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 pre-operatively 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. AHM can work with inhibin and be used to differentiate GCTs from patients of endometrioma and other types of cancer. The objective is to validate this information to allow there to be an efficient way for early, accurate, and minimal invasive diagnosis and provide optimal patient care. There have been many studies done to further prove this, including one where 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. The levels of the different hormones were recorded using receiver operating characteristic (ROC) graphs, and analyzed by calculating and comparing the area under the curves (AUC) of the different markers. The results showed that the combination of inhibin B and AMH increased the accuracy compared to either marker alone (sensitivity, 100%; specificity, 93%). It was concluded that inhibin B was the most effective single marker for detecting the presence and size of GCT but when differentiating from EOCs and ENDOs, inhibin B was best paired with AMH. In conclusion, research in circulating biomarkers can help improve early diagnosis and narrow the disparity between GCT and other types of cancer; it may even help with monitoring of patients with GCTs and follow ups reducing the risk of relapses.

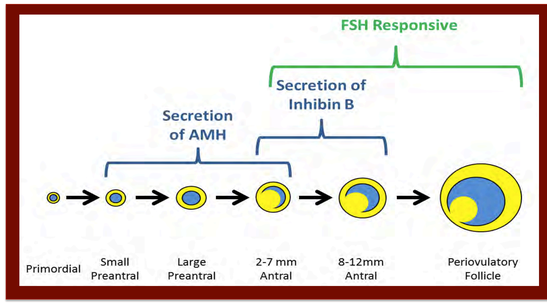

Figure 1: The interplay of follicular development and hormonal secretion. Image From: Chapters Archive - Page 6 of 32. (n.d.). Retrieved August 05, 2017, from www.endotext.org/chapter/page/6/

Methods and Materials

Traditionally, inhibin concentration peak of about 772±38 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 immunoreactive 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 order to acquire the optimal marker, the tumor marker levels in the 36 AGCT with disease (WD) samples were compared to the 99 AGCT disease free (DF) samples.

Serum and plasma samples were prepared and stored at −80 °C until the analysis. Serum inhibin B (ng/l) levels were analyzed using an enzyme-linked immunosorbent assay (ELISA) Inhibin B Gen II ELISA (A81303) and AMH (ng/ml) levels were analyzed from plasma samples with an ultrasensitive AMH ELISA (AL-105i). The levels of the different hormones were recorded using receiver operating characteristic (ROC) graphs, and analyzed by calculating and comparing the area under the curves (AUC) of the different markers. To aid the preoperative diagnosis of a potential AGCT, we recommend that inhibin B and AMH levels should be measured in premenopausal women and that inhibin B levels should be measured in postmenopausal women.

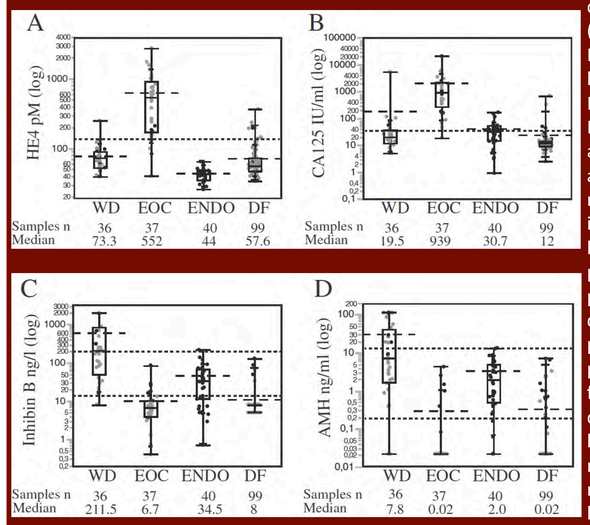

Figure 2: The concentrations of HE4 (A), CA125 (B), inhibin B (C), and AMH (D) levels in AGCT WD, EOC, ENDO, and AGCT DF patients. The data are shown as boxplots, and the dots represent measurements of individual patients, the black dots signifying premenopausal patients, and the gray dots denoting postmenopausal patients. Cut-off levels for each marker are depicted as dashed lines. The levels of the marker are represented on a logarithmic scale.[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).

Figure 3 (Table): Sensitivities and specificities of markers and marker combinations.[3]

	Sensitivity (%)	Specificity (%)
Inhibin B	92	98
AMH	83	92
inhB and AMH	100	92

Conclusion

Research in circulating biomarkers can help improve early diagnosis and narrow the disparity between GCT and other types of cancer. One can conclude that granulosa-cell tumors produce inhibin and serum inhibin levels reflect the size of the tumor. Inhibin B is the most accurate single marker for the diagnosis and follow-up of AGCTs. Adding AMH to inhibin B may be useful to differentiate AGCTs from ENDOs in premenopausal patients. Inhibin B and AMH may be a helpful and accurate way to monitor the disease. Further research is necessary because relapses of granulose cell tumors occur at an average postoperative period of five years.

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.

Acknowledgements

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. Patricia Winter for organizing each event, and Dr. Chang and Dr. Su for providing support and insight. Also, I would like to acknowledge every doctor and facility that gave us their time and let us visit.

Refrences

1.Färkkilä, A., Koskela, S., Bryk, S., Alfthan, H., Bützow, R., Leminen, A., . . . Unkila-Kallio, L. (2015). The clinical utility of serum anti-Müllerian hormone in the follow-up of ovarian adult-type granulosa cell tumors-A comparative study with inhibin B. *International Journal of Cancer, 137*(7), 1661-1671.
2. Geerts, I., Vergote, I., Neven, P., & Billen, J. (2009). The Role of Inhibins B and Antimüllerian Hormone for Diagnosis and Follow-up of Granulosa Cell Tumors. *International Journal of Gynecological Cancer, 19*(5), 847-855.
3.Haltia, U., Hallamaa, M., Tapper, J., Hynninen, J., Alfthan, H., Kalra, B., . . . Färkkilä, A. (2017). Roles of human epididymis protein 4, carbohydrate antigen 125, inhibin B and anti-Müllerian hormone in the differential diagnosis and follow-up of ovarian granulosa cell tumors. *Gynecologic Oncology, 144*(1), 83-89.
4.Jobling, T., Mamers, P., Healy, D. L., Maclachlan, V., Burger, H. G., Quinn, M., . . . Day, A. J. (1994). A Prospective Study of Inhibin in Granulosa Cell Tumors of the Ovary. *Gynecologic Oncology, 55*(2), 285-289.
5.Lappohn, R., Burger, H., Bouma, J., Bangah, M., Krans, M., & Bruijn, H. D. (1990). Inhibin as a marker for granulosa-cell tumors. *International Journal of Gynecology & Obstetrics, 31*(4), 384-385.

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The Effects of Embryonic Stem Cells on Ovarian Cancer

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Objective

Each year, more than 22,000 cases of ovarian cancer occurs. There is no cure, however there has been recent studies in developing human embryonic stem cells (hESCs) to inhibit the growth of tumors. The purpose of this poster is to demonstrate the data and research that shows how hESCs can prolong the growth of tumors and survivability in mice and rats. This poster will also show similarities between cancer and embryonic cells and how this discovery can change treatments of cancer.

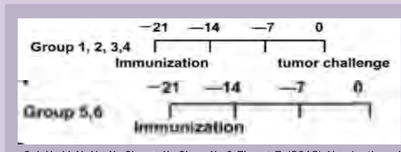
Abstract

The leading cause of death in gynecologic malignancy is ovarian cancer. Most women that are diagnosed with ovarian cancer are 55-64 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 after giving them ovarian cancer that was derived from murine cells. In one case study, mice and rats were used. The mice were vaccinated with H9 cells (hESCs), IVP-ES1 (mouse ESCs), ID8 cells, or phosphate-buffered saline (PBS) then inoculated with viable ID8 cells, mouse ovarian surface epithelium that resembles human epithelial ovarian cancer. The rats were vaccinated with H9 cells, NuTu-19 cells, or PBS then inoculated with viable NuTu-19 cells, rat ovarian cancer cell. A similar study was done with just rats. Controlled studies were done to compare the data and results had shown tumor antigen expression of nm23, p53, C-myc, and HER-2 in both animals. In the H9 vaccinated rat models, tumor growth and metastasis were prolonged compared to the controlled groups (no duration of time was provided) and fewer amounts of tumors were found. Tumor antigens, markers, and genes are expressed in embryonic stem cells presenting a possible relationship between the two. These studies support the hypothesis that oncofetal antigens are expressed in 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). They compared hESC line H9, mESC line IVP-ES1, and ID8 cells in the mice while comparing NuTu-19 cells and H9 in the rats. 56 mice were divided into six groups evenly. Groups 1 - 4 received vaccinations subcutaneously of IVP-ES1 ($5x10^6$) or H9 ($5x10^6$) or ID8 ($5x10^6$) phosphate-buffered saline (PBS). Each week, all were injected three times and live ID8 cells were inoculated one week after the final vaccination. Groups 5 and 6 received pre-irradiated H9 ($5x10^6$) or IVP-ES1 ($5x10^6$) injections, three times at one week intervals, two weeks after the final vaccination orbital venous blood was taken. 24 rats were divided evenly into four groups. Groups R1 - R3 received injections of pre-inactivated H9 ($1x10^7$) or NuTu-19 ($1x10^7$) or PBS, three times each week, and alive NuTu-19 cells ($1x10^7$) were inoculated one week after the final vaccination. Group R4 only received pre-irradiated H9 ($1x10^7$), repeated six times each week intervals, blood tests were done after the final vaccination.

The second study used 344 Fischer female rats (100-125 g). 42 rats were divided evenly into seven groups. Group 1 vaccinated with pre-irradiated hESCs ($1x10^7$), three times each week and inoculated one week after final vaccination with NuTu-19 cells. Group 2 vaccinated with pre-inactivated mitotic NuTu019 ($1x10^7$), three times each week and NuTu-19 cells ($1x10^6$) one week after the final vaccination. Group 3 vaccinated with PBS (100 ul) three times each week and one week after the final vaccination, NuTu-19 cells ($1x10^6$) were inoculated. Group 4 vaccinated with pre-irradiated H9 ($1x10^7$) three times each week and 4 weeks after the final vaccination, NuTu-19 cells ($1x10^6$) were inoculated. Group 5 vaccinated with pre-inactivated mitotic NuTu-19 ($1x10^7$) 3 times each week and NuTu-19 cells were injected 4 weeks after the final vaccination. Group 6 vaccinated with PBS (100 ul) 3 times each week and NuTu-19 cells ($1x10^6$) were inoculated 4 weeks after the final vaccination. Group 7 vaccinated pre-irradiated H9 ($1x10^7$) 6 times each week. Blood tests, for all, were done before inoculation.



Cui, H., Li, Y., Ye, X., Chang, X., Chen, X., & Zhang, Z. (2012). Vaccination with embryonic stem cells generates effective antitumor immunity against ovarian cancer. *International Journal of Molecular Medicine, 31*(1), 147-153.

Figure 1. For the mice, vaccinations of H9, IVP-ES1, and PBS were received three times each week for Groups 1-4. Groups 5-6 received vaccinations three times a week of H9 and IVP-ES1. [1]

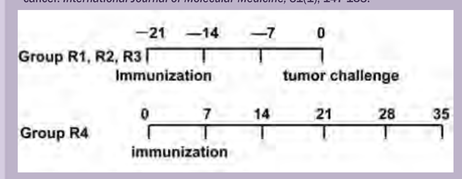
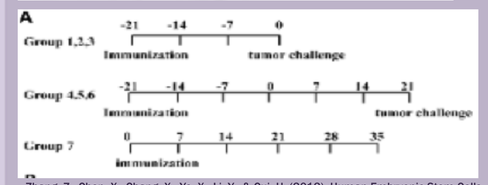


Figure 2. For the rats, Groups R1-R3 received inactivated H9, NuTu-19, and PBS three times each week. Group R4 received H9 six times a week. [1]



Zhang, Z., Chen, X., Chang, X., Ye, X., Li, Y., & Cui, H. (2012). Human Embryonic Stem Cells - a Potential Vaccine for Ovarian Cancer. *Asian Pacific Journal of Cancer Prevention, 13*(9), 4295-4300.

Figure 3. H9 immunizations and tumor inoculation for each group. Groups 1 and 4 vaccinated with H9. Groups 2 and 5 with pre-inactivated mitotic NuTu-19. Groups 3 and 6 with PBS. Group 7 with pre-irradiated 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 a longer survival time compared to the controlled groups. In Group R1, only one tumor >0.5mm was found and metastatic lesions were found in the diaphragm, peritoneal wall, intestine, mesentery and omentum. In the other groups, the lesions had spread to the kidney, liver, and lung with most tumors >0.5mm. Researchers had also found Th1/Th2/Th17 cytokine levels increased in the H9 and IVP-ES1 vaccinated groups. Some tumor markers were found in the H9 cells, such as HER-2, C-myc, p53, nm23, and PTEN. All blood levels were relatively normal except for four cases.

In the second study, similar results as the study above proved that H9 cells helped elongate the rat's survival and inhibited the tumors from metastasizing. 34kDa, 42kDa, 52kDa, and 80kDa molecules were discovered by H9-immunized rats and it was detected in NuTu-19 ovarian cancer cells, suggesting that an antitumor antibody response was produced. Both studies did not show significant side effects, except for those vaccinated with ID8 and NuTu-19 cells (the cancer cells).

Metastatic organs	Group R1 (H9) Diaphragm, peritoneal wall, intestine, mesentery, omentum	Group R2 (NuTu-19) Diaphragm, peritoneal wall, intestine, mesentery, omentum	Group R3 (PBS) Diaphragm, peritoneal wall, intestine, mesentery, omentum, kidney, liver surface and parenchyma lung
Liver and parenchyma metastasis	0% (0/6)	16.7% (1/6)	33.3% (2/6)
Lung metastasis	0% (0/6)	33.3% (2/6)	50.0% (3/6)
Metastatic tumor size >0.5 mm3	16.7% (1/6)	66.7% (4/6)	83.3% (5/6)

Cui, H., Li, Y., Ye, X., Chang, X., Chen, X., & Zhang, Z. (2012). Vaccination with embryonic stem cells generates effective antitumor immunity against ovarian cancer. *International Journal of Molecular Medicine, 31*(1), 147-153.

Figure 4. Results for first study.[1]

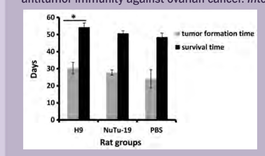


Figure 5. Data showing the tumor formation time and survival time of each vaccinations on the rats.[1]

Group	Group1,4 (H9)	Group2,5 (MMC-NuTu-19)	Group3,6 (PBS)
Vaccine route	S.C	S.C	S.C
Vaccine interval	Per week, 3 times	Per week, 3 times	Per week, 3 times
Tumor cells injection route	i.p	i.p	i.p
NO. rat	12	12	12
Metastatic organs	Diaphragm, peritoneal wall, intestine, mesentery, and omentum	Diaphragm, peritoneal wall, intestine, mesentery, omentum, kidney, liver surface and parenchyma, lung	Diaphragm, peritoneal wall, intestine, mesentery, omentum, kidney, liver surface and parenchyma, lung
Liver parenchyma metastasis (%)	0% (0/12)	16.7% (2/12)	33.3% (4/12)
Lung metastasis (%)	0% (0/12)	33.3% (4/12)	50.0% (6/12)
Metastatic tumor size >0.5mm3 (%)	25.0% (3/12)	75.0% (9/12)	83.3%(10/12)

Zhang, Z., Chen, X., Chang, X., Ye, X., Li, Y., & Cui, H. (2012). Human Embryonic Stem Cells - a Potential Vaccine for Ovarian Cancer. *Asian Pacific Journal of Cancer Prevention, 13*(9), 4295-4300.

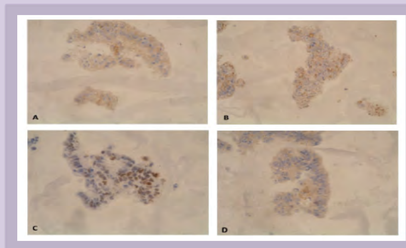
Figure 6. Results for second study.[5]

Conclusions

The data that has been acquired, so far, suggests that hESCs can cause significant effects on tumors found in rats and mice. Studies show that the survivability in the rats vaccinated with H9 was greater than those vaccinated with NuTu-19 and PBS. Studies also showed several genes or markers correlated with tumorigenesis, tumor growth and metastasis in hESCs, such as p53 which has connected to prognosis of ovarian cancer and this raises questions concerning if hESCs can be used for immunotherapy. Could this possible immunotherapy be used for pre-established cancer? Without a clinical trial, no one will ever know if this treatment will be harmful or beneficial to humans. This could potentially be the new and improved way of treating ovarian cancer, possibly leading to more options for other cancers as well.

Applications to Biotechnology

Without immunohistochemical staining, researchers would have never found tumor suppressing genes, oncogenes, 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 prognosis. PTEN, p53, and C-myc are very important to the process of carcinogenesis. Immunohistochemical staining helped scientists come to the conclusion that tumor antigens resonate from the hESCs providing them a foundation in examining the significance of tumor markers.



Zhang, Z., Chen, X., Chang, X., Ye, X., Li, Y., & Cui, H. (2012). Human Embryonic Stem Cells - a Potential Vaccine for Ovarian Cancer. *Asian Pacific Journal of Cancer Prevention, 13*(9), 4295-4300.

Figure 7. Immunohistochemical staining of tissue, showing expression of each protein. **(A) nm23, (B) C-myc, (C) p53, (D) PTEN.** [5]

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I would like to extend my greatest thanks to Dr. Ericka, for her continuous support and wonderful insight. I would like to thank Dr. Tracy and Dr. Kina for their 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

1. Cui, H., Li, Y., Ye, X., Chang, X., Chen, X., & Zhang, Z. (2012). Vaccination with embryonic stem cells generates effective antitumor immunity against ovarian cancer. *International Journal of Molecular Medicine, 31*(1), 147-153.
2. Dong, W., Du, J., Shen, H., Gao, D., Li, Z., Wang, G., . . . Liu, Q. (2010). Administration of embryonic stem cells generates effective antitumor immunity in mice with minor and heavy tumor load. *Cancer Immunology, Immunotherapy, 59*(11), 1697-1705.
3. R., T., G., D., H., S., & H. (1996). Development and characterization of a clinically useful animal model of epithelial ovarian cancer in the Fischer 344 rat. *American Journal of Obstetrics and Gynecology, 175*(3), 593-599.
4. Schwab, C. L., English, D. P., Roque, D. M., Pasternak, M., & Santin, A. D. (2014). Past, present and future targets for immunotherapy in ovarian cancer. *Immunotherapy, 6*(12), 1279-1293.
5. Zhang, Z., Chen, X., Chang, X., Ye, X., Li, Y., & Cui, H. (2012). Human Embryonic Stem Cells - a Potential Vaccine for Ovarian Cancer. *Asian Pacific Journal of Cancer Prevention, 13*(9), 4295-4300.

Viability of Autotransplantation of the Whole Ovary for Women at risk for POF

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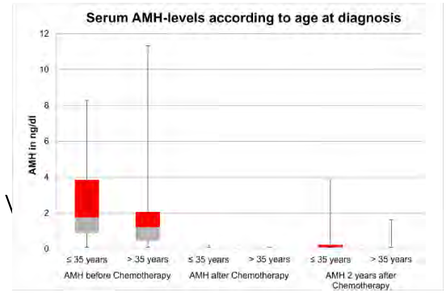
Objective/Background

One fourth of women diagnosed with some form of cancer are of fertile age and may require chemotherapy. The treatment has greatly increased survival rates but it also causes Premature Ovarian Failure or the loss of ovarian function before the age of 40. POF primarily affects women of reproductive age who are diagnosed with cancer. 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.



Serum AMH can be used to measure female fertility. The decline after chemotherapy is visible in this graph

McLaren, J. F., & Bates, W. (2012). Fertility preservation in women of reproductive age with cancer. American Journal of Obstetrics and Gynecology, 207(6), 455-462.[3]

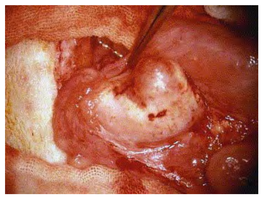
Abstract

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 developing POF. In fact, in one study, 50.6% of chemotherapy patients experienced permanent ovarian failure. If the ovaries were to be taken out before treatment and autotransplanted after treatment, this percentage could potentially go down. In terms of viability, autotransplantation of the whole ovary has not yet occurred in humans; there has, however, been success with similar types of autotransplantation. There have been reports of 26 successful births through autotransplantation of ovarian tissue. Subsequently, there has been success in animal studies with the procedure. In a foreign study, 4 out of 9 sheep regained luteal function and one of the four sheep was able to conceive spontaneously after the procedure. In addition, there has been a successful case in which a monozygotic twin donated her ovary to her sister who was eventually able to reproduce. These cases show that the viability and restorative potential of whole ovary autotransplantation.

Methods and Materials

Study 1: Since autotransplantation of the whole ovary is still in the experimental phase, there has not been any human trials. There has however been 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.

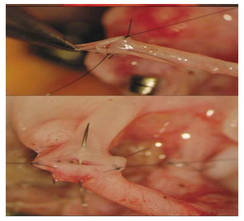
Study 2: Although no human trials have occurred, there have been several animal trials. In a study done at the General Hospital of Vienna, Austria, nine ewes, six months of age underwent a laparotomy unilateral oophorectomy. Afterward, the ovaries were transferred to a freezer and stored at -196˚ C in 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.



The ovary of sheep no. 8 at final explantation, exposing a mature follicle three months after regrafting.

Imhof, M., Bergmeister, H., Lipovac, M., Rudas, M., Hofstetter, G., & Huber, J. (2006). Orthotopic microvascular reanastomosis of whole cryopreserved ovine ovaries resulting in pregnancy and live birth. Fertility and Sterility, 85, 1208-1215.[1]

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.



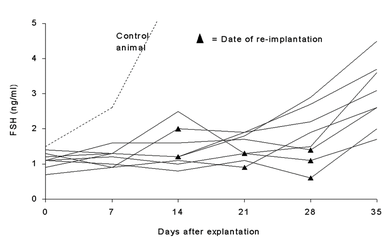
The donor's ovarian artery and ovarian vein are anastomosed to the recipient's ovarian artery and vein.

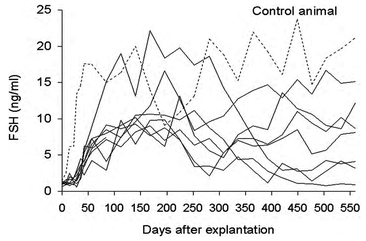
Silber, S. J., Grudzinskas, G., & Gosden, R. G. (2008). Successful Pregnancy after Microsurgical Transplantation of an Intact Ovary. New England Journal of Medicine, 359(24), 2617-2618. [6]

Results and Interpretations

Study 1: Across 13 different studies, there here 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 ischemia 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.





Imhof, M., Bergmeister, H., Lipovac, M., Rudas, M., Hofstetter, G., & Huber, J. (2006). Orthotopic microvascular reanastomosis of whole cryopreserved ovine ovaries resulting in pregnancy and live birth. Fertility and Sterility, 85, 1208-1215.[1]

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.

Acknowledgements

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

1. Imhof, M., Bergmeister, H., Lipovac, M., Rudas, M., Hofstetter, G., & Huber, J. (2006). Orthotopic microvascular reanastomosis of whole cryopreserved ovine ovaries resulting in pregnancy and live birth. Fertility and Sterility, 85, 1208-1215.
2. Macklon, K. T., Jensen, A. K., Loft, A., Ernst, E., & Andersen, C. Y. (2014). Treatment history and outcome of 24 deliveries worldwide after autotransplantation of cryopreserved ovarian tissue, including two new Danish deliveries years after autotransplantation. Journal of Assisted Reproduction and Genetics, 31(11), 1557-1564.
3. McLaren, J. F., & Bates, W. (2012). Fertility preservation in women of reproductive age with cancer. American Journal of Obstetrics and Gynecology, 207(6), 455-462.
4. Rosendahl, M., Wielenga, V. T., Nedergaard, L., Kristensen, S. G., Ernst, E., Rasmussen, P. E., . . . Andersen, C. Y. (2011). Cryopreservation of ovarian tissue for fertility preservation: no evidence of malignant cell contamination in ovarian tissue from patients with breast cancer. Fertility and Sterility, 95(6), 2158-2161.
5. Salama, M., & Woodruff, T. K. (2015). New advances in ovarian autotransplantation to restore fertility in cancer patients. Cancer and Metastasis Reviews, 34(4), 807-822.
6. Silber, S. J., Grudzinskas, G., & Gosden, R. G. (2008). Successful Pregnancy after Microsurgical Transplantation of an Intact Ovary. New England Journal of Medicine, 359(24), 2617-2618.

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Restoring Fertility in Cancer Survivors with Biosynthetic Ovary Implants

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UC San Diego Health Sciences

the Oncofertility™ Consortium
Saturday Academy

Background

Reproductive-aged female cancer patients may be left infertile or with reduced ovary function as a result of gonadotoxic treatment.[3] However, as the field of oncofertility advances, there are now options for young women with cancer to preserve their fertility. Upon diagnosis, patients can cryopreserve oocytes or embryos.[3] Unfortunately, these are not practical options 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-tranplantation.[5] Thus, there is still an unmet need for fertility preservation in cancer patients requiring immediate treatment. This poster will demonstrate the viability 3D-printed biosynthetic ovaries as a novel therapy for this target group.

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.[1] The goal of this study is to show that 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 immature murine follicles. The researchers observed ovulation of fully mature eggs and steroidogenesis *in vitro*. When the grafts were implanted into previously ovariectomized mice for *in vivo* study, orderly folliculogenesis was proven by the presence of primordial follicles; primary, secondary, and antral follicles; and corpora lutea. The researchers also examined the fertility of the mice with ovary implants through natural mating. Three out of seven mice with bioprosthetic ovaries yielded live births, while zero out of two mice with sham controls had pups. All pups from these matings fed from their lactating mothers, demonstrating hormonal restoration. The porosity of the implant allowed for sufficient nutrient diffusion and revascularization.[4] Moving forward, further refining the pore geometry can optimize implant function, as another study on cardiac cell scaffolds has demonstrated.[2] Future 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.

Application to Biotechnology

3D ink preparation: The 3D ovary scaffold was printed with gelatin ink, since gelatin is derived from collagen, a protein that is naturally found in human and murine ovaries, and has cell adhesion sites for the follicles to bind to. When the gel was printed a liquid state (>33˚C) the filaments would spread, resulting in poor layer resolution, and when the gel was printed in a fully crosslinked state (<25˚C), the filaments were clumpy, resulting in inconsistent pore size. The ideal gelatin ink temperature was 30˚C, which produced a partially-crosslinked gel solid enough to hold its shape but soft enough to be ejected smoothly from the nozzle.

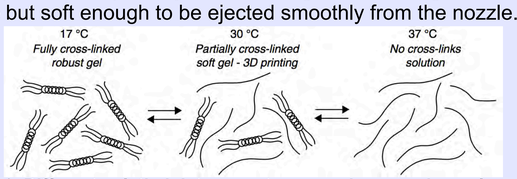

Figure 1: Different gelatin ink temperatures produce varying gel properties. *Retrieved from Laronda, M. M., et. al. (2017).[4]*

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

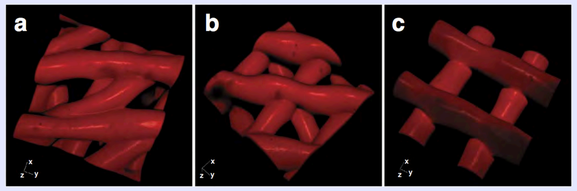

Figure 2: 3D-printed scaffolds with pore geometry of 30˚ (a), 60˚ (b), and 90˚ (c). *Retrieved from Laronda, M. M., et. al. (2017).[4]*

Materials and Methods

After the scaffolds were printed, the researchers performed three experiments summarized in the table below to examine their function. 2-3 mm biopsy punches were taken from the printed scaffolds for these studies. *Note: OVX = ovariectomized*

Experiment	Follicle #, size	Scaffold size, angle	Duration	#, type of mice
1. *In vitro*: test pore geometry on survival and *in vitro* maturation	3-4 150-180 µm per scaffold	2-3 mm, 30˚, 60˚, 90˚	2-8 days	N/A
2. *In vivo*: test vascularization and hormone restoration	40-50 small follicles (up to 180 µm)	2 mm (to fit in bursa), 60˚	1 or 3 weeks post-surgery	16 OVX + implant, 5 OVX
3. *In vivo*: test restorative ovary function	40-50 small follicles (up to 180 µm)	2 mm (to fit in bursa), 60˚	8-10 weeks post-surgery	7 OVX + implant, 2 OVX

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

Experiment 1: Scaffold biopsy punches were seeded with three or four 150-180 µm follicles. Two scaffolds per geometry (30˚, 60˚, 90˚) with four follicles each (24 follicles total) were analyzed with light microscopy and confocal fluorescence microscopy to measure follicle survival. The researchers also tested for steroidogenesis, which occurs within the theca and granulosa cells of ovarian follicles and produces hormones necessary for reproduction. In theca cells they stained for 3-β hydroxysteroid dehydrogenase (3βHSD), an enzyme involved in the steroid hormone pathway, and in spent growth media they measured levels of estradiol, a hormone produced by steroidogenesis.

Experiment 2: 60˚ scaffolds (2 mm diamter, 1.5 mm thick) were seeded with 40-50 small follicles (primordial, primary, small secondary up to 180 µm) isolated from green fluorescent protein positive (GFP+) mice and cultured four days *in vitro* to allow follicles to form cell-matrix interactions. These bioprosthetic ovaries were then implanted into 16 adult ovariectomized mice. Sham scaffolds that did not contain any follicles were implanted into five ovariectomized control mice.

Experiment 3: Seven ovariectomized mice with bioprosthetic ovaries and two ovariectomized mice with sham controls from the previous experiment were examined for fertility. These nine mice were mated with males who had previously sired pups (When surgically removing the ovaries, the reproductive tract was left intact to test fertility through natural mating).

Results

Experiment 1: Follicles in 90˚ scaffolds spread along struts, and by day 8 of culture about half died due to dissociation of granulosa cells from the oocyte. Follicles in 30˚ and 60˚ scaffolds had significantly higher survival (*see table below*).

Pore geometry	Follicle survival
30˚	78.57±3.57%
60˚	75.89±4.04%
90˚	48.47±8.31%

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

Follicles in 30˚ and 60˚ scaffolds had a greater 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.

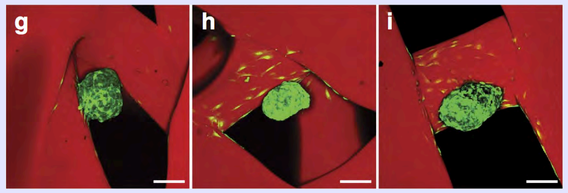

Figure 5: Follicles contacting struts in 30˚ (g), 60˚ (h), and 90˚ (i) scaffolds. *Retrieved from Laronda, M. M., et. al. (2017).[4]*

Further results only pertain to 30˚ and 60˚ scaffolds: Staining revealed 3βHSD activity in theca cells. Estradiol levels in spent growth media also increased from day 2 (1.26±0.36 pg/ml) to day 8 (255.19±114.21 pg/ml). Follicles ovulated fully mature MII eggs with normal polar bodies through scaffold pores.

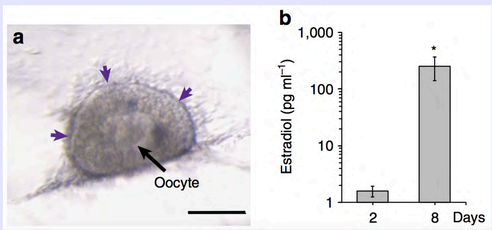

Figure 6: (a) Purple stain indicates 3βHSD activity. (b) Increased levels of estradiol in spent growth media from day 2 to day 8. *Retrieved from Laronda, M. M., et. al. (2017).[4]*

Experiment 2: The bioprosthetic ovaries became vascularized within one week of implantation, and the distribution and density of vessels were comparable to those in natural ovaries. Three weeks post-surgery, orderly folliculogenesis was demonstrated by the presence of primordial follicles; primary, secondary, and antral follicles; and corpora lutea. Anti-Müllerian hormone (AMH) and inhibin A, which are secreted by growing follicles, were detected at increased levels in the serum of mice with bioprosthetic ovaries (AMH, 1.51±0.34 ng/ml, inhibin A 32.31±11.33 pg/ml) compared to little or undetectable levels in sham controls.

Experiment 3: Three of seven mice with bioprosthetic ovaries had litters of one or two pups each, while zero of two mice with sham controls had pups. All pups from these matings fed from their lactating mothers, indicating the mothers' corpora lutea were producing enough progesterone to trigger prolactin and produce milk. After the pups grew to adulthood, they too sired or gave birth to their own litters.

Conclusion

These 3D-printed ovary scaffolds supported hormone function, oocyte maturation, and ovulation *in vitro* and *in vivo*, and live birth *in vivo,* demonstrating their potential in restoring fertility and endocrine function in cancer patients.[4] Moving forward, further refining of pore geometry can optimize implant function, as another study on cardiac cell scaffolds has demonstrated.[2] Manipulating stiffness and pore size for different compartments of the scaffold can create an implant that more closely resembles native ovary tissue, which is necessary for transplant longevity.[4] Future 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 of this technology 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 reintroducing malignant cells with ovarian tissue transplantation.[3,6] This technology has profound implications on the future of oncofertility and 3D-printed tissue engineering.

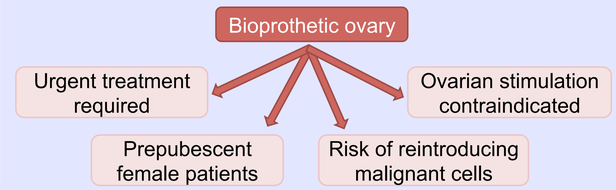

Figure 7: Potential applications of 3D-printed bioprosthetic ovaries

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A big thanks to Dr. Ericka Senegar-Mitchell for her unstoppable motivation and genuine passion for science and sisterhood; Dr. Kina Thackray for helping me review my research topic; Dr. Jeffrey Chang, Dr. Irene Su, and all of our other amazing guest lecturers for sharing their knowledge with us; and Ms. Patricia Winter for organizing the program. Of course, thanks to my family for supporting me along the way, and my ROSA sisters for an amazing summer and beyond.

References

1. Cancer Stat Facts. (n.d.). Retrieved August 03, 2017, from https://seer.cancer.gov/statfacts/
2. Engelmayr, G. C., Jr., Cheng, M., Bettinger, C. J., Borenstein, J. T., Langer, R., & Freed, L. E. (2008). Accordion-Like Honeycombs for Tissue Engineering of Cardiac Anisotropy. *Nature Materials,7*(12), 1003-1010.
3. Jeruss, J. S., & Woodruff, T. K. (2009). Preservation of Fertility in Patients with Cancer. *The New England Journal of Medicine,360*(9), 902-911.
4. Laronda, M. M., Rutz, A. L., Xiao, S., Whelan, K. A., Duncan, F. E., Roth, E. W.,…Shah, R. N. (2017). A bioprosthetic ovary created using 3D printed microporous scaffolds restores ovarian function in sterilized mice. *Nature Communications,8*, 15261.
5. Liu, J., Elst, J. V., Broecke, R. V., & Dhont, M. (2002). Early massive follicle loss and apoptosis in heterotopically grafted newborn mouse ovaries. *Human Reproduction,17*(3), 605-611.
6. Vanacker, J., Luyckx, V., Dolmans, M., Rieux, A. D., Jaeger, J., Langendonckt, A. V.,…Amorim, C. A. (2012). Transplantation of an alginate–matrigel matrix containing isolated ovarian cells: First step in developing a biodegradable scaffold to transplant isolated preantral follicles and ovarian cells. *Biomaterials, 33*(26), 6079-6085.

The Potential for Avian Newcastle Disease Virus (NDV) As an Additional Treatment For Cancer

Sathya Krishnasamy – Canyon Crest Academy - SDUHSD

Background

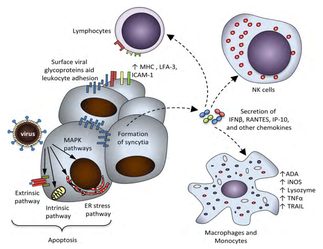
Cancer is a disease that is growing in prevalence, with the National Cancer Institute estimating that 38.5% of people will get cancer. Most individuals undergo chemotherapy, however, there is a chance of the cancer gaining drug resistance. Drug resistance may cause a relapse or neutralization of the treatment. Oncolytic virotherapy provides an alternative to chemotherapy that could be more effective in the treatment of solid tumors, especially for individuals with drug resistance.

The concept of immuno virotherapy for cancer was first conceived when it was observed that tumor regression occurred when an individual was infected. Studies attempted to use known human viruses to induce the regression of tumors. While this was successful, it ended up giving rabies to the individuals treated with this early form of oncolytic virotherapy.

The Avian Newcastle Disease Virus (NDV) is the ideal candidate for oncolytic virotherapy as it has a low level of cytotoxicity within mammalian cells. NDV is naturally occurring and can be obtained in the wild and has been well studied

The goal of oncolytic virotherapy is to cause lysis of the tumor cells. This poster will examine the mechanism and success of using NDV oncolytic virotherapy.

Figure 1
This figure shows the intrinsic and extrinsic pathways that NDV can take to lead to apoptosis. The direct mechanism causes stress to the ER while the indirect method attracts NK cells
Zamarin, D., & Palese, P. (2012). Oncolytic Newcastle disease virus for cancer therapy: old challenges and new directions. *Future Microbiology, 7*(3), 347-367. [6]



Abstract

One of the major challenges of chemotherapy is the chance of cancerous cells gaining drug resistance after a patient's initial exposure. Drug resistance may cause a relapse or neutralization of the treatment. Oncolytic virotherapy provides an alternative to chemotherapy that could be more effective in the treatment of solid tumors, especially for individuals with drug resistance. Furthermore, the use of Avian Newcastle Disease Virus (NDV) in the treatment of solid tumors could increase life expectancy for cancer patients. 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 intravenous form of NDV that is used for human stem cell lines and treatment of humans in clinical trials. Studies were conducted in human glioblastoma cells, non-small cell lung cancer, melanomas, and HeLa stem cell lines, as well as pc12 stem cell lines in rats using MTH-68 and P73. Studies show that NDV does not discriminate on the p53 gene, a tumor suppressor, and induces programmed cell death (apoptosis) in pc12 and HeLa cervical cells. NDV treatment has induced the regression of tumors by 50-90% both in vivo and in vitro and has increased life expectancy, dependent on the cancer and stage. The quantity and concentration of NDV also seems to play a significant role with the MOI for different cancers ranging from 1:1 to 100+:1. Bcl-xl, an antiapoptotic protein that aids tumor growth, initially was unresponsive to NDV, but after increasing the amount of the virus administered, apoptosis began to occur in correlation with the increased concentration of NDV. NDV treatments have been shown to be successful in increasing life expectancy when compared to chemotherapy, which suggests NDV may be a viable alternative treatment for cancer in the future.

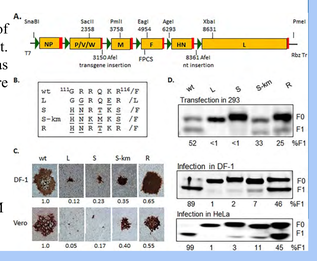
Methods and Materials

Recombinant forms of NDV from embryonated eggs or infected poultry were used to generate two forms of NDV, MTH-68 and P73 in the laboratory. In two cases, these strains were purchased from labs in the US and Europe. Research has largely used MTH-68, an intravenous form of NDV that is used for human stem cell lines and treatment of humans in clinical trials. Studies were conducted in human glioblastoma cells, non-small cell lung cancer, melanomas, astrocytes, and HeLa stem cell lines, as well as pc12 stem cell lines in rats using MTH-68 and P73.

The experiments were set up by first getting access to either MTH-68 or P73and the antigenome of the virus was constructed using fragments of subgenomic cDNA.

For in vitro studies, the cell cultures were washed with 1 mL phosphate buffered saline solutions and collected by centrifuge at 1500 rpm for five to ten minutes at four degrees Celsius. These were flash frozen until the virus was prepared. They were then placed on well plates 24 hours prior to infection. Immunoblot analysis using antibodies against the proteins was performed according to established protocols. A bioassay was used to detect IFN (interferon 1) induction. Small interfering RNA (siRNA) targets were identified using a selection server. For in vivo studies, the virus was prepared and then given to the in vivo studied subjects via IV or inhalation form.

Figure 2
This figure shows the generation of recombinant NDV 73T and other forms of it. Viral gene cassettes including start and end as well as cDNA assembly and insertions are included.
Cheng, X., Wang, W., Xu, Q., Harper, J., Carroll, D., Galinski, M. S., . . . Jin, H. (2016, June 01). Genetic Modification of Oncolytic Newcastle Disease Virus for Cancer Therapy. Retrieved August 02, 2017, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4934751/ [1]



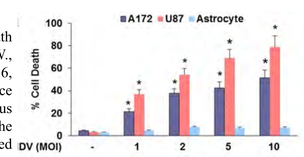
Results

These studies show that NDV can cause regression of the tumor or at least inhibit tumor growth. This is critical as the direct mechanism causes tumor regression without the immune system.

MTH-68 was able to kill a variety of types of transformed cancerous cells. Studies show that NDV does not discriminate on the p53 gene, a tumor suppressor. NDV destroys both types of cancerous cells that were both overexpressing p53 and those that were depleted. It also induces apoptosis in pc12 and HeLa cervical cells, where viral replication also occurred. All the in vitro targeted cells were destroyed in this treatment, the human glioblastoma cells and the HeLa cells. However the NDV treatment only induced regression of the pc12 cells in the rats, which was a negative result as it could not lyse all of it. Endoplasmic reticulum stress was noted, indicating that NDV caused tumor regression via the direct pathway.

With the glioma cell lines, a current study found conflicting information with a small clinical trial conducted in 2003. This found that NDV, working in concert with stem cells was not able to eliminate the tumor. While it did cause regression, there was a minimal amount of cell death that occurred without stem cells.

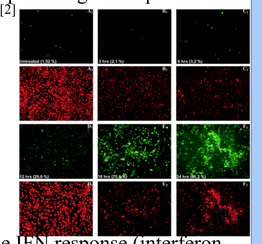
Figure 3
This figure shows the percentage of cell death relative to the MOI . Kazimirsky, G., Jiang, W., Slavin, S., Ziv-Av, A., & Brodie, C. (2016, October 10). Mesenchymal stem cells enhance the oncolytic effect of Newcastle disease virus in glioma cells and glioma stem cells via the secretion of TRAIL. Retrieved August02,2017,from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5057491 / [4]



This could be due to the use of neurospheres as this research was done in vitro, but it supports the general conclusion that an oncolytic treatment could not work on its own.

Another major factor was the amount of NDV used. The quantity and concentration of NDV also seems to play a significant role with the MOI for different cancers ranging from 1:1 to 100+:1. Bcl-xl, an antiapoptotic protein that aids tumor growth, initially was unresponsive to NDV, but after increasing the amount of the virus administered, apoptosis began to occur in correlation with the increased concentration of NDV. NDV overcame resistance to apoptosis in Bcl-xl overexpressing cells. [5] Caspase 3 activity was measured and there was a noted significant increase in activity that correlates with a higher number of cells being infected and following cycles of NDV replication. The susceptible cancer cell types represent a wide range of cancer cells from various origins in the body, indicating that the MTH-68/H strain of NDV utilizes a similar molecular mechanism to kill all of the different tumor cells. However, substantial differences in the sensitivities of tumor cell lines toward MTH-68/H were found, emphasizing the importance of the genetic background of cancer cells. [2]

Figure 4
The figure shows dying HeLa cells treated with MTH-68 during a 24 hour interval. Fabian, Z., Csatary, C. M., Szeberenyi, J., & Csatary, L. K. (2007). P53-Independent Endoplasmic Reticulum Stress-Mediated Cytotoxicity of a Newcastle Disease Virus Strain in Tumor Cell Lines. *Journal of Virology, 81*(6), 2817-2830.[2]



Most importantly, the NDV activated the IFN response (interferon 1), indicating lysis by an indirect mechanism. NDV infected tumor cells stimulated NK cells that produced the lymphokines gamma interferon and tumor necrosis factor alpha which also helped the antitumor effects brought about by NDV. This caused tumor regression even in the presence of antibodies.

Conclusion

There are many factors and mechanisms that allows NDV to induce lysis that are not fully understood such as how tumor regression continues with the presence of antibodies to NDV.

NDV treatments have been shown to be successful in inducing apoptosis for both in vitro studies on tumor cells and in vivo studies. This has occurred by causing tumor growth inhibition, thus increasing life expectancy of the rats when compared to chemotherapy alone, which suggests NDV may be a viable additional treatment for cancer in the future. While some of the results have not been as positive as was expected such as the study with astrocytes, genetic modification and the addition of stem cells and secretion of TRAIL better targeted glioblastomas and astrocytes while reducing the cytotoxicity that NDV could have on healthy cells. NDV may not be able to cause full tumor regression on its own, but working in concert with chemotherapy or radiation, an oncolytic treatment could lengthen the expected life span of an individual.

Using oncolytic virotherapy could be a powerful tool when treating cancers such as glioblastomas, where the life expectancy is about 14 months with traditional forms of treatment In the future, Oncolytic virotherapy also has the potential of reducing the risk to fertility for both men and women by using a lower dosage of chemotherapy drugs in concert with NDV. At present, more research is required to examine the exact mechanics behind tumor cells lysis in order to ensure that cytotoxicity is low and increase NDV's effectiveness in treating solid tumors.

Relevant Applications to Biotechnology

While oncolytic virotherapy is a new biotechnology in itself, major new improvements to existing technologies has 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 cDNA to the needs of cancer patients is an immense 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 creating 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 tumors and carry out in vitro experiments and in vivo experiments of human tumors xenografted into animals. Technologies to modify the outer structure of NDV and change human insertion has powerful implications for making cytotoxicity nonexistent when treating solid tumors with NDV. There is also potential for new models to study in. It has been proposed that the neurospheres 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.

Acknowledgements

I would like to thank Dr. Senegar-Mitchell for all her help, guidance, and support for the duration of this program, Dr. Tracy for her knowledge, feedback, and advice, and Ms. Winter for organizing this program. I would also like to acknowledge Dr. Chang and Dr. Su as well as all the other doctors, professors, and presenters who took the time to talk to us and share with us amazing knowledge and experiences. I would like to thank my family for their continuous support and my ROSA sisters for making this summer an incredible experience.

References

1. Cheng, X., Wang, W., Xu, Q., Harper, J., Carroll, D., Galinski, M. S., . . . Jin, H. (2016, June 01). Genetic Modification of Oncolytic Newcastle Disease Virus for Cancer Therapy. Retrieved August 02, 2017, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4934751/
2. Fabian, Z., Csatary, C. M., Szeberenyi, J., & Csatary, L. K. (2007). P53-Independent Endoplasmic Reticulum Stress-Mediated Cytotoxicity of a Newcastle Disease Virus Strain in Tumor Cell Lines. *Journal of Virology, 81*(6), 2817-2830.
3. Jarahian1, M., Watzl6, C., Fournier3, P., Arnold3, A., Djandji2, D., Zahedi3, S., . . . And, V. S. (2009, August 01). Activation of Natural Killer Cells by Newcastle Disease Virus Hemagglutinin-Neuraminidase. Retrieved August 02, 2017, from http://jvi.asm.org/content/83/16/8108.full
4. Kazimirsky, G., Jiang, W., Slavin, S., Ziv-Av, A., & Brodie, C. (2016, October 10). Mesenchymal stem cells enhance the oncolytic effect of Newcastle disease virus in glioma cells and glioma stem cells via the secretion of TRAIL. Retrieved August 02, 2017, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5057491/
5. Mansour, M., Palese, P., & Zamarin, D. (2011). Oncolytic Specificity of Newcastle Disease Virus Is Mediated by Selectivity for Apoptosis-Resistant Cells. *Journal of Virology, 85*(12), 6015-6023.
6. Zamarin, D., & Palese, P. (2012). Oncolytic Newcastle disease virus for cancer therapy: old challenges and new directions. *Future Microbiology, 7*(3), 347-367.

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Genetic Abnormalities Linked to Premature Ovarian Failure

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Coronado High School

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 amenorrhea, sterility, and menopausal symptoms, such as urogenital syndrome 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 chromosomal anomalies 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.

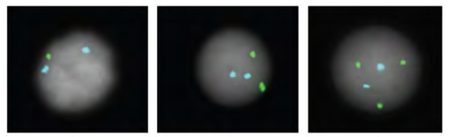
Materials and Methods

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-banding 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 homozygote and a true premutation.

Further studies were carried out by other parties to pinpoint BMP15 and PGRMC1 as genes associated with the disease. Polymerase chain reaction (PCR) fragments of the BMP15 gene were coded for in 166 unrelated Caucasian women with idiopathic POF. 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.

Figure 1. FISH analysis on interphase nuclei. FISH was performed using alpha satellite probes of X (green) and 18 (aqua) chromosomes. The images show the three different categories of signals detected: (a) X monosomy (two 18 signals and one X signal); (b) X disomy and (c) X trisomy.[2]



Baronchelli, S., Conconi, D., Panzeri, E., Bentivegna, A., Redaelli, S., Lissoni, S., . . . Dalprà, L. (2011). Cytogenetics of Premature Ovarian Failure: An Investigation on 269 Affected Women. *Journal of Biomedicine and Biotechnology,2011*, 1-9.

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 abnormality. Logistic regression was used to determine whether clinical information collected at first encounter could predict cytogenetic abnormality, but no single clinical feature was significantly associated with cytogenetic abnormality.

Three groups of women 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 excessive copies of the X chromosome. It was found that 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. Unfortunately, she defaulted from further follow-up studies.

In the separate studies, genetic analysis revealed that 5.42% of POF patients had a genetic mutation on the BMP15 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.

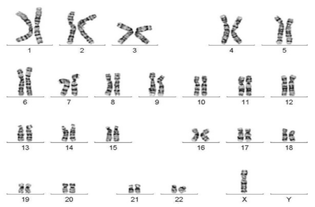


Figure 2. G-banded karyotype showing a cell line with monosomy X. Hemmat, M., Wang, B. T., Warburton, P. E., Yang, X., Boyar, F. Z., Naggar, M. E., & Anguiano, A. (2012). Neocentric X-chromosome in a girl with Turner-like syndrome. *Molecular Cytogenetics,5*(1), 29.

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.

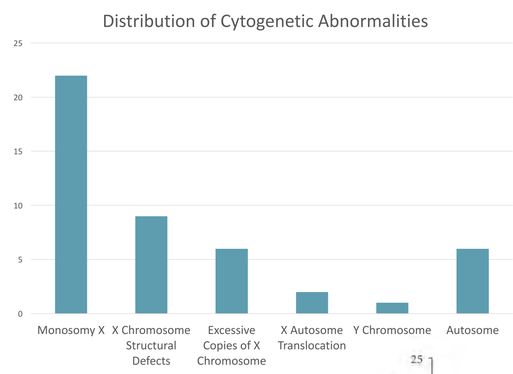
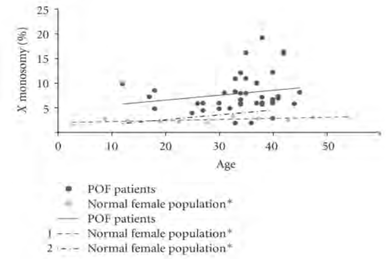


Figure 3. Distribution of cytogenetic abnormalities among the 46 women with an abnormal cytogenetic study. Monosomy X is the most common abnormality, accounting for almost 50% of all abnormalities found. Data from TK, et al.[7]

Figure 4. Percentage of X monosomy related to aging. The graph shows the linear trend of X monosomy both in POF patient group and in two different reference groups described in two reports from the literature: *(1) Guttenbach et al. (1995) [10] and (2) Lakhal et al. (2010) [25].



Baronchelli, S., Conconi, D., Panzeri, E., Bentivegna, A., Redaelli, S., Lissoni, S., . . . Dalprà, L. (2011). Cytogenetics of Premature Ovarian Failure: An Investigation on 269 Affected Women. *Journal of Biomedicine and Biotechnology,2011*, 1-9.

Relevant Applications to Biotechnology

Advancements in biotechnology have helped in genome sequencing and the study of genetics. The Human Genome Project made huge strides in this field, and now CRISPR is drawing attention to the idea of genetic modification. In order for this technology and future technology to be utilized successfully, more research, such as the genetic screening of women with POF, must be done to help characterize genes and solve the mystery behind diseases with unknown etiologies.

Acknowledgements

I would like to thank Dr. Ericka for her mentorship, inspiration, and guidance throughout the program. A special thanks to Sandra Davis and Bill Lemei for their support and help in furthering my education.

References

1. Ayed, W., Amouri, A., Hammami, W., Kilani, O., Turki, Z., Harzallah, F., . . . Slama, C. B. (2014). Cytogenetic abnormalities in Tunisian women with premature ovarian failure. Comptes Rendus Biologies, 337(12), 691-694.

2. Baronchelli, S., Conconi, D., Panzeri, E., Bentivegna, A., Redaelli, S., Lissoni, S., . . . Dalprà, L. (2011). Cytogenetics of Premature Ovarian Failure: An Investigation on 269 Affected Women. *Journal of Biomedicine and Biotechnology,2011*, 1-9.

3. Gersak, K., Franic, D., Veble, A., Pajnic, I. Z., Teran, N., & Writzl, K. (2010). Premature ovarian failure with FMR1 premutation, X chromosome mosaicism and blood lymphocyte microchimerism. Climacteric, 14(2), 289-293.

4. Mansouri, M. R., Schuster, J., Badhai, J., Stattin, E., Lösel, R., Wehling, M., . . . Dahl, N. (2008). Alterations in the expression, structure and function of progesterone receptor membrane component-1 (PGRMC1) in premature ovarian failure. *Human Molecular Genetics, 17*(23), 3776-3783.

5. Pasquale, E. D., Rossetti, R., Marozzi, A., Bodega, B., Borgato, S., Cavallo, L., . . . Persani, L. (2006). Identification of New Variants of HumanBMP15Gene in a Large Cohort of Women with Premature Ovarian Failure. *The Journal of Clinical Endocrinology & Metabolism, 91*(5), 1976-1979.

6. Qin, Y., Jiao, X., Simpson, J. L., & Chen, Z. (2015). Genetics of primary ovarian insufficiency: new developments and opportunities. Human Reproduction Update, 21(6), 787-808.

7. TK, L., IF, L., WK, C., TM, T., & ST, L. (2005). Chromosomal abnormalities and FMR1 gene premutation in Chinese women with premature menopause. Hong Kong Medical Journal, 11(4), 243-250. Retrieved July 22, 2017, from https://www.ncbi.nlm.nih.gov/pubmed/16085940.

Indicator Species Signifying Emasculation in Brazilian Men

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Mater Dei Catholic High School Class of 2018

Objective

This research attempts to address the indication of a significant reduction of reproductive performance occurring in fish that is now seen in humans. Additionally, to raise awareness of the environmental impact estrogen is 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.

Results of the epidemiological studies dealing with observed time trends in Brazilian male fertility disorders show a deterioration of sperm quality, a rise in testicular cancer, and an increase in cryptorchidism. Data is collected from a comparison of sperm count, motility, and morphology of 2300 semen samples from 2000 to 2002 and 2010 to 2012. In Ceara, Brazil, data was collected by water samples analyzing and identifying four estrogenic hormones in five biological wastewater treatment plants, resulting E2 and EE2 both with a 52% occurrence.

Data shows the average sperm concentration/ml decreased significantly from 61.7 million in 2000-2002 to 26.7 million and in 2010-2012, the total sperm concentration decreased significantly from 183.0 million to 82.8 million. 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 redirecting waterways that lead to the ocean onto farms that grow inedible crops because estrogen has a very positive affect on the growth of plants while filtering out the estrogen from the water.

Organisms		Experienced
Pipefish		Feminized male liver
Zebra Fish		Female characteristic in male fish
Fathead Minnows		Productivity of vitellogenin
Cricket frogs		Ovotestis

Figure 1 Examples of organisms who experienced negative effects of endocrine disruptors. These organisms are indicator species.

Methods and Materials

Semen samples were evaluated for sperm count, motility and morphology after liquefaction for 30 minutes. The evaluation of number of sperm and motility were performed through the counting chamber manufacturer (Makler counting chamber). Sperm count was completed in 10 squares of the chamber and motility was assessed in 100 random spermatozoa by marking them as rapid progressive motility (grade A), progressive motility(grade B), non progressive motility(grade C), and immotile(grade D) and motility was articulated as percentage. Sperm morphology was evaluated on air-dried smears, secure and stained by the quick-stain technique.

Wastewater samples were collected from five full-scale WWTPs located in the State of Ceará, Brazil. Each WWTP, five influent and effluent samples were analyzed in order to determine the estrogens quantities.

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. In Brazil, a total of 764 human sperm samples were analyzed in 2000-2002 and 1536 in 2010-2012. Days of abstinence and progressive sperm motility were similar between the 2000-2002 and 2010-2012 groups. Over time, the mean sperm concentration/ml decreased significantly from 61.7 million in 2000-2002 to 26.7 million in 2010-2012.

Frequency and Percentage of the Changes Found in the Sperm Analysis

Variable	% (n)
Diagnosis	
Oligozoospermia	2.3 (3)
Teratozoospermia	44.7 (59)
Oligoasthenoteratozoospermia	20.5 (27)
Oligoteratozoospermia	15.9 (21)
Azoospermia	7.6 (10)
Asthenozoospermia	2.3 (3)
Asthenoteratozoospermia	6.8 (9)

Figure 2 This table shows the percentage of changes found in the analysis of sperm. Seven types of diagnosis were made. The most frequent diagnosis is teratozoospermia with a 44.7 occurrence rate.[1]

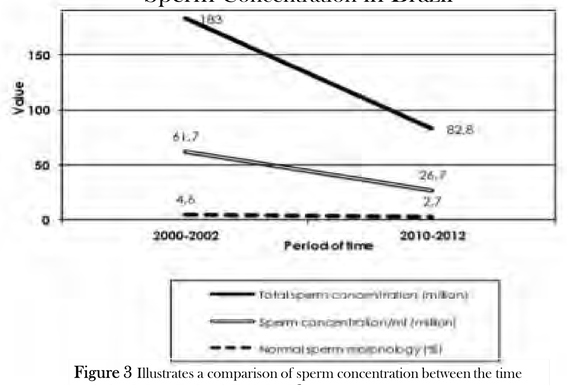

Figure 3 Illustrates a comparison of sperm concentration between the time periods of 2000-2002 and 2010-2012.[3]

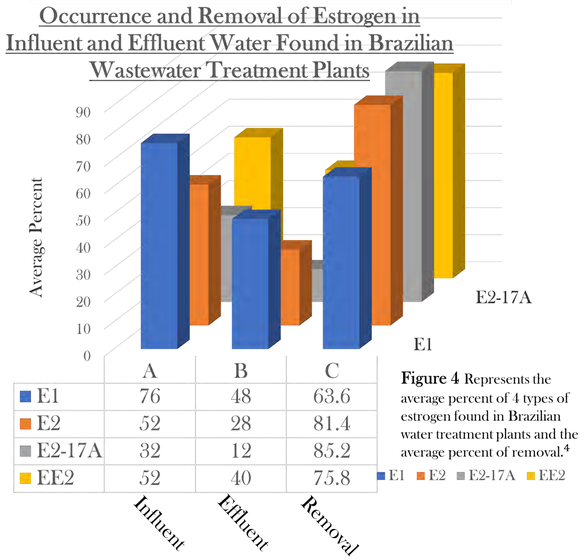

Figure 4 Represents the average percent of 4 types of estrogen found in Brazilian water treatment plants and the average percent of removal.[4]

Application to Biotechnology

The Sefi Makler Counting Chamber was utilized to determine concentration of spermatozoa in million/ml. The quantity of spermatozoa counted in any strip of 10 squares of the grid reflects the concentration and does not need additional calculation. This device is economical because it is reusable as it is easily cleaned with a disinfectant solution.

Conclusion

Indicator species are significant to understand undergoing complications found in the environment. 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.

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References

1. Eertmans, F., Dhooge, W., Stuyvaert, S., & Comhaire, F. (2003). Endocrine disruptors: effects on male fertility and screening tools for their assessment. Toxicology in Vitro Pflieger-Bruss, S., Schuppe,
2. H., & Schill, W. (2004). The male reproductive system and its susceptibility to endocrine disrupting chemicals. Andrologia
3. Jr., E. B., Setti, A. S., Daniela Paes De Almeida Ferreira Braga, Figueira, R. D., & Jr., A. I. (2015). Decline in semen quality among infertile men in Brazil during the past 10 years. International braz j urol,
4. Pessoa, G. P., Souza, N. C., Vidal, C. B., Alves, J. A., Firmino, P. I., Nascimento, R. F., & Santos, A. B. (2014). Occurrence and removal of estrogens in Brazilian wastewater treatment plants. Science of The Total Environment
5. Rose, E., Flanagan, S. P., & Jones, A. G. (2015). The Effects of Synthetic Estrogen Exposure on the Sexually Dimorphic Liver Transcriptome of the Sex-Role-Reversed Gulf Pipefish.
6. Silva, Manoela Cássia Budni Da, Leticia Burato Wessler, Kristian Madeira, and Caroline Calice Da Silva. "Male infertility profile in an assisted human reproduction clinic from the south of Santa Catarina, Brazil, from 2012 to 2014." Reprodução & Climatério".

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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

Academy of Our Lady of Peace

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.[2] 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.[3] 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 breastfeed less than other ethnicities (Figure 1).[6] 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 breastfeed, and 43.2% had ever breastfeed (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.

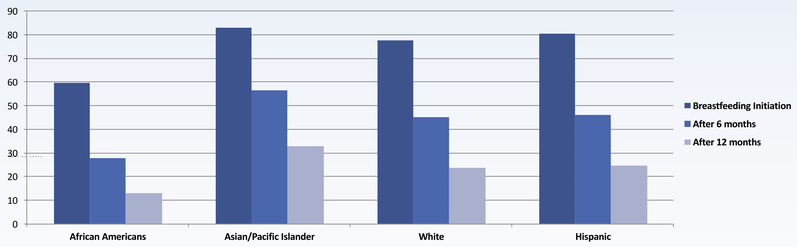


Figure 1. Healthy People 2010 and 2020 Goals and Centers for Disease Control and Prevention Data from 2007 on Racial and Ethnic Breastfeeding Initiation and Continuation. Jones, K. M., `& Power, M. L. (2015, May 01). Racial and Ethnic Disparities in Breastfeeding.

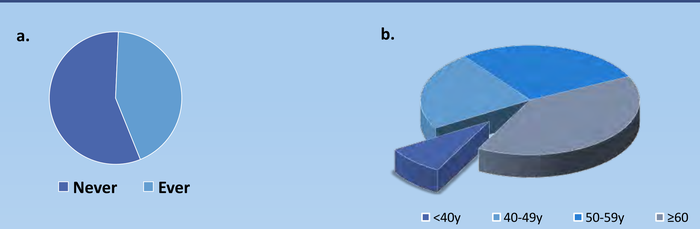


Figure 2. a| Lactation Among Parous Women **b|** Age at Diagnosis
Palmer, J. R. (2014, September 15). Parity, Lactation, and Breast Cancer Subtypes in African American Women: Results from the AMBER Consortium | JNCI: Journal of the National Cancer Institute | Oxford Academic.

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.[5] 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 polychotomous 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 BRCA1 mutation.[3] These few studies have been done with small groups and not tested in a large scale.

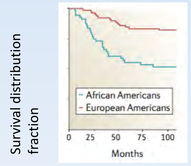


Figure 3: TNBC survival rate based on race. Dietze, E. (2015, February 12). Triple-negative breast cancer in African-American women: disparities versus biology.

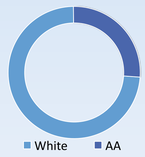
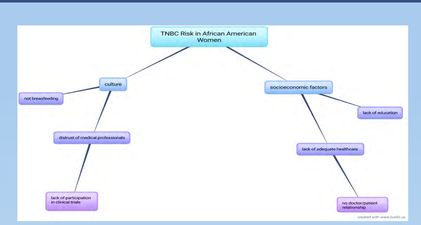


Figure 4. Case Participants by Race. Ma, H. (2017, January 13). Reproductive factors and the risk of triple-negative breast cancer in white women and African-American women: a pooled analysis.

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 minorities.[1] Thus, an even smaller fraction of studies have included African American women in breast cancer research. Cultural and socioeconomic factors have proven to be just as damaging as genetic or biological factors. These disparities within the AA community have become the downfall of many women. African American women are dying from the disease of economic and racial inequality.



Application to Biotechnology

Due to improvements in breast imaging technology, low income areas should participate in trials that test this technology. These new techniques should be equally available to all women, no matter one's socioeconomic status or racial identification.

Conclusions

The Tuskegee project is only one example of how African Americans have been mislead 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. Areas such as Washington D.C., where 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.

*photo via Centers for Disease Control and Prevention

*photo via Urological Sciences Research Foundation

Acknowledgements

Thank you Dr. Ericka and Dr. Tracy for being so patient with me and helping me find my research focus. I also would like to thank all of my ROSA sisters for making this journey so special in that I bonded with each and every one of you. Last but not least I would like to thank my friends and family for encouraging and supporting me throughout this process.

References

1. Bichell, R. E. (2015, December 16). Clinical Trials Still Don't Reflect The Diversity Of America. Retrieved August 04, 2017, from http://www.npr.org/sections/health-shots/2015/12/16/459666750/clinical-trials-still-dont-reflect-the-diversity-of-america
2. Brown, D. L. (2017, May 16). 'You've got bad blood': The horror of the Tuskegee syphilis experiment. Retrieved August 04, 2017, from https://www.washingtonpost.com/news/retropolis/wp/2017/05/16/youve-got-bad-blood-the-horror-of-the-tuskegee-syphilis-experiment/?utm_term=.b2711e077bb0
3. Dietze, E. (2015, February 12). Triple-negative breast cancer in African-American women: disparities versus biology. Retrieved July 22, 2017, from http://www.nature.com/nrc/journal/v15/n4/full/nrc3896.html?foxtrotcallback=true
4. Jones, K. M., & Power, M. L. (2015, May 01). Racial and Ethnic Disparities in Breastfeeding. Retrieved August 06, 2017, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4410446/
5. Ma, H. (2017, January 13). Reproductive factors and the risk of triple-negative breast cancer in white women and African-American women: a pooled analysis. Retrieved August 03, 2017, from https://breast-cancer-research.biomedcentral.com/articles/10.1186/s13058-016-0799-9
6. Palmer, J. R. (2014, September 15). Parity, Lactation, and Breast Cancer Subtypes in African American Women: Results from the AMBER Consortium | JNCI: Journal of the National Cancer Institute | Oxford Academic. Retrieved August 03, 2017, from https://academic.oup.com/jnci/article-lookup//10.1093/jnci/dju237

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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) as well as other such as 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 getting post-reproductive, young-onset breast cancer.

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 rise of progesterone and estrogen. The objective is to find the correlation between younger women undergoing fertility treatments and the increased risk of breast cancer post-treatment.

In a cross-sectional study done with a little over 43,000 women, 8,963 were infertile: 1,576 went through COS, 1,429 had hormonal stimulation without COS and 5,958 didn't receive any hormonal fertility treatment), researchers found that women with a history of infertility and had COS 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 induced a pregnancy that lasted 10+ weeks. Out of 288 final participants, 193 took CC only, 29 took FSH only, and 66 took both. Though 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 progesterone and nulliparous women exposed to gonadotropins was found.

There are inconsistent findings in the case studies conducted due to various limitations, for example, the number of cases observed and the length of follow up. Future research should address these limitations while focusing on each drug and combination.

Methods and Materials

In a cross-sectional study done with 43,313 Swedish women, 8,963 were infertile: 1,576 went through COS, 1,429 had hormonal stimulation without COS and 5,958 didn't receive any hormonal fertility treatment. Using FFDM, breast density was measured and compared with fertility treatments between fertile and infertile women.

In a study with 1,669 women (control sisters), who had a sister (1,422 women: case sisters) 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 final participants, 193 took CC only, 29 took FSH only, and 66 took both. In a long-term, historic-prospective 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 1,211 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.

	Took CC and got Breast Cancer	Took CC and didn't get Breast Cancer
Reference 1	86/1422	107/1669
Reference 2	27/247	154/1089
Reference 3	140/844	unknown
Reference 5	16/780	unknown

Table 1. Women receiving clomiphene citrate (CC) who got breast cancer compared with women receiving clomiphene citrate (CC) who did not.

Note: references 1 and 2 had two control groups.

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 percent volume than those who hadn't received 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.[3]

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. An elevated risk in women who had used both CC and FSH [n=66], with first use after age 35 was also identified.[1]

In the same study that identified progesterone as a risk factor, CC was found to be structurally and functionally similar to tamoxifen, which is used in the treatment of breast cancer, and clomiphene could therefore potentially reduce the risk of breast cancer at the receptor level.

In a registry based cohort study, CC exposure, but not ART, was associated with an increased risk of breast cancer in parous women.[2&5]

In the long-term, historic-prospective study, women who used fertility drugs did not have an increased risk of breast cancer, which is contradictory to some of the other studies which was either inconclusive or found an increase.[7]

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, differences in the treatments observed, number of treatment cycles, timing of treatment (when treatment and follow up were 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 made possible by the advancements in reproductive technology. The process of in-vitro fertilization (IVF) and the medications 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.

Table 2. Associations of young-onset breast cancer with fertility drugs and stimulated pregnancies*

Variable	Control sisters (n = 1669) No. (%)	Case sisters (n = 1422) No. (%)
Model I		
Nonusers of fertility drugs	1511 (90.5)	1292 (90.9)
CC only	107 (6.4)	86 (6.0)
FSH only	12 (0.7)	17 (1.2)
CC and FSH	39 (2.3)	27 (1.9)
Stimulated pregnancy	69 (4.1)	72 (5.1)
Model II		
Nonusers of fertility drugs	1511 (90.5)	1292 (90.9)
Use of fertility drug(s)	158 (9.5)	130 (9.1)
Stimulated pregnancy	69 (4.1)	72 (5.1)

*young-onset refers to case sisters diagnosed with breast cancer ate age less than 50 years.[1]

Fei, C., DeRoo, L. A., Sandler, D. P., & Weinberg, C. R. (2012, July 03). Fertility drugs and young-onset breast cancer: Results from the two sister study | JNCI: Journal of the National Cancer Institute | Oxford Academic. https://academic.oup.com/jnci/article/104/13/1021/2516873/Fertility-Drugs-and-Young-Onset-Breast-Cancer

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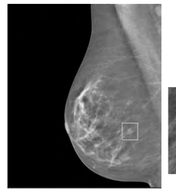


Figure 1. FFDM mammogram containing a 7mm malignant mass.[8]

Wei, J., Hadjiiski, L. M., Sahiner, B., Chan, H., Ge, J., Roubidoux, M. A., . . . Zhang, Y. (2008, June 1). Computer aided detection systems for breast masses: Comparison of performances on full-field digital mammograms and digitized screen-film mammograms. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2040166/

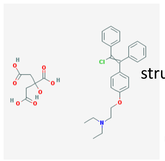


Figure 2. Chemical structure of the clomiphene citrate (CC) compound.[6]

PubChem. (n.d.). CLOMIPHENE CITRATE. https://pubchem.ncbi.nlm.nih.gov/compound/60974#section=Top

References

1. Fei, C., DeRoo, L. A., Sandler, D. P., & Weinberg, C. R. (2012, July 03). Fertility drugs and young-onset breast cancer: Results from the two sister study | JNCI: Journal of the National Cancer Institute | Oxford Academic. https://academic.oup.com/jnci/article/104/13/1021/2516873/Fertility-Drugs-and-Young-Onset-Breast-Cancer
2. Jensen, A., Sharif, H., Svare, E. I., Frederiksen, K., & Kjær, S. K. (2007, July 01). Risk of breast cancer after exposure to fertility drugs: Results from a large Danish cohort study. Cancer Epidemiology, Biomarkers and Prevention | American Association for Cancer Research. http://cebp.aacrjournals.org/content/16/7/1400
3. Lundberg, F. E., Johansson, A. L., Rodriguez-Wallberg, K., Brand, J. S., Czene, K., Hall, P., & Iliadou, A. N. (2016, April 13). Association of infertility and fertility treatment with mammographic density in a large screening-based cohort of women: A cross-sectional study. Breast Cancer Research | BioMed Central. https://breast-cancer-research.biomedcentral.com/articles/10.1186/s13058-016-0693-5
4. Planche, K., & Vinnicombe, S. (2003, September 22). Breast imaging in the new era. Cancer Imaging | St. Bartholomew's Hospital https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1434581/
5. Potashnik, G., Lerner-Geva, L., Genkin, L., Chetrit, A., Lunenfeld, E., & Porath, A. (1999, May). Fertility drugs and the risk of breast and ovarian cancers: Results of a long-term follow-up study. Fertility and Sterility | American Society for Reproductive Medicine. http://www.fertstert.org/article/S0015-0282(99)00085-0/fulltext
6. PubChem. (n.d.). CLOMIPHENE CITRATE. https://pubchem.ncbi.nlm.nih.gov/compound/60974#section=Top
7. Reigstad, M. M., Storeng, R., Myklebust, T. Å, Oldereid, N. B., Omland, A. K., Robsahm, T. E., . . . Larsen, I. K. (2017, January 20). Cancer risk in women treated with fertility drugs according to parity status—a registry-based cohort study. Cancer Epidemiology, Biomarkers and Prevention | American Association for Cancer Research. http://cebp.aacrjournals.org/content/26/6/953.long
8. Wei, J., Hadjiiski, L. M., Sahiner, B., Chan, H., Ge, J., Roubidoux, M. A., . . . Zhang, Y. (2008, June 1). Computer aided detection systems for breast masses: Comparison of performances on full-field digital mammograms and digitized screen-film mammograms. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2040166/

Investigating the Use of mTOR Inhibitor Compounds for the Promotion of T-regulatory Cells to Protect Fetal Development

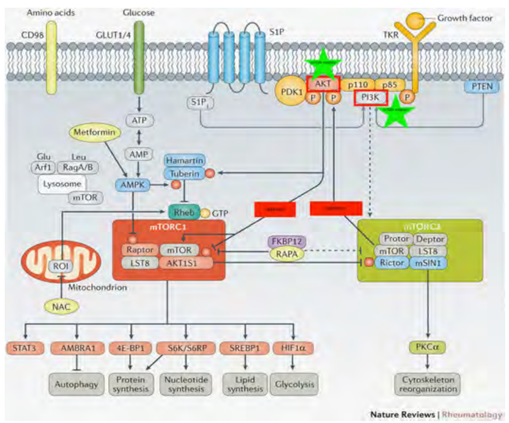
Smayra Ramesh • Westview High School

Objective

In recent years, there has been a great deal of investigation and progress in immunology as researchers explore its relevance in fertility issues within the female reproductive system. Currently, scientists believe that T- regulatory (Treg) cells are central to the solutions for complications in the early stages of pregnancy. The objective of this study is to connect two separate research areas: the mTOR gene-protein pathway and its relationship with pregnancy complications through T-regulatory cells. I would like to emphasize that my proposed solution of utilizing mTOR inhibitors to cure the pregnancy complications caused by downregulated Treg cell counts is based off of my own research of currently published studies since this particular use of mTOR inhibitor therapeutics has not been researched yet.

Abstract

This study is looking to show that the inhibition of the mTOR pathway can improve fertility in women who face pregnancy complications due to a lack of T regulatory cells. In patients with fertility problems due to low Treg cell counts, one major cause could be the over-expression of mTOR proteins. This excess of mTOR transcription/translation is often caused by over-activation of kinase receptors within the cell, specifically, AKT and P13K.[7] AKT acts by promoting transcription proteins within the mTORC1 complex while P1K3 is heavily involved in promoting transcription proteins within the mTORC2 complex. First-generation mTOR inhibitors have been shown to effectively inhibit the AKT and P1K3 pathways in clinical trials. By targeting both kinases at the same time, these inhibitors effectively inhibit the mTOR pathway, and, in response, an upregulation of T regulatory cells is likely to occur. The use of rapamycin in cell cultures for the expansion of Treg cells is allows for a selective expansion of T cells with regulatory properties while inhibiting proliferation and survival of non-Treg cells.[1] Investigating the inhibition of mTOR to promote Treg cells can help solve some of the issues such as infertility, miscarriage and preeclampsia that are linked with numerical and functional deficiency of T regulatory cells.[5]



Perl, A. (2015). Activation of mTOR (mechanistic target of rapamycin) in rheumatic diseases. Nature Reviews Rheumatology, 12(3), 169-182. Retrieved July 20, 2017. Green stars represent the kinase receptors that are inhibited through the mTOR inhibitor. Red blocks indicate the pathways that are blocked through inhibition.[6] (With edits)

Methods and Materials

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.[8] A Treg cell assay was developed to membrane stain CD25 and CD4 and was followed by intracellular staining for the FoxP3.[8] Each was associated to fluorescent markers. Then, cytotoxicity 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– then throughout their pregnancy were tested for T regulatory cell levels.[8]

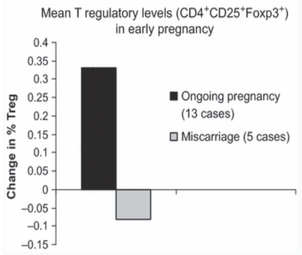


Figure 1

Change in T regulatory levels ($CD4^+$ $CD25^+$ $Foxp3^+$) in early pregnancy: *Ongoing pregnancy:* Mean Treg change +0.33 ± 0.32 (13 cases) *Miscarriage:* Mean Treg change −0.08 ± 0.28 (five cases) $P = 0.02$. Mean pregnancy day of first blood draw: 38.0 ± 19.7. Mean pregnancy day of second blood draw: 62.5 ± 23.8.[8]

As seen from ROC curve (Fig 2), quantification of peripheral blood T regulatory cells can be used to predict pregnancy outcome. Moreover, 100% (9/9) of patients whose Treg values were ≥1.0 in early pregnancy experienced pregnancy success, whereas 83% (5/6) of the patients whose Treg values were ≤0.05 experienced miscarriage ($P = 0.002$).[8]

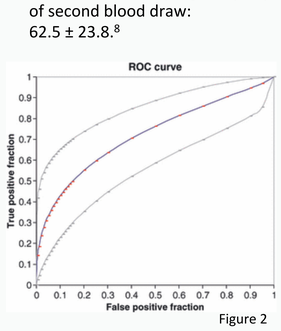


Figure 2

Figure 1 & Figure 2
Winger, E. E., & Reed, J. L. (2011). Low Circulating Foxp3 T Regulatory Cell Levels Predict Miscarriage Risk in Newly Pregnant Women with a History of Failure. American Journal of Reproductive Immunology, 66(4), 320-328. Retrieved July 24, 2017.

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.[1] The suppressive assay was then conducted. Responder T cells were stained with CFSE. Treg treated Rapamycin cells were added in equal number to stained Responder 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 T_{RPM} side (left) as this culture was exposed to the rapamycin inhibitor.[1]

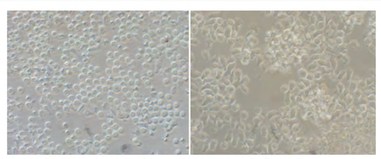


Fig. 5. Pictures of $CD25^-T_{RPM}$ (*left*) and $CD25^-T_{MED}$ (*right*) cells at the end of the expansion protocol.

Battaglia, M., Stabilini, A., & Tresoldi, E. (2011). Expanding Human T Regulatory Cells with the mTOR-Inhibitor Rapamycin. Methods in Molecular Biology mTOR,279-293. Retrieved August 1, 2017.

Results and Interpretations

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, IVF parameters and immune test results was performed using a one-way Anova calculator.[8] It has been demonstrated that both Treg cell number and function is associated with pregnancy loss. Fifty-four pregnant women with a history of immunologic infertility or pregnancy loss were evaluated (mean age: 36.7 ± 4.9 years, 2.8 ± 2.5 previous miscarriages; 1.5 ± 1.9 previous IVF failures). Twenty-three of these women experienced another first trimester miscarriage, and 31 of these women continued their current pregnancies past 12 weeks ('pregnancy success'). Patients with successful ongoing pregnancies experienced a mean Treg level of 0.72 ± 0.52%, while those that miscarried in the first trimester experienced a mean Treg level of 0.37 ± 0.29% ($P = 0.005$). First trimester $Foxp3^+$ Treg measurements may offer a new tool to identify need for early pregnancy intervention. [8]
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 of functional T-regulatory cells 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.[1]

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 cells, T-regulatory (Treg) lymphocytes act to suppress immune activation and thereby maintain immune homeostasis.[1]
The possibility of using Treg cells for the treatment of T-cell mediated diseases has recently gained increasing momentum.[1] Through the inhibition of mTOR, new infertility treatments could be created in order to solve pregnancy complications triggered by low T-regulatory cell counts. In the first experiment, it was found that decreased levels of T regulatory cells are correlated with higher incidence of pregnancy complications.[1] This finding suggests that early pregnancy assessment of T regulatory cells might have clinical utility.[1] The second experiment was able to demonstrate the effectiveness of using the mTOR inhibitor Rapamycin to selectively promote the proliferation of Treg cells while inhibiting the proliferation of both effector T cells and other potentially harmful cells.[1] Furthermore, there were extremely low toxicity levels detected from these mTOR inhibitor drugs used to inhibit the kinase receptors, further supporting the idea of testing this drug class for infertility rather than solely for cancer as it is currently.[8] Research into this process could help a large portion of 5-10% of women that deal with infertility issues due to preeclampsia and other unexplained conditions.8 This connection between mTOR and pregnancy complications through Treg cells is a novel field of study, but with enough research, it could be used to solve some of the causes of infertility.

Applications to Biotechnology

An array of different technologies were used to facilitate this series of experiments.
Firstly, different technologies were used to isolate the macrolide antibiotic Rapamycin from *Streptomyces hygroscopicus*. Rapamycin is an immunosuppressant and FRAP inhibitor and inhibits lymphokine-induced cell proliferation. Rapamycin works by forming a complex with FKBP12 and binding 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.[3] The isolation techniques used to acquire Rapamycin included the sequential use of electronic pipette systems, incubators, freezers, and centrifuges in order to generate enough inhibitor.[8]
For the separation and counting of the T-cells, a laser-based flow cytometer was used. Additionally, an autoMACS separation system was also utilized in different phases of the study for automated cell separation from blood at scale.[1]

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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 about Oncofertility. 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 everything you have done for the AYA patients!
Dr. Irene, you gave me the opportunity of a lifetime by allowing me to perform hands-on activities in your lab. You also gave me the chance to learn about a career path that I had never considered before. Thank you!
And most of all, I would like to give my utmost gratitude to Dr. Ericka. You gave me all of the tools necessary to understand my worth as a woman in science. I know never to back down.
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

1. Battaglia, M., Stabilini, A., & Tresoldi, E. (2011). Expanding Human T Regulatory Cells with the mTOR-Inhibitor Rapamycin. Methods in Molecular Biology mTOR,279-293. Retrieved August 1, 2017.
2. Cook, H., Snead, A., Yesayan, M., & Kahn, D. (2013). The role of regulatory T-cells and pregnancy outcomes. Fertility and Sterility, 100(3).
3. Chapman, N. M., & Chi, H. (2014). MTOR signaling, Tregs and immune modulation. Immunotherapy,6(12), 1295-1311.
4. Gedaly, R. (2015). MTOR Signaling in Regulatory T Cell Differentiation and Expansion. SOJ Immunology, 3(1). Retrieved July 23, 2017.
5. Guerin, L. R., Prins, J. R., Robertson, S. A. (2009). Regulatory T-cells and immune tolerance in pregnancy: a new target for infertility treatment? Human Reproduction Update, 15(5), 517-535.
6. Perl, A. (2015). Activation of mTOR (mechanistic target of rapamycin) in rheumatic diseases. Nature Reviews Rheumatology, 12(3), 169-182. Retrieved July 20, 2017.
7. Saito, S., Shima, T., Nakashima, A., Shiozaki, A., Ito, M., & Sasaki, Y. (2007). What is the role of regulatory T cells in the success of implantation and early pregnancy? Journal of Assisted Reproduction and Genetics, 24(9), 379-386.
8. Winger, E. E., & Reed, J. L. (2011). Low Circulating CD4 CD25 Foxp3 T Regulatory Cell Levels Predict Miscarriage Risk in Newly Pregnant Women with a History of Failure. American Journal of Reproductive Immunology, 66(4), 320-328. Retrieved July 24, 2017.

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 differentiating ethnicity 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 consequenting 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.

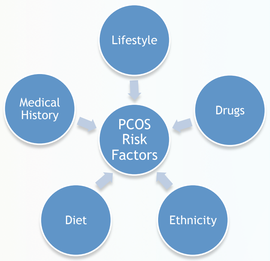

Figure 1 MD, A. (2016). PCOS Risk Factors [Infographic]. Retrieved from ttps:// anasbananas.com/professor-of-health/ prevention-and-treatment-pcos/[4]

Abstract

Increasingly, women of differentiating ethnicity 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 manifestos of PCOS and PCOS phenotypes. One such study explored the possibility of an Asian phenotype, where women from East Asia have been reported to have a lower BMI and a milder hyper androgenic 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.

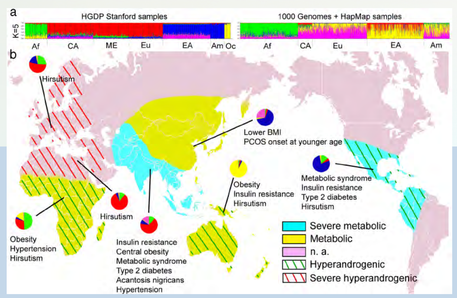

Figure 2 . Livio Casarinin and Giulia Brigante, (August 2014). *World distribution of the affinity to the genetic clusters and PCOS phenotypes prevalence.* [Inforgraphic]. Retrieved from URL (2017, August 2). https://academic.oup.com/jcem/article-lookup[3]

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 hyperandrogensim 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 used as it was necessary to use other studies focused on different ethnicities to create a large variation in ethnicities summarized in this poster. The second study derived data from the PPCOS 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 PPCOS II trial, to evaluate androgenic 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 not used in this 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 the both will help a) prove correlation between PCOS and ethnicity 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 Caucasian women with PCOS (11.9%), while Chinese women only had a (5.6%) prevalence[2]. (The Rotterdam criteria was used for this diagnosis).

Clinical variations in manifestation: There were three different clinical criteria/ representations 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%)[2].

Average %	Chinese	Caucasian	Non-Hispanic Black	Hispanic American
% of PCOS w/ hirsutism	37%	74.7%	82.7%	93.8%
% of PCOS w/ biochemical hyperandrogenimia	63.6%	67.7%	64.3%	75.8%
% of PCOS w/ PCOM	92.7%	74%	99%	100%

Table 1. Clinical Hormonal and Biochemical variables in PCOS for Chinese[2] Caucasian[2] Non-Hispanic Black[1] and Hispanic American[1] women with PCOS.

Prevalence: The prevalence of Hispanic Americans with PCOS is 13%[6] while the prevalence of Non Hispanic Blacks is unknown.

Clinical Variations: Hispanic women with PCOS had a higher prevalence of hirsutism (93.8%), biochemical hyperandrogenism (75.8%), and PCOM (100%), compared to the Non Hispanic Black women with PCOS who showed (82.7%) of hirusitism, (64.3%) of biochemical hyperandrogenism, and (99%) of PCOM[1]. A reason why the Hispanic subject population demonstrated the highest prevalence of all three variables could be because it was so small compared to the other ethnic subjects. Not all the clinical hormonal and biochemical variables of PCOS are represented in this poster as no studies were found to have focus on the same variables.

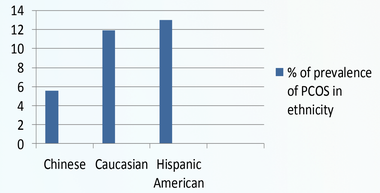

Chart 1. Prevalence of women with PCOS of different ethnicities (using East Asian and Caucasian ethnicities)[2] and Hispanic American women[1]

Conclusions & Discussion

Hispanic American women with PCOS had the highest prevalence, percent of hirsutism, hyperandrogenism and PCOM in comparison to 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 hyperandrogenism (75.8%) explains why there is an increase risk of higher hirsutism (93.8%) rates. The percent of women with hyperandrogensim 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 [2]. 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 ovary size in order to detect polycystic ovarian morphology (PCOM) which may occur in some PCOS patients. Various technologies are used to manage PCOS symptoms: Combined Oral Contraceptive Pill (COCP/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, advancements can be made to the criterions 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 oncofertility 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

1.Engmann, L., Legro, R., Jin, S., Sun, F., Polotsky, A. J., Hansen, K. R., . . . Santoro, N. (2013). Polycystic Ovary Syndrome Across Racial and Ethnic Groups. Ethnic Differences in Fertility and Assisted Reproduction, 185-199.

2.Huang, Z., & Yong, E. (2016). Ethnic differences: Is there an Asian phenotype for polycystic ovarian syndrome? Best Practice & Research Clinical Obstetrics & Gynaecology, 37, 46-55.

3. Livio Casarinin and Giulia Brigante, (August 2014). World distribution of the affinity to the genetic clusters and PCOS phenotypes prevalence. [Inforgraphic]. Retrieved from URL (2017, August 2). https://academic.oup.com/jcem/article-lookup/

4. MD, A. (2016). PCOS Risk Factors [Infographic]. Retrieved from ttps://anasbananas.com/ professor-of-health/prevention-and-treatment-pcos/

5.Williamson, K., Gunn, A. J., Johnson, N. and Milsom, S. R. (2001), The impact of ethnicity on the presentation of polycystic ovarian syndrome. Australian and New Zealand Journal of Obstetrics and Gynaecology, 41: 202–206.

6.Zhao, Y., & Qiao, J. (2013). Ethnic differences in the phenotypic expression of polycystic ovary syndrome. Steroids, 78(8), 755-760.

7.Zhang, H. Y., Guo, C. X., Zhu, F. F., Qu, P. P., Lin, W. J., & Xiong, J. (2012). Clinical characteristics, metabolic features, and phenotype of Chinese women with polycystic ovary syndrome: a large-scale case–control study. Archives of Gynecology and Obstetrics, 287(3), 525-531.

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Frequency and Types of Y Chromosome Microdeletions Among Infertile Men with Azoospermia and Severe Oligozoospermia

AnnMarie Walker — El Camino High School

Introduction/Objective

Y chromosome microdeletions in men with azoospermia or severe oligozoospermia leads to infertility. Azoospermia is the complete absence of sperm from the fluid ejaculated during 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 application to male infertility screening will also be discussed.

Abstract

The frequency and types of Y chromosome microdeletions among infertile men with azoospermia and severe oligozoospermia and the implications of this in the male infertility work-up will be demonstrated. The frequency and types of Y chromosome microdeletions can be influenced by geographical location. One study showed that Y chromosome deletions were detected in about 12.1% of Iranian infertile men. Another study took 1885 Iranian infertile men with azoospermia or severe oligozoospermia and tested them for microdeletions. Only 5.2% were diagnosed with microdeletions in the azoospermia factor. It is suspected the differences are due to ethnicity and the composition of the sample size and study populations. A study done on 3731 Chinese infertile men showed 9.14% had microdeletions in the AZF region. Another study done on 71 Indonesian men showed 15.49% had an AZFa microdeletion. Most of AZF microdeletions were found in the AZFa region for Indonesian men, whereas this was lower in Iranian men. Different frequencies and types of microdeletions can be explained by the differences in ethnicity. The genetics of each race or ethnicity can cause microdeletions in different AZF regions. Currently, microdeletion screening is not a part of the male infertility work-up. Screening should be advised for infertile men before using assisted reproduction treatments. Microdeletion screening should be a part of the male infertility work up.

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 ºC[5]. Subjects who did not have Y AZF microdeletions were screened for partial AZFc deletions. All negative PCR reactions were repeated for at least three times. Amplification started with an activation step of 15 min at 95 ºC, followed by 35 cycles of 30 s denaturation (94 ºC), 90 s annealing (57 ºC) and 60 s elongation (72 ºC), ended by an elongation step of 10 min and cooling to 4 ºC[5].

In another study, 3731 Chinese infertile men were studied. 2531 men had azoospermia, and 1200 men had oligozoospermia. Forty sperm donors were included as 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 of the Y chromosome. A novel semiconductor sequencing method was established to detect high-resolution AZFa microdeletions[4].

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 elongation phase end 72° C for 2 minutes. The cooling process was performed at 4° C. The 7 mL reaction products were separated by 2.5% of 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 of the AZFc region were detected. One subject had a microdeletion in the AZFb 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%.

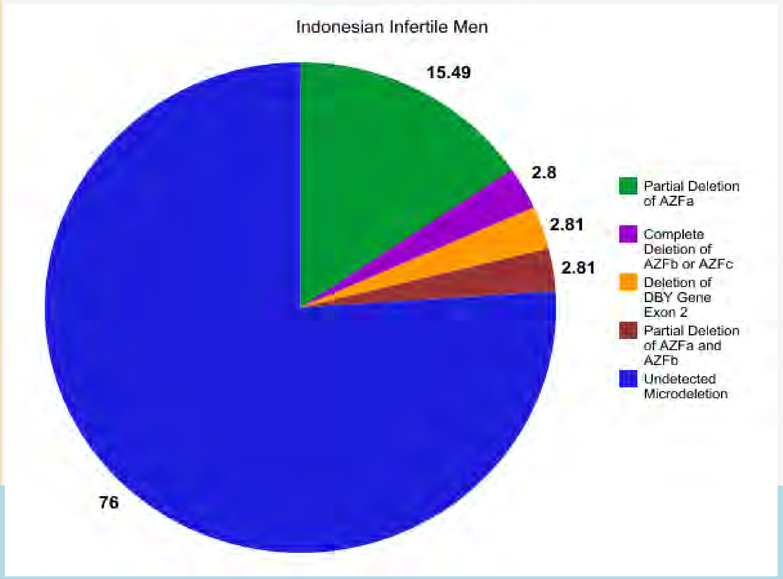
Conclusions

In conclusion, frequency and types of Y chromosome microdeletions vary from ethnicity to ethnicity. In Iranian men, microdeletions were found 7% of the time. In Chinese 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 infertile 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 biotechnology 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.

Indonesian Infertile Men

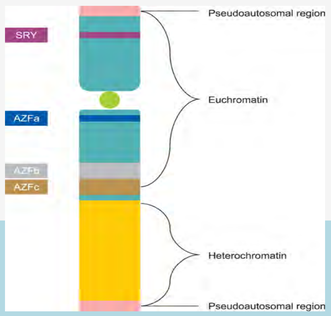


Graph representing AZF deletions in Indonesian infertile men.[2] Birowow, P., Putra, D. E., Dewy, M., Rasyid, N., & Taher, A. (2017). Y-Chromosomal Microdeletion in Idiopathic Azoospermic and Severe Oligozoospermic Indonesian Men. *Indones J Intern Med , 9*(1).

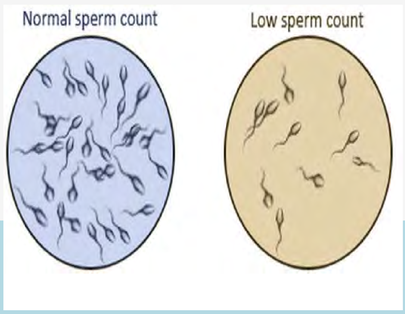
Iranian Infertile Men

Phenotype	n	Deletion(%)	Region
Azoospermia	70	6(6%)	AZFc,AZFb AZFc+AZFb
Oligozoospermia	30	1(1%)	AZFc
Total	100	7(7%)	

Table representing AZF microdeletions in Iranian infertile men.[5] Motovali-Bashi, M., Rezaei, Z., Dehghanian, F., & Rezaei, H. (2015). Multiplex PCR based screening for micro/partial deletions in the AZF region of Y-chromosome in severe oligozoospermic and azoospermic infertile men in Iran. *Iranian Journal of Reproductive Medicine*, *13*(9), 563–570.



AZF microdeletions locations along the Y chromosomes. Digital image. SpermHope.



Normal sperm count vs low sperm count. Digital image. Health Tap.

Acknowledgements

Thank you to Dr. Ericka for all the support she has given me 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

1. Asadi, F., Sadighi Gilani, M. A., Ghaheri, A., Roodgar Saffari, J., & Zamanian, M. (2017). The Prevalence of Y Chromosome Microdeletions in Iranian Infertile Men with Azoospermia and Severe Oligospermia . *Cell Journal (Yakhteh)*, *19*(1), 27–33.
2. Birowow, P., Putra, D. E., Dewy, M., Rasyid, N., & Taher, A. (2017). Y-Chromosomal Microdeletion in Idiopathic Azoospermic and Severe Oligozoospermic Indonesian Men. *Indones J Intern Med , 9*(1).
3. Kamaliyan, Z., Pouriamanesh, S., Amin-beidokhti, M., Rezagholizadeh, A., & Mirfakhraie, R. (2017). *HIWI2* rs508485 Polymorphism Is Associated with Non-obstructive Azoospermia in Iranian Patients. *Reports of Biochemistry & Molecular Biology*, *5*(2), 108–111.
4. Liu, X., Zhang, H., Pang, D., Xue, L., Yang, X., Li, Y., & Liu, R. (2017). Azoospermia factor a Microdeletions: Occurrence in Chinese Infertile Men and Novel Deletions Revealed by Semiconductor Sequencing. *Urology*.2017.04.024
5. Motovali-Bashi, M., Rezaei, Z., Dehghanian, F., & Rezaei, H. (2015). Multiplex PCR based screening for micro/partial deletions in the AZF region of Y-chromosome in severe oligozoospermic and azoospermic infertile men in Iran. *Iranian Journal of Reproductive Medicine*, *13*(9), 563–570.
6. Yousefi-Razin, E., Nasiri, M. J., & Omrani, M. D. (2016). Frequency of Y Chromosome Microdeletions Among Iranian Infertile Men with Azoospermia and Severe Oligozoospermia: A Meta-analysis. *Journal of Reproduction & Infertility*, *17*(4), 208–212.
7. Zaimy, M. A., Kalantar, S. M., Sheikhha, M. H., Jahaninejad, T., Pashaiefar, H., Ghasemzadeh, J., & Zahraei, M. (2013). The frequency of Yq microdeletion in azoospermic and oligospermic Iranian infertile men. *Iranian Journal of Reproductive Medicine*, *11*(6), 453–458.

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

Kylie Williams 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)*.

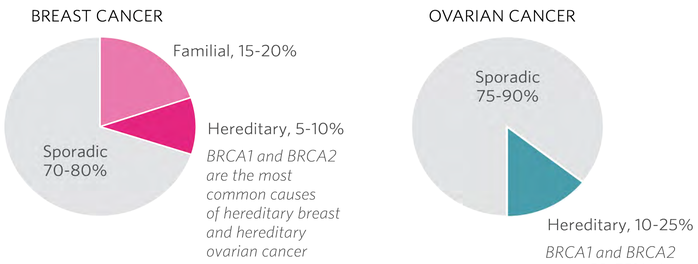

Figure 1. Percentage of inherited, developed cases of cancer between two gynecologic cancers that are known to be linked to the BRCA1 gene mutation. Charts from Ambry Genetics, *What is Hereditary Cancer?* (California, 2016).

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 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 human 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 CRISPR-Cas9 could provide are immense. Couples that struggle with reproduction due to PCOS or another linked disease that choose to undergo IVF could have embryonic gene editing as an option, and ensure that their offspring will not develop an inherited similar disease.

Abstract

About 1 in every 500 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 an inherited 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, once one or more viable embryos that 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 programmed DNA.

CRISPR-Cas9 utilizes guide RNAs 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 cancer gene from a human embryo in order to decrease the statistic of developed cancers among males and females.

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 embryo. The RNA-enzyme complex then splits apart the sequence and attaches to the corresponding DNA, where it is able to cleanly 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 procedure, 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 3)*

Experiment 1. Dr. Shoukhrat Mitalipov, a biologist at OHSU, created 58 viable embryos from 12 healthy female egg donors and one male donor who carried the MYBPC3 gene mutation, and sent a CRISPR-Cas9 line into each and every embryo.

Experiment 2. Oncologist Lu You at Sichuan University in Chengdu, China received ethical approval from a hospital review board after a hard-won legal battle. Dr. You's trial involved 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-Cas9 gene line itself costs Harvard and HSCI Faculty around $15,200 per line, and around $19,100 per line for outside non-profit institutions.

In vitro fertilization takes a total of four to six weeks to complete one cycle, while the CRISPR gene editing method takes about six to seven months to repair one DNA sequence. This excessive time gap 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.

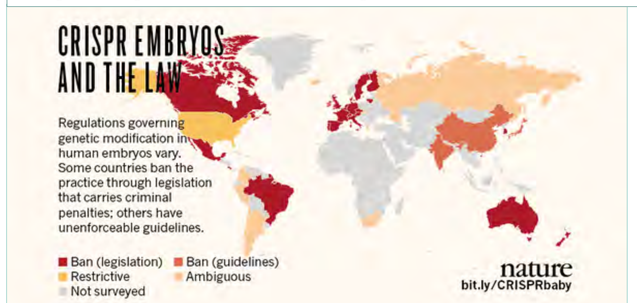

Figure 2. Colored world map and key depicting different regulations on CRISPR and embryonic gene editing across different countries, *Where in the World Could the First CRISPR Baby Be Born?* (2015)

Results and Interpretations

Experiment 1. As the embryos matured, Dr. Mitalipov confirmed that CRISPR-Cas9 was successfully able to cut the DNA sequence around the problematic gene MYBPC3, and repair those incision gaps with a healthy copy of the gene from the maternal donor. The 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 after the cycles of in vitro fertilization. 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 immune cells worked to attack and defeat the lung cancer, as Dr. You hypothesized. The participant is expected to receive a second injection sometime in the future, and the study is working to recruit at least ten more 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 cystic fibrosis patients, HIV resistance, and, most importantly, the removal of inherited diseases (specifically BRCA1). Over the years, the mention of CRISPR in PubMed publications has increased *(see Figure 4a)*, and overall funding for the investigation of CRISPR-Cas9 in genome editing has exponentially grown since the tool was first introduced *(see Figure 4b)*, which are both promising outlooks towards the future of CRISPR-Cas9 embryonic genome editing.

Conclusion

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% - 90% 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 recognized 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.

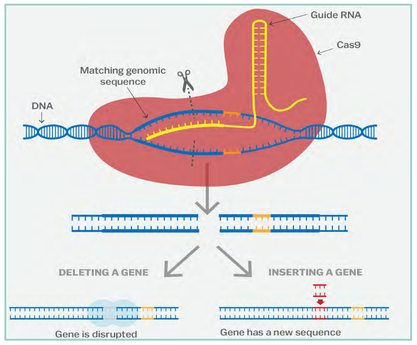

Figure 3. The anatomy of CRISPR and how it works, *Scientists successfully used CRISPR to fix a mutation that causes disease.* (2017)

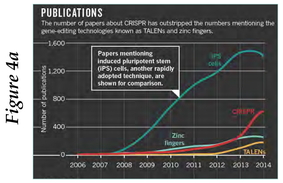

Figure 4a

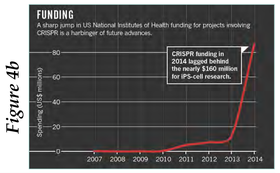

Figure 4b

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 complex that is 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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References

1. Barclay, E. (2017, August 02). Scientists successfully used CRISPR to fix a mutation that causes disease. This is huge. From https://www.vox.com/science-and-health/2017/8/2/16083300/crispr-heart-disease
2. BRCA1 & BRCA2: Cancer Risk & Genetic Testing. (n.d.). From https://www.cancer.gov/about-cancer/causes-prevention/genetics/brca-fact-sheet
3. Cha, A. E. (2017, August 02). First human embryo editing experiment in U.S. 'corrects' gene for heart condition. From https://www.washingtonpost.com/news/to-your-health/wp/2017/08/02/first-human-embryo-editing-experime-u-s-corrects-gene-for-heart-condition
4. CRISPR gene-editing tested in a person for the first time. (2016, November 15). From http://www.nature.com/news/crispr-gene-editing-tested-in-a-person-for-the-first-time-1.20988
5. Cui, J., Chew, S. J., Shi, Y., Gong, Z., & Shen, H. (2017). CRISPR system for genome engineering: the application for autophagy study. BMB Reports, 50(5), 247-256.
6. Genome Editing Services. (n.d.). From https://ipscore.hsci.harvard.edu/genome-editing-services -- Harvard University
7. Ledford, H. (2015, October 13). Where in the world could the first CRISPR baby be born? From http://www.nature.com/news/where-in-the-world-could-the-first-crispr-baby-be-born
8. Molteni, M. (2017, June 03). China Used Crispr to Fight Cancer in a Real, Live Human. From https://www.wired.com/2016/11/china-used-crispr-fight-cancer-real-live-human/

Figure 4a and 4b. CRISPR, the disruptor. (2015, June 3). Retrieved August 06, 2017, from https://www.nature.com/news/crispr-the-disruptor-1.17673