was also used in order to determine the E6 and p53 protein levels in the CaSki and SiHa cells. The antibodies used were: anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH served as an internal control), anti-p53, and anti-HPV16 E6.



Figures A and B: Representation of HPV16 *E6* editing using CRISPR.
Figure A provides a basic overview of how the CRISPR-Cas9 system work (explained above). Figure B demonstrates the approximate sites in which the *E6* oncogene was cut by CRISPR[1].



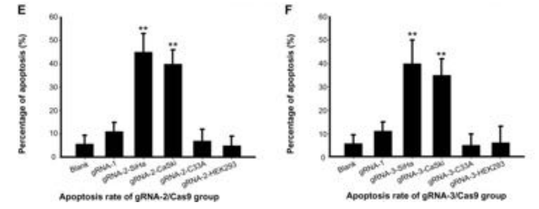
Figures C and D: Western Blot Analysis in SiHA and CaSki cells.
E6:GAPDH and p53:GAPDH ratios in the both the SiHa and CaSki cells were calculated. E6 levels were decreased by about 50% while p53 levels tripled in comparison to the "Blank" cells or the gRNA-1 group[1].

Results and Interpretations

The results of the corresponding four assays are as follows.

1. After transfecting the CaSki and SiHa cells with the three different gRNA/Cas9 plasmids, the T7E1 assay demonstrated that the gRNA-2 and gRNA-3 groups cleaved the E6 gene into products with approximately 200 - 300 bp. In the CaSki cells, cleavage occurred with a frequency of up to 19% (gRNA-3). In the SiHa cells, cleavage occurred with a frequency of up to 22% (gRNA-2). However, the products of the gRNA-1 group remained uncleaved. Therefore, the gRNA-2 and gRNA-3 groups were used for further testing, with gRNA-1 as an additional control.
2. Apoptotic rates were calculated via flow cytometry analysis. When treated with the gRNA-2/Cas9 and gRNA-3/Cas9 plasmids, the HPV16-positive SiHa and CaSki cell lines demonstrated approximately 35% greater apoptotic rates than the controls. Meanwhile, the apoptotic rates of the HPV16-negative C33A and HEK293 cell lines demonstrated no significant difference when compared to the untreated "Blank" cells.
3. The CCK-8 assay, used to determine the effects of CRISPR on cell proliferation or growth inhibition, demonstrated that the number of viable HPV16-positive (CaSki and SiHa) cells significantly decreased within the 96-hour time frame. On the other hand, there was no significant change between the viability of the transfected and untransfected HPV16-negative (C33A and HEK293) cells.
4. Western blot analysis demonstrated that the E6 protein levels decreased by approximately 50% in the transfected CaSki and SiHa cells as compared to the "blank" cells. The p53 protein levels in the transfected cells increased by approximately 300% as compared to the "blank" cells. The mutation of E6 allowed for the p53 protein to regain function and reverse the malignancy of the HPV16-positive cells.

In summary, the CRISPR-Cas9 system proved able to effectively cut the E6 gene and induce frameshift mutations, allowing the infected cells to regain the function of p53, inhibit cell proliferation, and trigger apoptosis—all the while leaving the HPV16-negative cells unharmed.



Figures E and F: CRISPR-Cas9 Induced Apoptosis.
Using gRNA-2 and gRNA-3, apoptotic rates in the SiHa cells on average increased by over 35% and over 30% in the CaSki cells as compared to the control[1].

Conclusion

Thus far the minimal cytotoxicity, great specificity, and proven effectivity of CRISPR, promise a bright future for this powerful technology. Cervical cancer is only the beginning, as all cancers are based in mutated DNA. CRISPR can be used to mutate or replace genes *in vivo* or *in vitro*, meaning that a person's genes can be edited at any time and permanently. As it is cheap, easy to use, precise, and successful, CRISPR is the future of targeted medicine. CRISPR has yet to be tested on human cells *in vivo*. Therefore, there are concerns regarding the off-target effects. Off-target effects, like the mutation of the incorrect genes, must be either reduced to nearly zero or controlled entirely before it can be even considered for FDA approval. Further studies should use CRISPR *in vivo* in model organisms in order to determine the side effects of genome editing. Although more trials must be conducted and modifications made, CRISPR offers a hopeful solution for an array of genetic diseases and abnormalities.

Applications to Biotechnology

CRISPR is a great breakthrough for the medical community. As the CRISPR-Cas9 systems were only discovered in 2012, there are still many tests to be done in order to perfect the technology. As of now, CRISPR can be used for genome editing and cancer modeling. It has the potential to transform future cancer research, therapy, and prevention. CRISPR is currently being used to model cancer in mice both *in vivo* and *in vitro*, which has many implications for the future study of cancer and the testing of chemotherapeutics. CRISPR is also in the preliminary stages of being used for systematic genetic screening in mammalian cells crop modification, making them pest-resistant or more health-beneficial.

Acknowledgments

I would like to thank my parents for their unconditional love and support. They have taught me so much and I would not be where I am without them. I would also like to thank Dr. Ericka, Mrs. Winter, Sheriden and everyone else involved in the Oncofertility Saturday Academy for giving me this amazing opportunity and experience. Lastly, I would like to thank my OSA sisters for creating lifelong memories.

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The Role of Fertility Sparing Surgery in Early Stage Epithelial Ovarian Cancer

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Introduction

Ovarian cancer is a disease that impacts not only the many women that it threatens, but also their fertility. To combat the disease, new methods of treatment, such as fertility sparing surgery (FSS) have emerged. FSS aims to preserve patient fertility. To accomplish this, FSS requires only a unilateral oophorectomy, where one ovary is removed while the other ovary and the uterus remain, as opposed to a more traditional bilateral oophorectomy and hysterectomy where both ovaries and the uterus are removed. Although this is relatively new method of treatment, significant effects have already been observed. This poster will examine the impact of standard treatments on patient fertility in relation to treatment effectiveness in managing early stage epithelial ovarian cancer.

Abstract

As a top concern for young cancer patients, fertility preservation must be addressed. Ovarian cancer is among those cancers whose treatment threatens fertility. 21,290 women are diagnosed with ovarian cancer annually.[5] However, through fertility sparing surgery (FSS), patients have greater ability to maintain fertility while treating cancer. The purpose of this study is to demonstrate the potential of FSS to help preserve patient fertility while effectively treating early stage epithelial ovarian cancer. To evaluate the success of various cancer treatments in preserving fertility, while curing disease, researchers compared women, less than 50 years of age, who had early stage, low-grade, non-clear cell epithelial ovarian cancer and received treatment. Patients were assigned to one of two study groups: women who underwent bilateral oophorectomy or women who received FSS. Researchers found rates of recurrence between 33% in a 109 patient study and 100% in a 3 patient study. [3,4] Researchers also found 90% to 100% of patients resumed normal menses and pregnancy success rates between 38% and 71%. [4,1,5] Beyond this, ultimately, researchers found that the survival rates of patients who underwent fertility sparing surgery were not significantly impacted. One study reported that the five year survival rate for patients who had undergone fertility sparing treatment was 84%, and the five year survival rate for patients treated with standard, more radical surgery was 82%.[4] As a result, it is clear that fertility sparing surgery, in the place of other procedures with the potential to severely limit fertility, should be considered for patients with early stage epithelial ovarian cancer. By offering FSS to patients, they will have potential to better preserve fertility and have a higher quality of life. Future studies with increased numbers are necessary for future evaluation.

Methods and Materials

All patients underwent staging surgery and were classified using the FIGO system. Histology, stage, and grade were evaluated. Patients included in the study had epithelial, stage 1A or 1C, grade 1 or 2, non-clear cell ovarian cancer, and were less than 50 years of age. Patients were assigned to one of two groups: those receiving bilateral oophorectomy or those receiving FSS. Patients considered to be at high risk of recurrence, with a grade higher than 1 or stage higher than 1A received platinum-based chemotherapy. Five year survival and recurrence rates were calculated, as well as rates of amenorrhea and pregnancy.

Results and Interpretations

In studying FSS, reproductive function, recurrence, effects of additional chemotherapy, and five-year survival rate were monitored. Overall, FSS allowed continued reproductive function, without increasing rates of recurrence or decreasing five year survival rates, in cases with or without additional chemotherapy.

Effects of Chemotherapy:
Chemotherapy was found not to change the reproductive outcomes in FSS patients. One study found chemotherapy didn't cause permanent amenorrhea and didn't impact conception or age of menopause. [2] In another with 15 patients who resumed menstrual function, seven of the fifteen had received adjuvant chemotherapy. [4]

Reproductive Function:
After FSS, most patients resumed menstruation. One study found that 15 of 17 patients who had received FSS subsequently experienced regular menstruation. [4] A second study reported 94% of patients continued to have menstrual function. [5] In one study, all FSS patients resumed regular menses. [1] Another study reported that 72% of the women treated with FSS attempted to become pregnant, but only 38% of them actually succeeded with full-term delivery. [1] However, two other studies reported much higher success rates of 68% and 71%. [2,5]

Group	Oophorectomy	Ovarian Preservation
5-Y Survival, % (No.)	91 (754)	94 (432)
95% CI, %	88-93	91-96
Stage IA		
5-Y Survival, % (No.)	94 (551)	95 (370)
95% CI, %	91-96	91-97
Stage IC		
5-Y Survival, % (No.)	83 (203)	88 (62)
95% CI, %	76-88	71-96

Figure 1: 5 year survival rate and confidence interval (CI) by stage and procedure[5]

Five-Year Survival Rate:
Overall, there was no significant difference in five-year survival rates for women who had FSS and women who didn't have FSS. One study found a five-year survival rate of 84% in the fertility-sparing group and an 82% five-year survival rate in the standard surgery group. [4] Figure 1 also demonstrates this; the overall five-year survival rate of women who underwent a bilateral oophorectomy was 91%, while women who had ovarian preservation had a similar survival rate of 94%.[5] However, it is possible that there is an initial, more rapid drop in survival in women who underwent FSS while women who had more radical surgery showed a less extreme, more gradual drop in survival. This is depicted in Figure 2. As stated though, by the five year mark, survival rates are the same. [1]

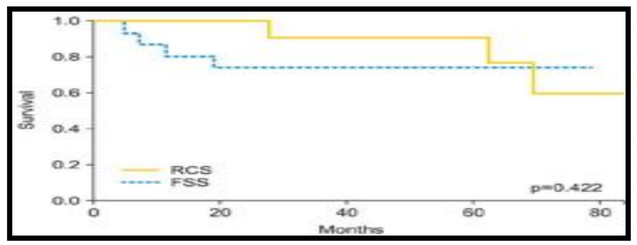

Figure 2: Survival rate over time in patients who under went FSS and RCS (bilateral oophorectomy)[1]

Recurrence:
Rates of recurrence for women who underwent standard surgery and women who underwent conservative surgery were similar. Two studies found recurrence rates of 22% and 16% in the FSS group and rates of 16% and 17% in the bilateral oophorectomy group, statistically insignificant differences. [1,4] However, in women who had FSS, recurrences tended to occur in the residual ovary. One study reported that of a group of 109 patients with relapses, 33% had relapse in the remaining ovary. [3] In other much smaller studies, two out of four patients with recurrence experienced recurrence in the residual ovary, while another found all three of patients with recurrence in the remaining ovary. [1,4] Furthermore, researchers found that FSS patients with recurrence experienced such recurrence significantly earlier, at an average of 10.3 months, as compared to the relapsed standard surgery group which experienced recurrence at an average of 53.3 months. [1] However, authors of two studies hypothesized that most recurrences could be cured with secondary recovery surgery and chemotherapy.

Conclusion

This poster reports on treatment of early stage, low grade epithelial ovarian cancer. Bilateral oophorectomy results in sterility and early menopause. In FSS, one ovary is removed and the residual ovary provides for continued ovarian function. FSS doesn't harm reproductive function. Although recurrent cancers tend to occur in the remaining ovary, FSS patients' overall rates of recurrence don't differ from that of patients who had standard bilateral oophorectomy. If there is recurrence in the residual ovary, researchers hypothesize that salvage surgery may be successful. Recurrence may even be prevented by having a contralateral oophorectomy after completion of childbearing. Overall, it is clear that FSS does not lead to increased recurrence or diminished five-year survival, while retaining acceptable hormone status and rate of reproduction. Consequently, FSS treatment should be offered to women with early stage, low grade, non-clear cell epithelial ovarian cancer.

Relevant Applications to Biotechnology

1. Current laparoscopic techniques are sufficiently advanced to be utilized for FSS. Laparoscopic surgery is less invasive than open surgery and results in significantly decreased postoperative morbidity.

2. Current advanced assisted reproductive techniques may used if required to achieve pregnancy. Should surviving patients require these techniques, such as IVF or ICSI, FSS will have left an egg-containing ovary from which autologous eggs can be harvested.

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I owe thanks to Dr. Ericka and Mrs. Winter for their insight and constant dedication to the academy. I'm grateful to Sheriden Smith and my OSA sisters for their help and friendship. I also am grateful to our steady mentor, Dr. Chang, for being more inspirational than he will ever know and each of the other doctors for their enthusiasm and willingness to share their knowledge. Above all, I also owe thanks to my mom, for her warm encouragement and support in all my endeavors, and my dad, Dr. Joel Sokoloff, for always inspiring me to pursue medicine, whether at age 4 by bringing me syringes to play with in the bathtub, or at age 17 with his willingness to share his expertise.

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Ovarian Stimulation for Premenopausal Breast Cancer Patients: Aromatase Inhibitors vs. Selective Estrogen Receptor Modulators

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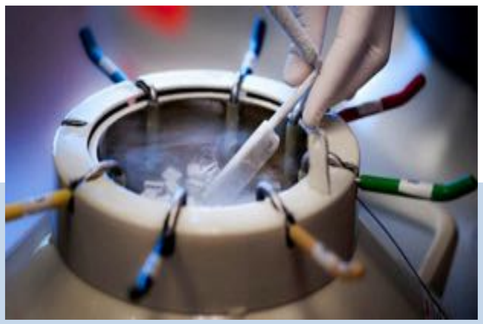
Canyon Crest Academy

Background

Aromatase inhibitors(AI) and selective estrogen receptor modulators (SERM) are both classes of drugs which contribute to keeping estrogen levels low, however they use different mechanisms to go about it. Aromatase inhibitors competitively bind to the active site of the enzyme aromatase, a cytochrome P450 enzyme which catalyzes the reaction that converts androgens to estrogens. This prevents the enzyme from being able to catalyze the reaction and create estrogen, thus keeping estrogen levels low. SERMs are high-affinity ligands, which, depending on the tissue with which they interact, can act as either estrogen antagonists or estrogen agonists. For breast tissue, they act as estrogen antagonists. Specifically to the focus of this project, aromatase inhibitor, letrozole, and selective estrogen receptor modulator, tamoxifen, will be discussed. Letrozole is a third generation AI which was first created in the early 1990's, and tamoxifen is a first generation SERM which exhibits antiestrogenic effects on breast tissue by binding to estrogen receptors and blocking RNA transcription. By looking closely at these two drugs, a conclusion may be drawn for the efficacy of AI vs SERM.

Abstract

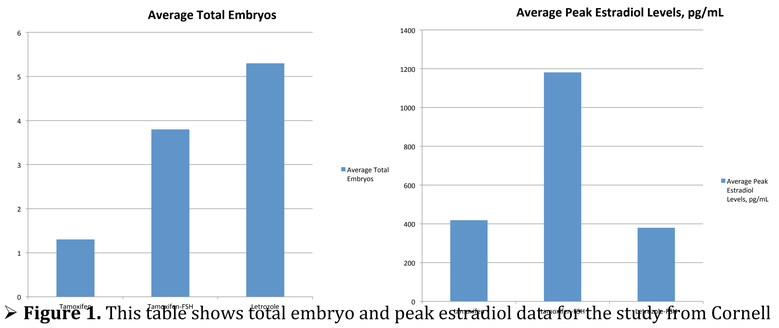
About 15% of breast cancer patients are within ages 15-44, and knowing that after treatment they may no longer be fertile, many breast cancer patients have chosen to undergo embryo cryopreservation. However, the rise in estrogen levels that accompanies the use of common ovarian stimulants is dangerous for breast cancer patients, as estrogen plays a significant role in tumorigenesis of breast tissue. This study will evaluate two solutions to this problem: aromatase inhibitors and selective estrogen receptor modulators. They each work differently, and a comparison will be conducted to determine their relative efficacy and their positive and negative aspects. Representing each of these groups, one study with 60 patients compared letrozole(AI) and tamoxifen(SERM). Patients did not exceed stage three breast cancer, and were between the ages of 24 and 43. Experimental groups took 60mg/d tamoxifen, 60mg/d tamoxifen with FSH, or 5mg/d letrozole with FSH. After egg retrieval and IVF, the embryos were cryopreserved. In total, the tamoxifen group resulted with 13 cycles in 12 patients, the tamoxifen-FSH group had 9 cycles in 7 patients, and the letrozole-FSH group had 11 cycles in 11 patients. Tamoxifen-FSH and Letrozole-FSH produced the highest embryo yield, but Tamoxifen-FSH resulted in very high E_2 levels. Therefore, Letrozole-FSH was found to be the best combination for successful egg retrieval while not threatening estrogen levels. Furthermore, cancer recurrence rates were about the same in the IVF group as the controls, with three in twenty-nine and three in thirty-one, respectively. From this study, letrozole-FSH, the aromatase inhibitor, was concluded to be the most effective. However, both aromatase inhibitors and selective estrogen receptor modulators are successful in keeping estrogen levels low, and because there are other factors they contribute to for successful ovarian stimulation for breast cancer patients, both should be considered in making a personalized decision for every patient.



➢ **Figure 2. An image of embryo cryopreservation.** Istanbul / Turkey | In Vitro Fertilization, low cost - affordable IVF Treatment. (n.d.). Retrieved August 8, 2015.

Methods and Materials

For this topic, a study from the Center for Reproductive Medicine and Infertility at Weill Medical College of Cornell University conducted a controlled comparison between tamoxifen and letrozole for ovarian stimulation in breast cancer patients, representing the SERM and aromatase inhibitors, respectively. Human, female subjects were used, all were between the ages of 24-43 and none exceeded stage III breast cancer. This study included three experimental groups: a group of 12 women who received tamoxifen, a group of 7 who received tamoxifen with FSH, and a group of 11 who received letrozole with FSH. The control group consisted of 31 eligible women who elected natural cycle IVF(NCIVF) and went without ovarian stimulation. Reasons for this decision include cost and a lack of time to conduct this process before cancer treatment was needed. For those in the tamoxifen group, baseline FSH, LH, and E_2 levels were recorded, and on the second to third day of menstruation, 60 mg/d of tamoxifen was taken until FSH levels were below the baseline. For those in the tamoxifen-FSH group, along with the 60 mg/d of tamoxifen was a 150 U injection of recombinant FSH. This was continued daily until hCG was administered. For those in the letrozole-FSH group, an oral 5 mg/d of letrozole was initiated on the second to third day of menstruation, and after two days 150U/d of FSH was added. Like the tamoxifen-FSH group, this was continued daily until hCG was administered. If, 3 days after oocyte retrieval, E_2 levels were above 250 pg/mL, letrozole was continued until that levelwas below 50 pg/mL. For all of the patients, FSH, LH, and E_2 levels were recorded every one or two days until the oocytes were removed. If E_2 levels exceeded 250 pg/mL, a GnRH antagonist was released to avoid a premature LH surge. When the lead follicle reached an average diameter of 17-18 mm, hCG was administered, oocytes were retrieved 36 hours later, IVF was performed by ICSI, and embryos were frozen at the 2-pronuclear stage.



➢ **Figure 1.** This table shows total embryo and peak estradiol data for the study from Cornell University comparing tamoxifen, tamoxifen-FSH, and letrozole-FSH. Oktay, K., Buyuk, E., Libertella, N., Akar, M., & Rosenwaks, Z. (2005). Fertility Preservation in Breast Cancer Patients: A Prospective Controlled Comparison of Ovarian Stimulation With Tamoxifen and Letrozole for Embryo Cryopreservation. *Journal of Clinical Oncology*, 7.

Results and Interpretations

In the results of the study, a variety of comparative data was recorded. The tamoxifen group resulted with 13 cycles in 12 patients, the tamoxifen-FSH group had 9 cycles in 7 patients, and the letrozole-FSH group had 11 cycles in 11 patients. There was an exception for the tamoxifen group, which had two cycle cancellations due to spontaneous ovulation right before the oocytes were retrieved. The age of IVF and control patients was similar, with the average being 37.7±0.8 years of age for controls and 36.5±0.7 years of age for IVF patients. Letrozole-FSH and tamoxifen-FSH groups both had higher numbers of mature oocytes and oocytes retrieved than the tamoxifen group, thus they resulted in higher embryo counts as well. Average total embryos per patient for tamoxifen, tamoxifen-FSH, and letrozole-FSH were 1.3±0.2, 3.8±0.8, and 5.3±0.8 respectively. However, tamoxifen-FSH resulted in much higher peak estradiol than other groups, at an average of 1,182±271pg/mL. Letrozole-FSH was able to maintain peak estradiol levels very well, averaging at 380±57pg/mL, and tamoxifen alone peaked at 419±39pg/mL. The letrozole-FSH group proved to have the shortest follow-up time as well, averaging at 272±31 days versus 609±89, 418±109, and 660±71 days for tamoxifen, tamoxifen-FSH, and controls, respectively. Fortunately, there was no difference in recurrence rates between controls and IVF patients.

Discussion

The purpose of this project was to determine the efficacies of AIs in comparison to SERMs. From the data above, it can be concluded that letrozole-FSH, the AI, was more effective in keeping estradiol levels low while stimulating the ovaries than tamoxifen-FSH or tamoxifen, the SERM groups. However, this does not rule out tamoxifen for ovarian stimulation. Both aromatase inhibitors and selective estrogen receptor modulators have proved to minimize estrogen levels with their different mechanisms. Furthermore, the small numbers used forthis study indicate that further research should be conducted to confirm these findings. Another SERM that was not brought up in the study is clomiphene citrate; this is the standard drug for IVF in infertile patients. According to a clinical pharmacology review, letrozole has become more and more common and could potentially replace clomiphene. Therefore, not only is letrozole, and thus AI, good for fertility preservation in breast cancer patients, but also fertility preservation for general infertile patients. In addition, tamoxifen, as a partial agonist, is associated with an increased incidence of uterine cancer while letrozole is not. Perhaps the scale is leaning in favor of the aromatase inhibitors.. However, while tamoxifen is antiestrogenic to breast cells, it is an estrogen agonist in bone tissue, and extended use of it can prevent postmenopausal osteoporosis. As conveyed, there are a number of benefits and risks that accompany the use of these drugs. It is important to involve both options when discussing fertility preservation with breast cancer patients so that a decision can be made that best fits to their personalized care.

Relevant Application to Biotechnology

With advancements in biotechnology, measures should be taken to increase the options that patients are presented with when interested in preserving their fertility. Furthermore, more research should be conducted to continue opening doors into more personalized patient care and to ensure that fertility options are available to all people no matter their state of health.

Acknowledgements

I would like to extend a special thank you to Dr. Chang and his incredible talents, Dr. Ericka Senegar-Mitchell for all of the help and support she provided throughout this experience, and to Mrs. Winter, for making all of this possible. Furthermore, a loving hug goes out to my wonderful OSA sisters and sister-in-science, Sheriden, for always being fun and supportive and making this experience worthwhile. This academy has brought together some really amazing ladies in science and I am so glad I was able to be a part of it!

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The Effect of Testicular Sperm Extraction (TESE) During Adolescence on the Reproductive Success of Males with Klinefelter Syndrome

Vivien Vaucher

San Dieguito Academy

Objective

This poster aims to to identify how having Klinefelter Syndrome (KS) can affect a male's fertility; one of the more viable options, testicular sperm extraction, that has been tested for fertility preservation; and how utilizing sperm from adolescent KS males obtained through testicular sperm extraction affects their reproductive success in the future.

Abstract

Klinefelter Syndrome (KS) is a disorder that occurs when a male has any number of additional X chromosomes to the X chromosome in the XY set that determines his sex. This syndrome is one of the most common sex chromosome disorders, affecting an estimated one in 500-1,000 males at birth, however, many cases go undiagnosed because the symptoms often present mildly. Most KS males face infertility and a deficit in testosterone, and if diagnosed, patients can be put on testosterone treatments to regulate hormone levels in the body for the development of secondary sex characteristics. Males with KS often seek assisted reproductive technology, a common method being testicular sperm extraction (TESE), where a biopsy is performed to retrieve testicular tissue, and it is then cryopreserved for future sperm extraction. Due to the knowledge that the success of TESE decreases as testosterone increases, it is ideal for this procedure to be performed before the onset of puberty when testosterone does not have any effect. This particular study was conducted on eight adolescent males with Klinefelter Syndrome who served as an experimental group to examine the success of TESE on KS males. Following an analysis of a semen sample to detect azospermia, these males were given the option to have testicular tissue cryopreserved. After examination of the testicular tissue extracted, one patient was found to have viable spermatozoa in one testis, and another was found to have a low, but present amount of germ cells in one testis. Seven of the males were shown to have azospermia. TESE was used as the preservation method due to it having proven to allow for successful spermatogenesis of the sperm retrieved, and additionally, egg fertilization through ICSI in vitro fertilization procedure. Thus far, TESE has proven effective in the fertilization of eggs via in vitro fertilization, but it has not yet been tested enough to determine statistics regarding the prevalence of full reproductive success. However, the results of testicular tissue biopsies performed on adolescent KS males show potential for the future reproductive success of the patients.

Methods and Materials

In a French study conducted from 2008 to 2011, eight adolescent males diagnosed with Klinefelter Syndrome were evaluated for their fertility and served as an experimental group for the possible fertility preservation method of testicular sperm extraction, or TESE. A group of four males with tissue cryobanked via TESE prior to gonadotoxic treatment served as the control group. Each KS male was asked to provide a semen sample in order for the researchers to detect azospermia. Following the analysis, these males were then given the option to have testicular tissue cryopreserved. TESE was used as the method of preservation due to it having proven to allow for successful spermatogenesis of the sperm that are retrieved, and additionally, fertilization of eggs through the ICSI in vitro fertilization procedure.

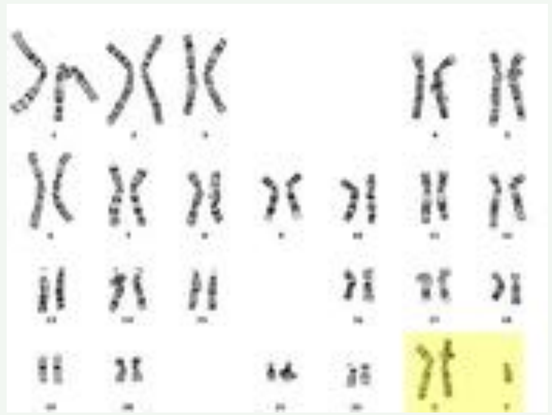

Figure 1. Highlighted is the genetic karyotype for the XY chromosome pair of a male affected by Klinefelter Syndrome that contains an extra X chromosome. *Goetsch, A. (2014, June 5). Klinefelter syndrome. Retrieved August 5, 2015.*

Results

When tested, all of the patients had hypospermia, which causes lower sperm count in semen. In addition, seven of the males had azospermia, and therefore a complete absence of motile sperm in their semen. This made the extraction during adolescence essential, since testosterone levels are highest during puberty and therefore will have the greatest effect on the sperm of young adult males. After analysis of the testicular tissue extracted, one patient was found to have viable spermatozoa in the right testis, and another was found to have a low, but present amount of spermatids in the right testis. All of the patients' seminiferous tubules were empty or degenerative, and therefore were not producing any gametes that would allow for the production of viable spermatozoa. Overall, spermatozoa count was significantly lower in the experimental group of KS males observed in the study compared to any other control group involved in similar studies.

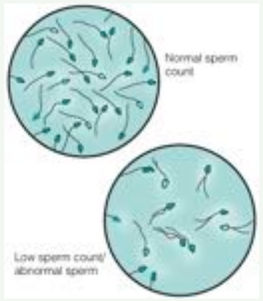

Figure 2. Often males with Klinefelter Syndrome will have fertility problems that come from hypospermia, or low sperm count as is pictured on the right. *Low sperm count. (2015). Retrieved August 11, 2015*

Relevant Applications to Biotechnology

Due to Klinefelter Syndrome being the most common sex chromosome disorder to affect males, and the symptoms so often going unnoticed, there should be a genetic test that is performed along with the screenings performed on a fetus in utero. A test could be of great use to parents so they can be made aware of the implications of the disorder, and have time to educate themselves on fertility preserving options for their son. In addition, it would give the parents more time to consider ways in which they could go about discussing fertility preservation with their son at the onset of puberty, since fertility is often not of great concern to adolescents

Conclusions

So far, results have not been entirely conclusive on how preserving fertility through TESE has affected the reproductive success of males with Klinefelter Syndrome since the males in the study have not yet sought out assisted reproduction to conceive. However, past attempts at conception through the ICSI in vitro fertilization procedure using sperm extracted via TESE have shown to be successful in the fertilization of eggs and creation of embryos. Further studies would be required to get adequate information regarding the effect of utilizing TESE to preserve the fertility of KS males on their future reproductive success.

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First and foremost, I would like to thank my wonderful mentors, Dr. Ericka Senegar-Mitchell, and Sheriden Smith for all of their help and encouragement throughout the research process. In addition, I extend my thanks to Ms. Patricia Winter for all of her hard work that goes into making this academy such an incredible opportunity for girls to further enrich their studies in science. I also give many thanks to my friends for enduring countless hours of science jargon and research ideas, and for pushing me to explore all of my interests and to find my place in the scientific world. Finally, I would like to thank my parents for all of their support throughout this academy, and with all of my scientific endeavors.

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The Enhancement of Cancer Immunotherapy with Gold Nanoparticles

Paulina Wells
San Ysidro High School

Objective

The objective of this poster is to analyze the effects of utilizing AuNPs in cancer immunotherapy (cancer vaccines) with the intention of reducing tumor growth. It is important to investigate this correlation in order to formulate new cancer treatment plans that will reduce toxicity to the patient while increasing the eradication rate of the cancer and ultimately increasing long term survival.

Abstract

Cancer immunotherapy is a cancer treatment that utilizes adjuvants and antigens to stimulate the immune system to detect and eradicate cancer cells. By utilizing immunotherapy, tumor growth can be suppressed and may facilitate other cancer therapies, thus reducing toxicity, decreasing time of cancer eradication, and increasing survival rates. Certain cancer vaccines have been developed to inhibit malignant tumor growth, but these vaccines have a weak immune-stimulating capacity of creating tumor antigen-specific responses to stop the spread of cancer. However, according to various studies, the emergence of gold nanoparticles (AuNPs) has presented advantages to cancer vaccines.[1]In three studies, researchers investigated the efficacy of AuNPs conjugated with antigens and/or adjuvants in inhibiting tumor growth by injecting tumor bearing mice with either this mixture, a free antigen/adjuvant mixture, or a saline solution (control). One study investigated the efficiency of AuNPs conjugated with EDB (tumor-associated self-antigen) and $OVA_{257-269}$ (ovalbumin peptide antigen) on the inhibition of breast cancer tumor growth, while another investigation focused on the suppression of B16-OVA tumor through the combination of AuNPs with OVA and CpG (adjuvant). [2, 3] A third investigation researched the suppression of RFP-expressing tumors with AuNPs conjugated with CpG and RFP (model antigen). [5] Evidently, the results were similar. The AuNP/EDB-$OVA_{257-269}$ (~ 480 mm^3) repressed tumor growth compared to the free antigen (~900 mm^3) and the control (~1100 mm^3). [2] Similarly, AuNP/OVA (~5-7 mm^2) and AuNP/OVA+AuNP/CpG (~10 mm^2) dramatically inhibited tumor growth compared to OVA (~85 mm^2) and the control (~110 mm^2). Compared to RFP (~900 mm^3) and the control (~850 mm^3), CpG/RFP/AuNP (~200 mm^3) significantly suppressed the tumor. [6] It was concluded that AuNPs helped the delivery of antigens and adjuvants to the targeted site, thus having an enhanced therapeutic effect. By utilizing AuNPs in combination therapy with cancer vaccines, cancer can be more efficiently eradicated.

Methods and Materials

Many studies have been conducted to analyze the effect of utilizing gold nanoparticles (AuNPs) in cancer vaccines with the intention of suppressing tumor growth. In one study, researchers investigated the efficacy of AuNP/EDB-$OVA_{257-269}$ vaccines in tumor bearing mice. Most cancer-associated antigens are endogenous antigens and are difficult to induce cytotoxic T cell responses. Therefore, the effectiveness of vaccines against cancer antigens has been limited because malignant cells have evolved "immune surveillance." Since many studies haven't focused on the enhancement of cytotoxic T cell responses through cross presentation, the EDB was chosen for this investigation, as it is a tumor associated self-antigen highly expressed in tumor tissue. In order to facilitate cross presentation, the researchers presented a CTL epitope-containing peptide of chicken ovalbumin ($OVA_{257-269}$) to the C terminus of the EDB. The 4TI murine breast cancer cell line was selected, as it is an EDB-overexpressing tumor model, and was implanted in BALB/C mice. When tumors reached approximately 30 mm^3, saline (control), an IFA/EDB-$OVA_{257-269}$ mixture, AuNP/EDB-$OVA_{257-269}$, or free EDB-$OVA_{257-269}$ was injected three times in a weekly basis into the footpad of each mouse for this ~28 day study. [3] Another investigation researched the immune distribution of AuNPs in the delivery of the OVA antigen and the CpG adjuvant, and their enhanced therapeutic effect on tumor growth. CpG, a synthetic oligodeoxynucleotide that binds to toll-like receptor 9 (TLR9) in the endosomes of antigen presenting cell, was used as the adjuvant in this study as it has been demonstrated that AuNP delivery facilitates uptake into endosomes, thus producing a strong immune response with anti-tumor activity. In this research, mice were subcutaneously inoculated with 5 x 10^5 B16-OVA cells and the tumors were grown for 5 days to the size of 5 mm^2. The mice were then given their first equivalent doses of free OVA, OVA+AuNp-CpG, AuNP-OVA, and AuNP-OVA+AuNP-CpG on day 5 and then given a booster shot on day 12.[2] Another study investigated the therapeutic efficacy of the AuNP-based vaccines on RFP-expressing tumors. Red fluorescent protein (RFP) was chosen as the model antigen, and CpG was utilized as the adjuvant for this study because it is necessary to use a strong adjuvant that enhances antigen-specific T cell responses. Mice were inoculated with RFP-expressing B16F10 cells on day 0. Once the tumors reached approximately 40 mm^3 in size (on day 10 after inoculation), the mice were injected five times with PBS (control), RFP, AuNPs, RFP/AuNPs, or CpG/RFP/AuNPs or CpG/RFP/AuNPs according to a schedule (arrows in Figure 1). [6]

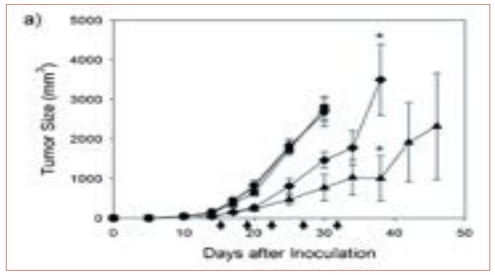

Figure 1. RFP-expressing tumor after immunization. The mice were immunized with five injections of PBS (control, •), AuNPs (▾), RFP (▪), RFP/AuNPs (◆), or CpG/RFP/AuNPs (▴) .[3]

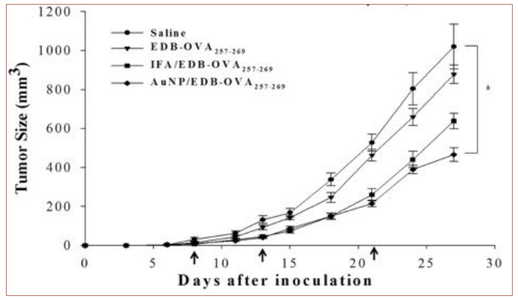

Figure 2. Changes in the size of EDB-expressing 4TI cell tumors after immunization. Mice were injected with saline (control), EDB-$OVA_{257-269}$, IFA/EDB-$OVA_{257-269}$, or AuNP/EDB-$OVA_{257-269}$. [3]

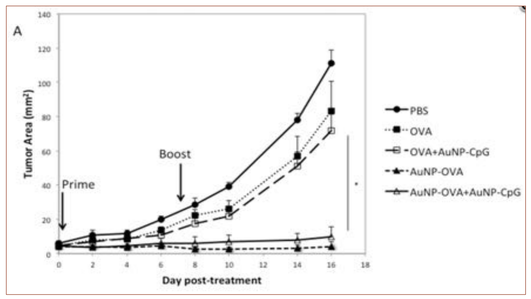

Figure 3. Growth in B16-OVA tumor bearing mice. Mice were injected with PBS, OVA, OVA+AuNP-CpG, AuNP-OVA, and AuNP-OVA +AuNP-CpG.[2]

Results and Interpretations

All three studies concluded that AuNPs helped enhance cancer vaccines by inhibiting tumor growth. In the first study, tumor growth in mice treated with AuNP/EDB-$OVA_{257-269}$ was suppressed (46%) compared with the saline/control (100%) and free antigen groups (86%). [2] After ~ 27 days, the tumor in mice treated with AuNP/EDB-$OVA_{257-269}$ vaccines was suppressed to ~480 mm^3. However, the tumor in mice injected with the free antigen (EDB-$OVA_{257-269}$) experienced growth up to ~ 900 mm^3 while the control group had tumors that grew up to ~1100 mm^3. These results indicate that the AuNP-based vaccine was effectively delivered to the local lymph nodes and created an effective antigen-specific cytotoxic T-cell reaction via cross-presentation of resident dendritic cells, thus inhibiting tumor growth in the EDB-overexpressing breast tumor model (Figure 2). Similarly, the AuNP-infused cancer vaccines in the second study significantly inhibited tumor growth compared to the other experimental groups (Figure 3). Into 16 days of the study, the AuNP/OVA condition had the strongest response, suppressing the B16-OVA tumor to ~5-7 mm^2 compared to the free antigen (OVA) vaccine which experienced growth up to ~85 mm^2 and the PBS (control) solution (~110 mm^2). [3] Surprisingly, the addition of AuNP-CpG had practically no effect on the suppression of tumor growth combined with free OVA and AuNP/OVA. The AuNP/OVA+AuNP/CpG suppressed the tumor to a size of ~10 mm^2 while the mice injected with OVA+AuNP/CPG had a tumor growth of ~75 mm^2. The probable reason for why AuNP/CpG did not have a significant effect was due to perhaps the tumor being too advanced to be affected by the CpG alone. In addition, the AuNP/OVA+AuNP/CpG probably didn't benefit from the fact that the antigen and adjuvant were on separate particles as immune stimulation is more potent when both substances are co-localized. Nevertheless, all of the AuNP vaccines better suppressed tumor growth compared to the free antigen and control vaccines. This suggests that the AuNPs facilitated the delivery of the antigen and adjuvant, which led to a stronger antigen specific immune response. In the third investigation, the AuNP infused vaccines significantly suppressed tumor growth (Figure 1). The CpG/RFP/AuNP vaccine inhibited the tumor to ~200 mm^3 and RFP/AuNP suppressed tumor growth to ~230 mm^3 in 20 days of the study. Mice injected with RFP experienced tumor growth up to ~900 mm^3 while the control group had tumors ~850 mm^3. Even though AuNPs have shown to have an adjuvant effect on their own, the mice injected with bare AuNPs experienced tumor growth up to ~800 mm^3. [6] However, the AuNP vaccines conjugated with the antigen and/or adjuvant demonstrated a significant suppression of tumor growth due to the efficient delivery of AuNPs. As concluded by all three studies, the gold nanoparticles were able to enhance the cancer vaccines by efficiently delivering the substances to dendritic cells and activating CD8+ cells, which induced an antigen-specific immune response, thus inhibiting tumor growth.

Conclusion

It was concluded from these three studies that AuNPs helped enhanced the therapeutic effect of cancer vaccines. The AuNP-infused cancer vaccines efficiently inhibited tumor growth compared to the free antigen/adjuvant shots due to the fact that gold nanoparticles have several properties that enable efficient drug delivery. Through this breakthrough, the creation of new and efficient cancer vaccines can be possible. Even though cancer immunotherapy can be improved through the use of gold nanoparticles, more research needs to be conducted on the complete eradication of malignant cancer cells through cancer vaccines. Through further research, cancer immunotherapy can be utilized to eradicate cancer while reducing toxicity, increasing eradication rate, and potentially increasing long-term survival rates. As a result, the lives of many cancer patients can be positively impacted through cancer vaccines.

Application to Biotechnology

Due to the engineering of biocompatible gold nanoparticles, research on cancer immunotherapy and its enhancement through gold nanoparticles was made possible. This research reveals that cancer therapies can become more efficient through the use of a gold nanoparticles, thus reducing toxicity, decreasing time of cancer eradication, and increasing long-term survival. By utilizing the information and data gathered from this research, improvements in the delivery and function of various cancer therapies, such as radiation therapy, can occur (Figure 4). Not only can the effectiveness of other cancer treatments improve, but new forms of combination therapies utilizing gold nanoparticles that efficiently target the cancer can be developed. Through this research, the lives of cancer patients can be significantly impacted by the development of new and more effective combination therapies and cancer treatments.

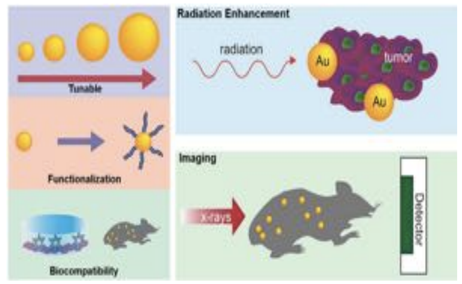

Figure 4. The use of gold nanoparticles in radiation therapy and imaging. AuNPs are biocompatible and can be easily changed into different sizes and shapes. [4]

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