The Use of Inhibin B and Anti-Müllerian Hormone as a Diagnosis Marker for Granulosa Cell Tumors (GCTs)

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Objective

Granulosa Cell Tumors account for a minor amount of ovarian cancer but for those who may encounter it; it’s a major problem. This research gives patients of a rare disease a voice in attempting to find an accurate, minimal-invasive, early diagnosis technique. The objective of this poster is to validate the use of Inhibin B and Anti-Müllerian Hormone as biochemical markers for diagnosis of granulosa cell tumors and differentiation from other types of cancer to detect the disease preoperatively and postulate optimal patient care.

Abstract

Diagnosis of ovarian cancer is challenging especially when considering the rarity of GCTs. Granulosa cell tumors make up 5-7 percent of ovarian cancer and symptoms are vague and diagnosis and follow up are essential. Inhibin is believed to be the most accurate marker for granulosa cell tumors because, normally, ovarian granulosa cells produce inhibin. In GCTs the serum inhibin levels reflect the size of the tumor. AMH should be measured in premenopausal women and that inhibin B levels should be measured in premenopausal women.

Methods and Materials

Traditionally, inhibin concentration peak of about 77238 U per liter in the follicular phase of the menstrual cycle and is undetectable in the serum of menopausal women. To see if inhibin can be a biomarker for the presence of GCTs, one study measured the serum inhibin concentrations (number of U per liter) in six women with granulosa cell tumors. In another study, one inhibin B, AMH, along with human epididymis protein 4 (HE4) and carbohydrate antigen 125 (CA125) were measured in 135 samples from AGCT patients, 37 epithelial ovarian carcinoma (EOC) patients, and 40 endometrioma (ENDO) patients. In the second study, inhibin B and AMH levels were also higher in AGCT WD patients when compared to those of EOC and ENDO patients. In the second study, inhibin B and AMH levels were also higher in AGCT WD patients when compared to DF samples, confirming their roles as markers in the diagnosis of AGCT patients. The receiver operating characteristic (ROC) curve analyses performed showed that in differentiating AGCTs from EOCs, all single markers were very accurate, with AUCs between 0.92 and 0.97, and combining the markers didn’t improve the accuracy. In distinguishing AGCTs from ENDOs, the single markers were less accurate, and combining the markers seemed to improve their performance. When evaluating the markers in the follow-up of AGCT patients, the accuracies of inhibin B and AMH were higher than those of the other single markers. This was similar to the sensitivity and specificity results, alone, both inhibin B and AMH served high sensitivity and specificity, when combined sensitivity rose to 100% (Figure 3).

Results

The results confirm that inhibin B and Anti-Müllerian Hormone can be used as a diagnosis marker for granulosa cell tumors. In the first study, five women experienced elevated inhibin levels. In two women, the serum inhibin levels were abnormally elevated 5 and 20 months before the clinical indicators of recurrence became evident. The maximum concentrations were about 3000 U per liter, which is three to four times the peak level for normal patients. The serum inhibin level remained undetectable in one patient who was disease-free for 11 years. After the removal of the tumor, inhibin levels in the patients stabilized. In the second study, Inhibin B and AMH levels showed significantly higher in AGCT WD patients when compared to those of EOC and ENDO patients (Figure 2). Inhibin B and AMH levels were also higher in AGCT WD samples when compared to DF samples, confirming their roles as markers in the diagnosis of AGCT patients. The receiver operating characteristic (ROC) curve analyses performed showed that in differentiating AGCTs from EOCs, all single markers were very accurate, with AUCs between 0.92 and 0.97, and combining the markers didn’t improve the accuracy. In distinguishing AGCTs from ENDOs, the single markers were less accurate, and combining the markers seemed to improve their performance. When evaluating the markers in the follow-up of AGCT patients, the accuracies of inhibin B and AMH were higher than those of the other single markers. This was similar to the sensitivity and specificity results, alone, both inhibin B and AMH served high sensitivity and specificity, when combined sensitivity rose to 100% (Figure 3).

Applications to Biotechnology

The mechanism used to detect the hormone levels, enzyme-linked immunosorbent assay (ELISA), has proved very helpful. There are variations of the ELISA tests, but the most utilized type consists of an antibody attached to a solid surface. This antibody has affinity for the substance of interest, such as bacteria, another antibody, or in this case, a hormone. The more substance of interest that is present in the test sample, the less linked enzyme will bind to the solid surface.

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I would like to thank the Reproductive and Oncofertility Science Academy for this opportunity of a lifetime. A special thank you to Dr. Ericka Senegar-Mitchell for making learning exciting. Ms. Pavia for organizing each event, and Dr. Chang and Dr. Su for providing support and insight. Also, I would like to acknowledge every doctor and faculty that gave us their time and let us visit.

References

The Effects of Embryonic Stem Cells on Ovarian Cancer
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Abstract
The leading cause of death in gynecologic malignancy is ovarian cancer. Most women that are diagnosed with ovarian cancer are 64 years old and are in the III or IV stage due to the lack of recognizable symptoms and insufficient screening techniques. In 2016, over 70% of women were diagnosed in late stages of ovarian cancer and the average survival rate was less than 5 years. With research and time, scientists have compiled data that suggests human embryonic stem cells (hESCs) can effectively inhibit the growth of tumors in ovarian cancer via vaccination. Both case studies have vaccinated rats and mice with stem cells that were derived from hESC line iPS-E1, mESC line iPS-E1, and ID8 cells in the mice while comparing NuTu-19 cells and H9 in the rats. This poster will also show similarities between cancer and embryonic cells, suggesting stem cell immunization might generate an immune response against gene products and tumor cells. This can further research and could be applied to women with pre-established ovarian cancer in clinical trials.

Methods and Materials
The first study used specific pathogen-free C57BL/6 female mice (20-25 g) and Fischer 344 female rats (100-125 g). The H9 and iPS-E1 line iPS-E1, mESC line iPS-E1, and ID8 cells in the mice while comparing NuTu-19 cells and H9 in the rats. 56 mice were divided into groups evenly, Groups 1-4 received vaccinations subcutaneously of iPS-E1 (5x10^6) or H9 or iPS-E1 (5x10^6) or ID8 (5x10^6) with phosphate-buffered saline (PBS) pre-treatment. Three times each week, and 4 weeks after the final vaccination, NuTu-19 cells (1x10^6) were inoculated one week after final vaccination. Groups 5 and 6 received pre-inactivated phosphate-buffered saline (PBS) 344 female rats (100-125 g). They compared hESC line H9, mESC line IVP-ES1, and ID8 cells into seven groups. Group 1 vaccinated with pre-irradiated hESCs (1x10^7), repeated six times each week, and 4 weeks after the final vaccination. Group 2 vaccinated with pre-inactivated mitotic NuTu-19 (1x10^7) 3 times each week and weeks Vaccinations were started on the third week of the experiment. Figure 1. For the mice, vaccinations of H9, iPS-E1, and PBS were received three times each week for Groups 1-4. Groups 5-6 received vaccinations three times a week of H9 and iPS-E1. 1

Figure 1. For the rats, Groups R1-R3 received inactivated H9, NuTu-19, and PBS three times each week. Group R4 received H9 six times a week. 1

Figure 2. 3 H9 immunizations and PBS inoculation for each group. Groups 1 and 4 with vaccinated H9. Groups 2 and 5 with pre-inactivated mitotic NuTu-19. Groups 3 and 6 with PBS. Group 7 with pre-inactivated H9. 5

Results
Both studies proved that H9 cells inhibit the growth of tumors in both mice and rats. In the first study, the tumor’s formation and growth was longer in the H9 and IVP-ES1 vaccinated mice than the ones vaccinated with ID8 and PBS. In the rats, H9 vaccinations resulted in longer survival by showing a possible relationship between the two. These studies support the hypothesis that oncofetal antigens are expressed in cancer and embryonic stem cells, suggesting stem cell immunization might generate an immune response against gene products and tumor cells. This can further research and could be applied to women with pre-established ovarian cancer in clinical trials.

Applications to Biotechnology
Without immunohistochemical staining, researchers would have never found tumor-surfacing genes, antigens, and metastasis-related genes in the tissue of the mice and rats. Such markers included nm23, p53, C-myc, HER-2, PTEN, and CK. Both nm23 and HER-2 correspond negatively to tumor metastasis and progression. PTEN, p53, and C-myc are very important to the process of tumor genesis. This hypothesis helped scientists come to the conclusion that tumor antigens resonate from the hESCs providing them a foundation in examining the significance of tumor markers.

Acknowledgements
I would like to extend my greatest thanks to Dr. Ericka, for her continuous support and wonderful insight. I would like to thank Dr. Tzukendel for her amazing help. Dr. Chang for his knowledge and kindness. Most of all, I would like to thank all of the doctors and researchers for spending their time with us and helping further our education in the field of medicine. To my parents, I could not have done this without your unconditional love. And to my amazing ROSA sisters, you all are so beautiful and I am so happy to have met you. I cannot wait to see what the future holds for all of us.

References
Objective/Background

One fourth of women diagnosed with some form of cancer are of fertile age and may require chemotherapy. One way to potentially lower the rate of POF in female cancer patients is autotransplantation of the ovary. The primary objective of this research is to explore the viability of autotransplantation of the whole ovary for women who are at risk for POF.

Abstract

Females undergoing cancer treatments or other gonadotoxic treatments have a variety of options when it comes to fertility preservation. This research will focus on a new preservation technique: ovarian autotransplantation. Specifically, the objective is to explore the viability of autotransplantation of the whole ovary for women who are at risk for POF. In addition, this technique could prove beneficial because follicle atresia and ischemia are reduced, no ovarian stimulation is needed, and no delay in cancer treatment is necessary. Since ovaries have a great amount of plasticity, they can restore endocrine function after revascularization. Approximately 25% of all women diagnosed with cancer are of reproductive age. Furthermore, while chemotherapy has improved the survival rate, it has also increased the risk of permanent ovarian failure. If the ovaries were to be taken out before treatment and then reimplanted, the ovaries would be removed before treatment and then reimplanted. This research will focus on fertility preservation: no evidence of malignant cell contamination in ovarian tissue.

Methods and Materials

Study 1: Since autotransplantation of the whole ovary is still in the experimental phase, there has been no human trials. There has however seen several human trials in autotransplantation of cortical ovarian tissue. With this technique, one half of the ovary is excised through and then, the ovarian cortex is cut into small pieces for cryopreservation. When it is time for autotransplantation, the frozen tissue is attached to the remaining ovary through avascular grafting.

Study 2: Although no human trials have occurred, there have been several animal trials. In a study done at the General Hospital of Vienna, Austria, nine ewes, six months of age underwent laparotomy unilateral oophorectomy. Afterward, the ovaries were transferred to a freezer and stored at -196°C in liquid nitrogen. Between 3 and 5 weeks of the original procedure, the ewes underwent contralateral laparotomy. The vascular pedicle of the ovary was dissected under a microscope and then the frozen-thawed ovary was autografted through anastomosis.

Study 3: There has been one human case of ovary autotransplantation between 38 year old, monozygotic twins. One twin experienced POF at age 15, so her twin decided to donate an ovary to her. The ovary was laparoscopically removed from the fertile sister and then prepared for transplantation. The donor's ovarian veins and arteries were anastomosed to the recipient's ovarian veins and arteries.

Results and Interpretations

Study 1: Across 13 different studies, there have been reports of 26 successful births through autotransplantation of ovarian tissue. Although there has been success with this technique, there is also an increase in post-grafting ischemic and follicle atresia due to the grafting method. In fact, all of the women in these studies experienced some extent of atresia.

Study 2: The Austrian study had a great amount of success. After reimplantation, FSH levels rose for approximately 3 months and eventually reached normal levels after 6 months. Furthermore, four out of nine sheep regained luteal function and one of the four sheep was able to conceive spontaneously after the procedure.

Study 3: The case in which a monozygotic twin donated her ovary to her sister was successful. After a 100 minute ischemic period, normal-appearing blood flow was seen. Furthermore, 101 days after transplantation, the recipient had her first menstruation cycle in 22 years. Almost two years later, the recipient gave birth to a healthy baby girl.

Discussion

The objective of this research was to explore the viability of autotransplantation of the whole ovary for women who are at risk for POF. Viability is defined as the ability to restore menstrual cycle and potential for pregnancy. There has been success with similar methods, animal trials, and a few human cases. Through the results, it is clear that this is a viable method that will help women who are at risk for POF. Whole ovary autotransplantation could prove to be a revolutionary tool in restoring endocrine and reproductive functions. It could also ensure that women who urgently need cancer treatment do not experience a delay. In moving forward, phase one trials need to be done in humans. Phase 1 trials are typically done in healthy individuals to assess safety. From there, the method could get approved as an accepted method of fertility preservation and eventually be offered at hospitals throughout the world. Furthermore, this technique could potentially address a whole other set of fertility concerns in the future. Perhaps, the ovary could be extracted, modified or treated, and then reimplanted.

Relevant Applications to Biotechnology

This preservation technique would not be possible without the use of biotechnology. Before autotransplantation can occur, the ovaries must be removed through laparotomy. In a laparotomy, a surgical incision is made to gain access to the abdominal cavity. Once the incision has been made, the ovary is removed. In this case, the vascular pedicle of the ovary must be removed as well, so the ovary can be reimplanted successfully.

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I would like to thank Dr. Ericka Senegar-Mitchell for being my big sister in science and guiding me through this research project and academy. She has been a great mentor and has taught me many invaluable life skills. I am full of gratitude for Dr. Chang, Dr. Su, and all of the other medical professionals who have inspired me through their amazing teaching. Thank you to Dr. Kina for assisting me with my poster and abstract. Thank you to my family and friends for being understanding and supporting me through this entire academy. Finally, thank you to my fellow ROSA sisters for constantly pushing to be my best and making this an unforgettable summer.

References

Background

Reproductive-aged female cancer patients may be left infertile or with reduced ovary function as a result of gonadotoxic treatment.1 However, this is not a practical option for those in need of immediate treatment, since the ovarian stimulation cycles necessary for these procedures can take up to one month.3 Ovarian tissue cryopreservation is another option, but it is still in experimental stages since hypoxia due to delayed revascularization depletes the number of viable follicles post-transplantation.4 Thus, there is still an unmet need for fertility preservation in cancer patients requiring immediate treatment. This poster will demonstrate the viability of 3D-printed biosynthetic ovaries as a novel therapy for this target group.

Scaffold pore geometry: Another major component of this study was determining the optimal pore geometry for increased follicle-interaction. Three scaffold designs were printed with varying angles between each layer (30˚, 60˚, and 90˚) using an EnvisionTEC 3D Bio-Polymer. Scaffolds were chemically crosslinked with an EDC/NHS stabilization.

Abstract

In 2017 alone, it is estimated that upwards of 7,000 women in the U.S. under the age of 45 will be diagnosed with metastatic cancers, for whom there are no effective fertility preservation options. The goal of this study is to determine if a 3D biosynthetic ovary can benefit these women by mimicking the structure and function of a natural ovary to restore fertility post-treatment. In a recent study, researchers 3D printed microporous bioprosthetic ovaries made of gelatin ink and seeded them with 40-50 μm murine follicles. The researchers observed ovulation of fully mature eggs and steroidogenesis in vitro. When the grafts were implanted into mice, vascularization maintained the microvasculature, indicating follicular survival. The grafts showed increased levels of estradiol, a hormone produced by steroidogenesis.

Materials and Methods

After the scaffolds were printed, the researchers performed three experiments summarized in the table below to examine their function.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Follicle type</th>
<th>Scaffold pore geometry</th>
<th>Duration</th>
<th>Type of measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. in vitro test</td>
<td>live</td>
<td>30˚ scaffold</td>
<td>2-3 mm, 30˚, 60˚, 90˚</td>
<td>2-8 days</td>
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<tr>
<td>2. in vitro test</td>
<td>dead</td>
<td>30˚ scaffold</td>
<td>2 mm (to fit bursa), 60˚</td>
<td>1 or 3 weeks post-surgery</td>
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<tr>
<td>3. in vivo test</td>
<td>live</td>
<td>30˚ scaffold</td>
<td>2 mm (to fit bursa), 60˚</td>
<td>1 or 3 weeks post-surgery</td>
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Experiment 1: 3D-printed ovary scaffolds supported hormone function, as measured by increased levels of estradiol in spent growth media also increased from day 2 (1.26±0.36 pg/ml) to day 8 (2.55±0.11±4.21 pg/ml). Follicles ovulated fully matured eggs with normal polar bodies through scaffold pores.

Experiment 2: 3D-printed ovaries were used to determine the optimal pore geometry for increased follicle survival. The researchers also tested for stromal activity, with 3D scaffolds demonstrating increased levels of estradiol, primary, and secondary follicles in murine models. Three weeks after implantation, 3D-printed ovaries achieved fertility outcomes comparable to those in natural ovaries. In vivo, researchers found that 3D-printed ovaries significantly increased follicle survival rates compared to sham controls.

Experiment 3: 3D-printed ovaries were used to determine the optimal pore geometry for increased follicle survival. The researchers also tested for stromal activity, with 3D scaffolds demonstrating increased levels of estradiol, primary, and secondary follicles in murine models. Three weeks after implantation, 3D-printed ovaries achieved fertility outcomes comparable to those in natural ovaries. In vivo, researchers found that 3D-printed ovaries significantly increased follicle survival rates compared to sham controls.

Results

Follicles in 30˚ and 60˚ scaffolds had a higher chance of contacting two or three struts than did those in 90˚ scaffolds. Follicle survival was positively correlated with the number of strut contacts, implying follicles cultured in vitro require multiple strut contacts to maintain a spherical shape necessary for survival.

Figure 2: 3D-printed scaffolds with pore geometry of 30˚ (a), 60˚ (b), and 90˚ (c). Retrieved from Laronda, M. M., et. al. (2017).4

Figure 6: (a) Purple stain indicates 3HJDH activity. (b) Increased levels of estradiol in spent growth media from day 2 to day 8. Retrieved from Laronda, M. M., et. al. (2017).4

Conclusion

These 3D-printed ovary scaffolds supported hormone function, estradiol ovulation, and ovulation in vitro and in vivo, and live birth in vivo, demonstrating their potential in restoring fertility and endocrine function in cancer patients.5 Moving forward, further refinement of pore geometry can optimize implant function, as another study on cardiac cell scaffolds has demonstrated.6 Further study is also required to understand the effects of pore size on different compartments of the scaffold. The scaffold can create an implant that more closely resembles native ovary tissue, which is necessary for transplant longevity.4 In vivo, research should also focus on gathering more data on murine models and extending these studies to larger animal models before moving on to human applications. Other applications can expand to prepubescent girls diagnosed with childhood cancers, since they are unable to produce the mature oocytes necessary for egg or embryo cryopreservation; cases where ovarian stimulation is contraindicated, or when there is a risk of introducing malignant cells with ovarian tissue transplantation.2,3 This technology has profound implications on the future of oncology and 3D-printed tissue engineering.

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References


Figure 3: Summary of experimental set-ups used to examine function of 3D-printed ovaries. Adapted from Laronda, M. M., et. al. (2017).4

Figure 4: Follicle survival varies with pore geometry. Data adapted from text context by Laronda, M. M., et. al. (2017).4

Figure 5: Follicles contacting struts in 30˚ (g), 60˚ (h), and 90˚ (i) scaffolds. Retrieved from Laronda, M. M., et. al. (2017).4
Background

Cancer is a disease that is growing in prevalence, with the National Cancer Institute estimating that it will affect 1 in 3 people. Drug resistance remains a significant challenge as it allows cancerous cells to evade treatment. One promising approach is oncolytic virotherapy, which involves the use of viruses to kill cancer cells.

Methods and Materials

Recombinant forms of NDV from embryonated eggs or infected poultry can selectively bind to, enter, and use cancerous cells as hosts over normal cells. Research has largely used MTH-68, an NDV strain with a single point mutation in the M gene which allows it to evade the body's immune system.

Results

These studies show that NDV can cause regression of the tumor at chemotherapeutic levels, which could be more effective in the treatment of solid tumors, especially for individuals with drug resistance. The use of NDV has been shown to be successful in increasing life expectancy when compared to chemotherapy alone, which suggests NDV may be a viable alternative in cancer treatment.

Conclusion

There are many factors and mechanisms that allow NDV to induce cell death that are not fully understood such as how tumor regression continues with the presence of antibodies to NDV. While NDV is a powerful tool, there is still much to learn about how the virus can be better designed to target specific cancerous cells.

References


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Relevant Applications to Biotechnology

While oncolytic virotherapy is a new biotechnology in itself, new improvements and changes in virotherapy technologies have allowed for genetically modified and engineered forms of NDV to be used to greater success in oncolytic virotherapy treatment. The ability to modify and tailor NDV with ONA to the needs of cancer patients is increasingly step forward and these techniques must continue to be perfected in order to make significant steps forward. Cytotoxicity is a major concern when it comes to oncolytic virotherapy, but with more advanced technologies for designing recombinant forms of NDV, the virus can be better designed to target specific cancerous cells and reduce cell death. There have been major advances in the ability to study and control oncolytic virotherapy in a way that was not possible in experiments of human xenografted into animals.

Technologies to modify the outer structure of NDV and change human insertion has powerful implications for making cytotoxicity tolerant when treating solid tumors with NDV. There is also potential for new models to study it. It has been proposed that the neurotropism that astrocytes were studied in may have influenced the results. The future must allow for better models for testing NDV in vitro before moving to clinical trials.
Genetic Abnormalities Linked to Premature Ovarian Failure

Katie Larratt

Objective

The cause of many cases of premature ovarian failure was largely unknown until the idea of a genetic etiology was introduced. The goal of this research is to determine the prevalence of cytogenetic abnormalities in women with premature ovarian failure and locate genes that could potentially cause the disease.

Abstract

Premature ovarian failure (POF) is a condition which causes the cessation of ovarian function and the onset of menopause before age 40. POF can develop as early as the teen years, causing the failure of ovulation, infertility, and menopausal symptoms, such as an untimely menopause and osteoporosis. The chance of becoming pregnant with POF is around 5%. In fact, this disease leads to 10% of ovulatory female sterility. POF is already known to be caused by several factors, including autoimmune diseases, metabolic diseases, or cancer treatment; however, about 10-15% of women with POF have an affected first-degree relative, giving rise to the idea that the disease can also be caused by genetic abnormalities. Among these abnormalities, mutations in the X chromosome appear to play a key role in the development of POF. Karyotype, fluorescent in situ hybridization (FISH) analysis, and FMR1 testing was performed on several cohorts of women with POF to detect chromosomal abnormalities. Structural and numerical X chromosome abnormalities appeared in the results, and it was found that around 20% of POF cases are caused by genetic mutations. The most common mutations occur in the FMR1, BMP15, and PGRMC1 genes in the form of numerical defects, deletions, and translocations. Sex chromosome mosaicism also presents itself in about 20% of women with POF, although its impact on ovarian function and infertility is unknown. These studies highlight the importance of the X chromosome in POF etiology and show that the routine assessment of chromosomal anomalies is highly important as it provides information for reproductive management and genetic counseling. It has been established that the X chromosome and these three genes play a role in the development of POF, but further genetic screening and analysis is necessary for the understanding of the role that these three genes play in ovarian health.

Materials and Methods

A cohort of 295 women in China were studied at the Clinical Genetic Service, Department of Health, Hong Kong. Inclusion criteria were secondary amenorrhea for at least one year before age 40 and menopausal state confirmed either symptomatically, by a gynecologist, or by elevated follicle-stimulating hormone (FSH) levels. Normal FSH levels of women after puberty ranges from 4.7 IU/L to 21.5 IU/L. In this study, FSH levels were considered elevated if they were above 20 IU/L.

Karyotypes using the standard G-bandning technique were performed on all women using DNA from peripheral blood lymphocytes. FMR1 gene testing was used on 116 patients. GeneScan analysis was used to determine the relative size of the alleles. Women with two normal-sized alleles were considered normal, and those with only one normal-sized allele underwent Southern blotting analysis to differentiate between a normal homoygote and a true premutation. Further studies were carried out by other parties to pinpoint BMP15 and PGRMC1 as genes associated with disease. Polymerase chain reaction (PCR) fragments of the BMP15 gene were coded for in 166 unrelated Caucasian women with idiopathic POF. Further studies were carried out by other parties to pinpoint BMP15 and PGRMC1 as genes associated with disease. Polymerase chain reaction (PCR) fragments of the BMP15 gene were coded for in 166 unrelated Caucasian women with idiopathic POF. Further studies were carried out by other parties to pinpoint BMP15 and PGRMC1 as genes associated with disease. Polymerase chain reaction (PCR) fragments of the BMP15 gene were coded for in 166 unrelated Caucasian women with idiopathic POF.

Results and Interpretation

Of the 295 women studied, 46 (15.6%) had an abnormal cytogenetic study. 39 of the 46 women had abnormalities in some part of the X chromosome. Within those seven outliers, one woman had an abnormality involving the inclusion of a Y chromosome in a mosaic cell line while the other six had autosomal mutations. There appeared to be a correlation with cytogenetic abnormalities and some clinical features in women with POF. Those with chromosomal abnormalities had a younger mean age at menopause than those without (28.2 years vs 31.0 years). Also, women with chromosomal abnormalities were shorter in stature than those without (151.1cm vs 157.1cm). Dysmorphic features were present in 34% of those with confirmed cytogenetic abnormalities. Large-scale genetic information collected at first encounter could predict cytogenetic abnormalities, but no single clinical feature was significantly associated with cytogenetic abnormalities. These groups were compared: those with sex chromosome abnormalities, those with autosomal defects, and those with normal cytogenetic studies. Normal patients were used as a control. Significant differences were only detected between those with sex chromosome abnormalities and the control, suggesting that younger menopausal age, shorter stature, and higher prevalence of dysmorphic features are associated with mutations in the X chromosome.

Abnormalities in the X chromosome included monosomy X, structural defects, and epigenetic changes in the X chromosome. The prevalence of dysmorphic features was 56% in those with X chromosome structural defects compared to 13% for the controls. Of the 116 women who underwent FMR1 genetic testing, one had the fragile X premutation. In a normal FMR1 gene, the CGG repeats are less than 54, and full mutation occurs when CGG repeats are greater than 200. The patient had 92 CGG repeats, a positive family history of mental retardation, but a negative family history of premature menopause. Further genetic studies in the separate studies, genetic analysis revealed that 5.42% of patients had a genetic mutation on the PGRMC1 gene. Cytogenetic analysis and RNA expression studies done on the mother and daughter revealed reduced expression of PGRMC1 because of a translocation on the PGRMC1 gene.

Conclusions

This study is consistent with previous findings in suggesting that X chromosome abnormalities are the main contributors to the development of POF. These abnormalities account for the younger age at menopause, shorter stature, and higher prevalence of dysmorphic features. This study’s reported rate of FMR1 premutation carriers was 0.86%, much lower than the western estimate of 13.8%. This could be due to the identified differences in CGG structure between Chinese and Caucasian women. The mechanism that causes POF in premutation carriers remains unidentified.

BMP15 is necessary for the progression of folliculogenesis, suggesting that BMP15 defects are involved in the pathogenesis of POF in humans. It is also suggested that altered levels of PGRMC1 may cause POF through increased apoptosis of ovarian cells. It has been established that the X chromosome and these three genes play a role in the development of POF, but further genetic screening and analysis is necessary for the understanding of the mechanism which causes the disease as well as role that these three genes play in ovarian health.

Materials and Methods

A cohort of 295 women in China were studied at the Clinical Genetic Service, Department of Health, Hong Kong. Inclusion criteria were secondary amenorrhea for at least one year before age 40 and menopausal state confirmed either symptomatically, by a gynecologist, or by elevated follicle-stimulating hormone (FSH) levels. Normal FSH levels of women after puberty ranges from 4.7 IU/L to 21.5 IU/L. In this study, FSH levels were considered elevated if they were above 20 IU/L.

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Regarding the PGRMC1 gene, karyotypes, Southern blotting, and Western blotting tests were performed on a mother and daughter with POF to screen for cytogenetic abnormalities and for RNA expression of PGRMC1.
Objective
This research attempts to address the indication of a significant reduction in reproductive performance occurring in fish that is now seen in humans. Additionally, to raise awareness of the environmental impact of estrogen having throughout the animal kingdom. This will be done by tracing levels of estrogen in Brazilian waterways and analyzing semen quality to prove the negative impact of estrogen found in water quality.

Abstract
Indicator species are organisms that reflect a specific environmental condition through their biology. The importance of this research is to identify an indicator of infertility found in human males through gulf pipefish found in South America. This work attempts to raise awareness of the environmental impact estrogen is having throughout the animal kingdom. The cause of high levels of infertility on male humans, that is indicated in pipefish, through observing levels of estrogen found in Brazilian waterways. Epidemiological studies have been started to analyze the short and long-term effects of endocrine disruptors on human males.

Methods and Materials
Semen samples were collected from five full-scale WWTPs located in the State of Ceará, Brazil. Each WWTP, five influent and effluent samples were analyzed. The frequency of occurrence of estradiol (E2) and 17α-ethynylestradiol (EE2) both with a 52% occurrence.

Results
With the results, there is a link between the decrease in human and fish male fertility that derives from the E2 and EE2, endocrine disruptors occurrences in waterways. Through analyzing endocrine disruptors such as, 17β-estradiol (E2) and 17α-ethynylestradiol (EE2) and its negative effect on male fertility and sexual orientation of Gulf Pipefish, this problem could be solved through the use of biotechnology, a possible solution could be formulated to address the levels estrogen found in water to divert it away from human consumption through installing inedible plants that thrive on estrogenic chemicals onto waterways. The plants could filter the estrogen out bodies of water and this could regulate the fertility of animals affected by estrogenic chemicals.

Conclusion
Indicator species are significant to understand underlying complications found in the environment. Though analyzing endocrine disruptors such as, 17β-estradiol (E2) and 17α-ethynylestradiol (EE2) and its negative effect on male fertility and sexual orientation of Gulf Pipefish, this problem could be solved through the use of biotechnology, a possible solution could be formulated to address the levels estrogen found in water to divert it away from human consumption through installing inedible plants that thrive on estrogenic chemicals onto waterways. The plants could filter the estrogen out bodies of water and this could regulate the fertility of animals affected by estrogenic chemicals.

Acknowledgements
I would like to express my greatest gratitude to all those individuals who have shaped my character and for their continuous support. Thank you Mr. Gonzalez and Dr. Till for stimulating my passion for science and for your endless support. Dr. Ericka S. Mitchell and McLeom, thank you for building my confidence and for being such exceptional female models in the world of science. A special thanks to Dr. Chang for igniting my passion for medicine and for offering his time to educate the B.O.A interns, I am very grateful for The Oncofertility Consortium for testing my capabilities and unleashing my true potential.

References

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References
The Correlation between the Effects of Cultural and Socioeconomic Factors and a Higher Risk of Triple Negative Breast Cancer in African American Women

Olivia Lewis

Objective
The African American community has a dark past in the medical realm. Medical projects and trials such as the Tuskegee project has caused a high level of distrust within the African American community towards medical professionals. The researchers of the Tuskegee project led 600 black men to believe they were being treated for “bad blood” or syphilis in 1932. This so-called treatment lasted for 40 years, even after penicillin was found as a treatment for syphilis. The objective of this research project is to discuss the cultural and socioeconomic factors that put premenopausal African American women at risk of aggressive disease, specifically Triple Negative Breast Cancer. I hope to bring about awareness on the issue and remind medical professionals to not give up on the African American community. Lives are being lost.

Abstract
Breast Cancer is a disease that enters the lives of women of all races and ethnicities. However, Triple Negative Breast Cancer (TNBC) is an aggressive subtype that is prevalent among premenopausal African American (AA) women.1 Reasons for this include socioeconomic and cultural factors such as lack of breastfeeding, distrust towards non-black physicians, lack of a doctor/patient relationship, lack of adequate healthcare, and a lack of participation in clinical trials. Most of these factors have not been fully explored. However, reproductive factors such as lactation and parity have been assessed. AA women have been found to have more children and breastfed less than other ethnicities (Figure 1).2 A study done by the AMBER Consortium tested this by analyzing the reproductive factors of 3,698 AA women who were diagnosed with invasive breast cancer. Each participant was classified as ER+, PR+, or triple negative (ER-, PR-, HER2-). Data regarding the participant’s age at diagnosis, number of births, lactation, and age at first birth were collected and compared with each breast cancer subtype. 56.8% of the parous participants had never breastfed, and 43.2% had ever breastfed (Figure 2). The results revealed that parous women have an increased risk of ER- and TNBC, and that breastfeeding can reduce these risks. Choosing to not breastfeed can increase risk of breast cancer among all women. However, this is mainly an issue in the African American community due to cultural aspects along with a lack of education on the matter. In this case, a connection between reproductive factors and TNBC could be found among AA women. However, cultural and socioeconomic factors such as distrust and lack of adequate healthcare have yet to be investigated. More research that specifically targets and aims to aid and educate the AA community about the damaging effects of these factors is necessary.

Methods and Materials
TNBC has the highest prevalence lowest survivability among AA women (Figure 3). Late diagnosis could be a reason for this. The AMBER study revealed that only 8.7% of the participants were diagnosed before the age of 40 (Figure 2). However, AA participation in clinical trials is very low. A similar study also tested the association between reproductive factors and TNBC.3 There were 2,658 patients with breast cancer with 2,448 controls who were between the ages of 20-64 years. Each patient was a participant of one of three population based control studies. Multivariable polytomous unconditional logistic regression methods were used to do case control comparisons between breast cancer subtypes. The study found that Parous women who breastfed for at least one year had a 31% lower risk of TNBC than parous women who had never breastfed. AA women ages 20-44 who breastfed for 6 months or longer had an 82% lower risk of TNBC than AA women who had never breastfed. This study came to the same conclusion as the AMBER study. However, this study only had 26.3% AA participation as opposed to 73.7% white participants (Figure 4). A Genetic connection between AA women and TNBC has been investigated. However, it was found that less than 20-25% of African American women with TNBC have a germline BRCA2 mutation.4 These few studies have been done with small groups and not tested in a large scale.

Results
Due to late diagnosis, a lack of adequate healthcare, and lack of participation in clinical trials, AA women are at a disadvantage as far as TNBC survivorship. This may be due to lack of adequate healthcare in predominately AA and lower income areas. In the U.S., 60% of low-income women are screened for breast cancer while 80 percent of high-income women are screened. This directly effects the AA community because they account for 24% of Americans living below the poverty line. Many researchers have asked the question as to why TNBC is prevalent in AA women and have conducted studies. According to epidemiologist Sam Oh of the University of California, San Francisco Center for Genes, Environment and Health, only 2% of cancer studies have included enough women who had never breastfed. This study only had 26.3% AA participation as opposed to 73.7% white participants (Figure 4). A Genetic connection between AA women and TNBC has been investigated. However, it was found that less than 20-25% of African American women with TNBC have a germline BRCA2 mutation. These few studies have been done with small groups and not tested in a large scale.

Conclusions
The Tuskegee project is only one example of how African Americans have been misled and used for medical purposes. The black men who participated were taken advantage of due to their lack of education. For these reasons, AA have been wary of participating in medical studies. A level of trust must be built between medical professionals and the AA community in order to establish better doctor-patient relationships. With this improved relationship, more African Americans are likely to participate in research studies. Many studies base their finding off of Black vs. White data which has failed to distinguish the class differences within the AA community. The African American community, much like the town of Tuskegee, had an image which was not an accurate representation of the lives of African Americans living in the south. Prominent African Americans live in the same area as low income AAs, would be an ideal location for research. A way to execute this research would be to create a mobile app that can be accessed by AA women all over the country. The app would allow them to track their doctors visits or annual breast screenings. Special focus on data compiled from locations like D.C can be analyzed and used to track the medical patterns of African Americans within that community.

Acknowledgements
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References
The Correlation Between Infertility Treatments and Post-Reproductive Breast Cancer

Emily Potts • Francis Parker School

Objective

The question: Is there a correlation between infertility treatments, specifically clomiphene citrate (CC) and follicle stimulating hormone (FSH), and an increased risk of developing breast cancer? The purpose of this poster is to provide evidence for the hypothesis that women who use fertility drugs have an increased risk of breast cancer. The objective is to find the correlation between younger women undergoing fertility treatments and the increased risk of breast cancer post-treatment.

Abstract

Fertility drugs, clomiphene citrate (CC) and follicle stimulating hormone (FSH), are taken during controlled ovarian stimulation (COS) stage of in-vitro fertilization (IVF) to mimic the natural release of progesterone and estrogen. The hypothesis that women who used fertility drugs had a higher absolute dense and non-dense volume, possibly due to the effect of estrogen promoting excessive growth of breast tissue: fibroglandular tissue in the breast is the target for tumor development. Due to both the nature of the tissue and increased difficulty to screen, women with dense breasts have a 4-6x higher risk of breast cancer. In a study with 3,091 women (case and control) younger than 50 years old with a sister diagnosed with breast cancer, evaluations were done to see if treatment had induced a pregnancy that lasted 10+ weeks. Out of 288 participants, 193 took CC only, 29 took FSH only, and 66 took both. Although overall data suggests there wasn't a significant increased risk, women who used the fertility drugs and conceived were at a higher risk of getting young-onset breast cancer. In yet another study, a long-term risk after use of fertility drugs was found. Researchers found that women with a history of infertility and who had COS had a higher absolute dense, non-dense, and percent dense volume compared to fertile women, which was either inconclusive or found an increase. This may indicate that women who hadn’t received treatment. Due to both the nature of the tissue and increased difficulty to screen, women with dense breasts have a 4-6x higher risk of breast cancer.

Methods and Materials

In a cross-sectional study done with 43,313 Swedish women, 8,963 were infertile: 1,576 went through COS, 780 had cycles of hMG. Of those 780 women, 16 cases of breast cancer were identified. Follow up was 17.9 ± 5 years.

In a study with 1,669 women (control sisters), who had a sister (1,422 cases: women with younger than 50 years old and diagnosed with breast cancer, evaluations were done to see if treatment had induced a pregnancy that lasted 10+ weeks. Out of 288 participants, 193 took CC only, 29 took FSH only, and 66 took both. In a long-term, historic-constructive study with 1,197 infertile women, 417 were unexposed patients and 780 women were exposed to 3,978 cycles of CC and/or hMG: 603 received 2,187 cycles of CC, 161 received 2,111 cycles of CC followed by 531 cycles of hMG, and 16 received 49 cycles of hMG. Of those 780 women, 16 cases of breast cancer were identified. Follow up was 17.9 ± 5 years.

Results and Interpretation

Researchers in the cross-sectional study found that women with a history of infertility had a higher absolute dense, non-dense, and percent dense volume compared to fertile women. Additionally, among infertile women, those who went through COS had a higher absolute volume than those who didn’t receive treatment. Due to both the nature of the tissue and increased difficulty to screen, women with extremely dense breasts have a 4 to 6-fold higher risk of developing breast cancer compared to women having fatty or non-dense breasts. In the two-sister study, women who used the fertility drugs and conceived a 10+ week pregnancy were at a higher risk of getting young-onset breast cancer.

Conclusions

Though much of the evidence suggests that clomiphene citrate (CC) causes an increase in risk of getting breast cancer, the rise is not significant enough in the studies done to draw a parallel. There is no clear conclusion to whether or not infertility treatments lead to breast cancer, as the studies done have many limitations in terms of proper reference group, small number of cases observed, self-reporting, different in the treatments, observed, number of treatment cycles, timing of treatment when treatment and follow up started, length of follow up, and age group differentiation. It is also essential that patients get screened before and monitored throughout infertility treatment to ensure the findings are accurate. Additionally, there should be a consideration of the different genetic profiles of patients. Once more is known about the genomics of the patients as well as the pathways of CC, it may be easier to understand why some women react differently to the drug. In looking to confirm this theory, future research should address these limitations, especially by increasing the study size and adjusting the timing of treatment and follow up, and focus further on each drug and how it has affected patients in multiple situations and environments.

Relevant Applications to Biotechnology

The studies done to research this correlation have been possible by the advancements in reproductive technology. The process of in-vitro fertilization (IVF) and the technologies taken for hormonal treatment during and after IVF are continually being improved. Additionally, the technology available for breast-cancer screening is more advanced than ever before. With full field digital mammography (FFDM) and future advancements in 3D mammography, detection, identification, evaluation, and treatment management of breast malignancies have greatly improved and will continue to do so.

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References

Inhibitor therapies have not been researched yet.

Between February 1, 2009 and March 31, 2010, women with a history of immunologic infertility and/or pregnancy loss with first trimester T-regulatory-cell-levels assessed at the Laboratory for Reproductive Medicine and Immunology were identified. Fifty-four women were identified in this group. A T-reg assay was developed to membrane stain CDS and CD4 and was followed by intracellular staining for FoxP3. Each was associated to fluorescent markers. Then, cytobody was assessed by flow cytometry where labeled target cells were incubated with isolated patient mononuclear cells and propidium iodide to stain killed cells. Each of the patients were given one of four treatment to address their existing conditions—throughout their pregnancy were tested for T regulatory cell levels.

Methods and Materials

Multiple studies must be used to corroborate the correlation of T regulatory cell counts and pregnancy complications as well as the effectiveness of using Rapamycin to inhibit mTOR and increase T regulatory cell counts.

The first study collected data in a retrospective analysis, showing that T-regulatory-cell-levels can predict miscarriage risk in newly pregnant women. Statistical analysis of success rates was performed using Fisher’s exact test as well as the variance for patient characteristics.

The second study determined that the mTOR inhibitor rapamycin significantly reduces the undesired expansion of effector T cells. After the isolation, purification, and culturing of Treg cells from the blood samples, the inhibitor assay demonstrated that there is a 10-fold increase in Treg cell numbers when compared to the control group that was not exposed to the Rapamycin inhibitor. This in vitro result demonstrates that the mTOR pathway might be an effective target to increase Treg cell counts and ultimately treat pregnancy complications.

In order to test the effectiveness of inhibiting mTOR with Rapamycin, T regulatory cells were isolated from peripheral blood samples. After the Treg cells were purified, grown, and cultured in order to produce enough quantity to conduct many trials of the experiment. The suppressive assay was then conducted. Respondor T cells were stained with CFSE. Treg treated Rapamycin cells were added in equal number to response Stander T cells. After 7 days of culture, cell division was monitored by levels of CFSE dilution. In the image below, a larger quantity of cells are seen within the Treg side (left) as this culture was exposed to the rapamycin inhibitor.

In conclusion, the immune system is a complicated network of many different processes that interact with each other to protect against disease. Among the many immune system targets, T regulatory T lymphocytes act to suppress immune activation and thereby maintain immune homeostasis.

Results and Interpretations

Applications to Biotechnology

An array of different technologies were used to facilitate this series of experiments.

Firstly, different technologies were used to isolate the macrophages and monocytes used to acquire Streptomyces hygroscopicus. Rapamycin works by forming a complex with FRAP12 and finding mTOR, causing it to be inhibited. This antibiotic was key to the inhibition of mTOR. Selective mutagenesis of wild-type Streptomyces hygroscopicus was done using ultraviolet radiation in order to promote the expression of Rapamycin within the colonies.

The isolation techniques the many immune system targets, T regulatory T lymphocytes act to suppress immune activation and thereby maintain immune homeostasis.

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I would like to thank Dr. Chang for taking the time to make sure every single one of us understood everything he taught on Oncotelfity. A huge thank you also to Kara from The Children’s Hospital of Orange County. You have been doing amazing things and all of us ROSA sisters appreciate the kindness and encouragement you have given.

And finally, ROSA sisters thank you for making my summer the best it could be. I have smiled more, learned more, and loved more than I ever have before.

References


Increased correlation between ethnicity and Polycystic Ovarian Syndrome

Sofia Reyes
Bonita Vista High school

Background & Objective

Polycystic Ovarian Syndrome (PCOS) is the most common endocrine problem causing infertility for women of reproductive age. Symptoms of PCOS include: hyperandrogenism, insulin resistance, chronic anovulation, irregular hair growth, acne and polycystic ovarian morphology (PCOM). Increasingly, women of differing ethnicities have contracted PCOS in different rates and factors. In past studies PCOS in the general population has ranged from 2–20%. Recently studies have suggested that PCOS diagnosis is higher in certain ethnicities. Due to the increased knowledge of correlation between a patient’s ethnicity and potential of PCOS, a higher degree of variability in the clinical manifestation of PCOS has been noted. The objective of this poster is to explore the increase in correlation and the corresponding different effects of PCOS, taking into consideration how large of a factor a patient’s lifestyle, affects their health and PCOS risk factor; including their environment and ethnicity.

Abstract

Increasingly, women of different ethnicities have contracted PCOS in different rates and factors. The need for further research on the correlation regarding PCOS is important as PCOS holds high prevalence of metabolic syndrome, diabetes and cardiovascular disease. The objective of this poster is to first prove through case studies that there is a correlation between ethnicity and PCOS, why this predisposition exists and what it might mean for the patient and their treatment. This will also address how large of a factor a patient’s lifestyle, outside of medical practice, affects their health; including their environment and ethnicity. It is increasingly recognized that different ethnic backgrounds are likely contributors to different manifestations of PCOS and PCOS phenotypes. One such study explored the possibility of an Asian and Caucasian phenotype, women in East Asia have been reported to have a lower BMI and a milder hyperandrogenic phenotype, but with the highest prevalence of metabolic syndrome. Another study showed that South Asian women have a high prevalence of insulin resistance and metabolic syndrome, and are at a larger risk of type two diabetes. These two studies helped explore how even within the same geographic regions , a variation in ethnicity can cause different manifestations of PCOS. The poster will further explore this idea, comparing different ethnicities that are not so closely centered around geography to find if similar variations in manifestations can be noted. There is a need for studies to connect results and conclude if there are ethnic variations in the prevalence of PCOS and its clinical representation. Understanding the prevalence of ethnicity in PCOS women is important to help target the relevant populations to establish the most effective treatment that meets the patients’ needs.

Methods & Materials

This poster used result from two different case studies conducted in small regional areas where ethnically related women had PCOS. The first study regarded Chinese, Taiwanese, Japanese, Thai, Korean, Caucasian, Middle Eastern and South Asian women. It compiled data regarding prevalence of women with PCOS of different ethnicities; percent of hirsutism, and biochemical hyperandrogenism and PCOM. 15,924 total number of Chinese subjects were tested for prevalence of women with PCOS, using the Rotterdam Criteria; and 728 Caucasian subjects were used. Rotterdam criteria was also used to identify PCOM. To determine the percentage of PCOS women with hirsutism, the study used the Modified Ferriman-Gallwey (mFG) cut off score. To determine the biochemical hyperandrogenism total testosterone (nmol/L), and total androstenedione (ng/ml) was measured. Averages were taken from the data of Chinese and Caucasian women with PCOS. These two ethnicities from the study were the only as it was necessary to use other studies focused on different ethnicities to create a large variation in ethnicities summing up the information. This study derived the highest prevalence from the PPCS II Trial, which was a multicenter randomized controlled double-blind clinical trial conducted at 11 clinics within the United States, was a secondary analysis on the PPICS II trial. To determine if there was a difference and metabolic phenotype between different racial and ethnic groups. Women aged 18-40 with PCOS who met modified Rotterdam criteria were included. Out of the total 702 women in the secondary analysis, 476 were Non-Hispanic White (however in the poster as Caucasian ethnicity data was already obtained), 98 Non Hispanic Black, and 128 Hispanics.

Results

The results demonstrate both the comparison of ethnic prevalence regarding PCOS and the clinical differences in PCOS. Separately understanding both the will help a) prove correlation between PCOS and ethnic by highlighting its prevalence in certain ethnicities, and b) demonstrate which clinical traits do certain ethnicities have a higher manifestation of.

Prevalence: Between the Asian and Caucasian ethnicities, there was a larger prevalence of Chinese women (31.1%), while PCOM prevalence had a (5.6%) prevalence. (The Rotterdam criteria was used for this diagnosis). Clinical variations in manifestation: There were three different clinical criteria/ representation of PCOS that were compared across Chinese and Caucasian PCOS patients (hirsutism, hyperandrogenism and PCOM). Caucasian women had a higher rate of hirsutism (74.7%), Caucasian women also had a higher rate of biochemical hyperandrogenism (67.7%), however Chinese women had a higher rate of PCOM (92.7%).

Table 1: Clinical Hormonal and Biochemical variables in PCOS for Chinese Caucasian Hispanic American

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>% of PCOS w Hirsutism</th>
<th>% of PCOS w Biochemical hyperandrogenism</th>
<th>% of PCOS w PCOM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese</td>
<td>37%</td>
<td>63.6%</td>
<td>92.7%</td>
</tr>
<tr>
<td>Caucasian</td>
<td>74.7%</td>
<td>67.7%</td>
<td>92.7%</td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>82.7%</td>
<td>63.4%</td>
<td>99%</td>
</tr>
<tr>
<td>Hispanic American</td>
<td>93.8%</td>
<td>75.8%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Conclusions & Discussion

Hispanic American women with PCOS had the highest prevalence, percent of hirsutism, hyperandrogenism and PCOM in the Non-Hispanic Black, Caucasian and Chinese women with PCOS. The higher prevalence could be due to Hispanic populations high rate of obesity and 50% chance of diabetes. Additionally, the high rate of hirsutism and biochemical hyperandrogenism explains why there is an increase risk of higher hirsutism (93.6%) rates. The percent of women with hyperandrogenism is the data with most similarity, partially because PCOS is definitive for its effects on the bodies hormones. However the rest of the data reflects how each ethnicity greatly varied in how the women manifested their PCOS, and that some ethnicities had a higher prevalence of PCOS addressing that there is an increase in correlation. There is still a need for comparative studies across different ethnicities to establish epidemiological differences observed.

Helping physicians know of an increase in prevalence amongst a certain ethnicity may allow for more targeted measures of screening and more attention to educate the patient of their higher risk. By doing so, preventive treatment can be provided. Understanding the variability of how PCOS can present itself clinically in different ethnicities is also important as it can allow for application of treatment and drugs that work most effectively for specific ethnicities.

Applications to Biotechnology

Ultrasounds are used to count follicles present within the ovaries and investigate every size in order to detect polycystic ovarian (57%) (PCOM) which may occur in some PCOS patients. Various technologies are used to manage PCOS symptoms: Combined Oral Contraceptive Pill (COPC/birth control) used to manage irregular menstrual cycles, anti-androgen monotherapy for hormones: clomiphene, metformin, gonadotrophins, surgery and in vitro fertilization for infertility. Although there are many technologies available to treat PCOS, improvements and advancements can be made to the criteria used to diagnose PCOS because there is to much variability in each patient. Perhaps in the future instead of recognizing PCOS as one disease there will be different versions of PCOS diagnosis, and more specified gauges for specific ethnicities.

Acknowledgements

Thank Dr. Ericka for being a relentless advocate for science, the oncology consortium, women and community, which inspires me everyday. A huge thank you to all the mentors who also gave up their time and equipment to invest in educating the future of science, you are my heroes. Lastly, I would like to thank my family and my ROSA sisters for always encouraging me and making me laugh.

References

Y chromosome microdeletions in men with azoospermia or severe oligozoospermia leads to infertility. Azoospermia is the complete absence of sperm from the fluid ejaculated into the orgasm. Oligozoospermia is low sperm concentration in ejaculate. Because of their azoospermia or oligozoospermia, they are infertile. The poster will demonstrate the frequency and types of Y chromosome microdeletions among infertile men with azoospermia or severe oligozoospermia through genetic studies done on men of different ethnicities and locations across the globe. The implications of this in the male infertility work-up will also be discussed.

**Methods and Materials**

One study consisted of 100 infertile men and 100 fertile men. All of the subjects were Iranian. The infertile men were divided into two groups: 70 azoospermic men and 30 severely oligozoospermic men. Patient history was collected, including information on personal habits such as smoking, and medical history, including testicular injuries. Hormone levels were also collected. The control group consisted of 100 fertile men.

Blood samples were collected for DNA extraction method and amplified in multiplex polymerase chain reaction. Each subject was tested for four AZF loci. After an initial denaturation step of 5 min, each PCR reaction was carried out at the annealing temperature specific for each primer pair, ended by an elongation step of 10 min and cooled to 4°C. Subjects who did not have Y AZF microdeletions were screened for partial AZF deletions. All negative PCR reactions were repeated for at least three times. Amplification started with an activation step of 15 min at 95°C, followed by 35 cycles of 30 s denaturation (94°C), 90 s annealing (57°C) and 60 s elongation (72°C), ended by an elongation step of 10 min and cooling to 4°C.

In another study, 3731 Chinese infertile men were studied. 2531 men had azoospermia, and 1200 men had oligozoospermia. Forty six percent of patients included in a control group. Karyotype analysis was performed using G-band staining of peripheral blood lymphocytes. Polymerase chain reaction (PCR) amplification using specific sequence-tagged sites (STS) was performed to screen for AZF region microdeletions in the Y chromosome. A novel semiconductor sequencing method was established to detect high-resolution AZFa microdeletions.

In a third study, 71 Indonesian patients were found to have azoospermia or oligozoospermia. Patients were examined for testicular volumes, seminal analysis, and hormone levels. Five fertile men joined this study as a control group. PCR reactions were used to identify deletions in the AZF region. For DNA amplification, the process begins with the initial activation at a temperature of 95°C for 2 minutes, followed by 35 cycles of denaturation (94°C) for 15 seconds, annealing at 58°C for 30 seconds, the elongation phase at 72°C for 10 minutes, and ending with the reaction ended phase 72°C for 2 minutes. The cooling process was performed at 4°C. The 7 ml reaction products were separated by 2.5% agarose gel at 50 volt for 3.5 hours.

**Results**

In the study done on Iranian infertile men, no AZF microdeletions were found in the control group. Seven subjects had microdeletions. Six of them had azoospermia, and one had severe oligozoospermia. The overall frequency of microdeletions was 7%. In the azoospermic men with microdeletions, five deletions in the AZFb region were detected. One subject had a microdeletion in the AZFc region. One last subject had a microdeletion that occurred in the AZFbc region. No microdeletions were found in the AZFa region. In the study done on Chinese infertile men, 341 out of 3731 (9.14%) had microdeletions in the AZFa, AZFb, or AZFc region. 13 men (3.81%) had a deletion in the AZFa region. In the study done on Indonesian infertile men, 17 out of 71 (23.94%) had a microdeletion in their Y chromosome. The AZFa microdeletion was the most common, showing up in 15.49%.

**Conclusions**

In conclusion, frequency and types of Y chromosome microdeletions vary from ethnicity to ethnicity. In Iranian infertile men, 9.14% had microdeletions. In Indonesian infertile men, 23.94% had microdeletions. More research needs to be done on those of all ethnicities. This research should consist of microdeletion screening in fertile men with azoospermia and severe oligozoospermia. After we know the frequency and types of microdeletions in men across the world, we can implement screening techniques in infertility clinics.

**Applications to Biotechnology**

Biotechnology helps all those with Y chromosome deletions and azoospermia or severe oligozoospermia receive a reason for their infertility, instead of receiving the diagnosis of "unexplained infertility." As infertility advances, Y chromosome microdeletions won’t be a definite sentence for never having kids. New technology will help those with azoospermia and severe oligozoospermia to father kids.

**Acknowledgements**

Thank you to Dr. Erida for all the support and encouragement and my sisters throughout this six weeks. I’d like to also thank Dr. Sasha for answering all my reproductive science questions. Lastly, I would like to thank all my ROSA sisters for giving me the most important gift of all: friendship.

**References**


The Potential Application of CRISPR During the IVF Process In Order to Target the BRCA1 Gene

Pallavi Willms
Mission Hills High School

Objective
The purpose of this research is to investigate the potential application of CRISPR to genetically edit human embryos. More specifically, repairing the hereditary BRCA1 gene in these embryos, which is responsible for up to 30% of developed breast cancers, and around 15-25% of ovarian cancers (see Figure 1).

Methods and Materials
Once the programmed CRISPR-Cas9 component is inserted into a viable embryo, the Cas9 protein first forms a complex containing guide RNA that corresponds to the adjacent DNA sequence of the inherited BRCA1 gene. Any resulting DNA sequence that is redundant with the corresponding DNA, where it is able to easily cut the double strands at a specified location, and a certain part of the gene sequence can be removed and a complete strand of programmed DNA can be inserted in that cut.

In the interests of this specific research, the BRCA1 gene mutation would be located prior to the pronuclear stage, the DNA sequence of the embryo would be matched, and, using CRISPR/Cas9, the BRCA1 gene would be removed from the embryo, allowing healthy programmed DNA to replace it (see Figure 5).

Background
In the past, the CRISPR-Cas9 tool had only been used on mice in the United States due to federal restrictions (see Figure 2). However, since April of 2017, eastern countries such as China have been using CRISPR/Cas9 on live humans to remove tumors, and on embryos to edit genes; however, those embryos have not yet been used for reproductive purposes. August 2nd, 2017 marked the day that the CRISPR-Cas9 tool was used on an embryo in the Oregon Health and Science University. As popular and revolutionary as CRISPR-Cas9 is, it carries a massive ethical debate, which is why it has only recently been used on human embryos in the United States. CRISPR/Cas9 is also known for its potential off-target effects, which make it a potentially dangerous procedure. However, the possible benefits of CRISPR-Cas9 could provide immense. Couples that struggle with reproduction due to PCOS or another linked disease that cause to undergo IVF could have embryonic gene editing as an option, and ensure that their offspring will never develop an inherited similar disease.

Abstract
About 1 in every 200 people in the United States carries either the BRCA1 or BRCA2 gene. That totals to roughly 538,500 people in the U.S. alone that have inherited a risk of certain cancers. Potential offspring of these carriers have a 50% chance of acquiring BRCA1/2 gene mutations as well, and once the mutation becomes penetrative, have an 85% chance of actually developing breast cancer. This research poses a solution concerning the BRCA1 gene specifically – and conceiving through in vitro fertilization. An inherited BRCA1 gene mutation can be detected as early as the embryonic stage. According to this research, one or more of the embryos which contain an inherited BRCA1 gene mutation are produced through IVF, scientists will be able to use a world-renowned gene editing tool called CRISPR, along with the Cas9 enzyme, in order to locate the BRCA1 gene; cut at a desired location in the DNA sequence, and remove the genetic mutation, to be later replaced with healthy, functioning DNA. CRISPR-Cas9 utilizes guide RNA that correspond to DNA targets in order to edit at a high efficiency. This leads researchers to believe that CRISPR is now capable of more advanced genome targeting in medicine and biotechnology. CRISPR has only recently been used on human embryos, and the outcome was reasonably successful – yet ethical bans and restrictions on its use in certain countries have caused some complications between researchers and their respective governments. Although the concept is one of the near future – especially regarding ongoing ethical debates concerning experimental editing of human embryos – CRISPR-Cas9 could potentially be used to repair an inherited BRCA1 gene cancer from a human embryo in order to decrease the statistic of developed cancers among males and females.

Results and Interpretations
Once the programmed CRISPR-Cas9 component is inserted into a viable embryo, the Cas9 protein first forms a complex containing guide RNA that corresponds to the adjacent DNA sequence of the inherited BRCA1 gene. Any resulting DNA sequence that is redundant with the corresponding DNA, where it is able to easily cut the double strands at a specified location, and a certain part of the gene sequence can be removed and a complete strand of programmed DNA can be inserted in that cut.

In the interests of this specific research, the BRCA1 gene mutation would be located prior to the pronuclear stage, the DNA sequence of the embryo would be matched, and, using CRISPR/Cas9, the BRCA1 gene would be removed from the embryo, allowing healthy programmed DNA to replace it (see Figure 5).

Figure 1. Percentage of inherited, developed cases of cancer between two gynecologic cancers that are known to be linked to the BRCA1 gene mutation. Charts from Ambyr Genetics, What is Hereditary Cancer? (California, 2016).

Figure 2. DNA and RNA-enzyme complexes cut DNA, where it is able to easily cut the double strands at a specified location, and a certain part of the gene sequence can be removed and a complete strand of programmed DNA can be inserted in that cut. (see Figure 4a).

Experiment 1. As the embryos matured, Dr. You was successfully able to cut the DNA sequence around the problematic gene MYBPC3, and repair those mutations with healthy DNA sequence. This experiment resulted in 42 out of the 58 embryos with new mutation-free copies of the gene – a 72% success rate. Because of embryonic gene editing restrictions in the U.S., the embryos were destroyed, not able to be legally used for reproductive purposes.

These results are very promising, especially for patients who have a limited number of viable embryos at the time of their first treatment. In the future, scientists believe that CRISPR technology could fix genetic mutations in embryos that otherwise would be discarded, giving patients more embryos to transfer and a higher chance of getting pregnant. Experiment 2. According to oncologist Lu You, the initial treatment was successful. The edited embryos were worked to match and defect the lung cancer. You hypothesized that the participant is expected to receive a second injection sometime in the future, and the study is working to recruit as many as possible patients with aggressive cancers to participate in the trial. Those participants will receive 2-4 injections as well.

Due to this success, scientists are now beginning to investigate the CRISPR tool as a viable solution for treating both breast and ovarian cancers, with the help of a surgeon. Lu You’s trial included only one subject – an adult patient with an aggressive form of lung cancer (age and gender were not released due to patient confidentiality). Immune cells were taken via the patient’s blood, edited using CRISPR-Cas9, cultured, and reintroduced back into the body.

The national average cost for the in vitro fertilization process runs up to around $12,000+. This doesn’t include the cost of the medications that accompany the procedure, which add another three to five thousand dollars. The CRISPR gene editing itself costs Harvard and HSCI Faculty around $15,200 per line, and around $75,100 per line for outside non-profit institutions.

In vitro fertilization takes a total of four to six weeks to complete a cycle, while the CRISPR gene editing method takes about six to seven months to repair a DNA sequence. This excessive time causes no drastic issue in the entire process, however it does require the embryo to be preserved through freezing while CRISPR-Cas9 is being used.

Concluding
The use of CRISPR-Cas9 in order to repair the BRCA1 gene mutation in embryos created through in vitro fertilization will significantly decrease the cases of developed breast and/or ovarian cancer due to the BRCA1 gene later in life. Between 45%-50% of women possessing the BRCA1 gene mutation will develop breast cancer before the age of 70 – meaning that a simple CRISPR procedure during the embryonic stage can prevent the onset of breast cancer and improve quality of life for numerous women. The CRISPR-Cas9 enzyme now has recuperated potential that makes it a candidate for broader scientific issues, such as applying it to numerous and/or unexplained diseases, gender or trait selection, and even cloning in the future.

References
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Application to Biotechnology
Programmable DNA sequencing and replacing via CRISPR is considered simple, precise, and versatile – one of the best gene editing tools created up to date. CRISPR-Cas9 is a cost effective enzyme RNA that will be an entirely man-made technology that will be applied to live human embryos in order to locate and repair a BRCA1 gene mutation inside the embryo. CRISPR-Cas9 is a revolutionary biotechnology that can effectively and efficiently make precise corrections.

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