# *Natural Science*

# Pubertal Onset and Maintenance in FGFR3 Global Knockout Mice

Amanda Brown

The following is an excerpt from a longer piece. For full text, please visit https://scholar.colorado.edu/concern/undergraduate_honors_theses/w9505121v

**Lay Summary:**

Human reproduction relies on a cascade of hormones beginning in a part of the brain called the hypothalamus. The initial hormone, called gonadotropin releasing hormone (GnRH), initiates the hormonal cascade and allows for reproductive potential. The initial protein released, GnRH, is released from GnRH neurons in the hypothalamus. During fetal development, the production of GnRH neurons relies on certain receptors called fibroblast growth factors to direct the correct placement of GnRH neurons in the brain. If migration of the GnRH neurons fails, one cannot go through puberty and would be incapable of reproducing. One receptor that is not well studied for its role in GnRH neuron development and migration is fibroblast growth factor receptor 3 (FGFR3). This study aims to examine the role of FGFR3 in gonadal maturation and function by characterizing mice with a disruption in the FGFR3 gene, meaning that the FGFR3 receptors do not function well or at all in these transgenic mice. The onset of puberty was assessed by sex hormone-mediated events such as vaginal opening (VO) in females and balanopreputial separation (BPS) in males. Additionally, the reproductive potential of the adult mice was also assessed using viable sperm counts in males and reproductive hormone cyclicity in females. FGFR3 deficient females exhibited delayed vaginal opening and abnormal cyclicity. On the contrary, FGFR3 deficient male mice exhibited normal timing of BPS and normal sperm concentration compared to traditional mice. These results indicated that females were more adversely affected by FGFR3 deficiency than males. These results provide initial evidence that FGFR3 deficiency can disrupt female reproduction. Further, inactivating FGFR3 mutations may contribute to human reproductive disorders such as hypogonadotropic hypogonadism.

**Abstract:**

Vertebrate reproduction is driven by hormones from the hypothalamic-pituitary-gonadal axis. Previous studies showed that neurons from the hypothalamus secreting gonadotropin-releasing hormone (GnRH) require fibroblast growth factors (FGF) to develop and mature, but the specific role of fibroblast growth factor receptor 3 (FGFR3) in overall reproduction is unknown. This study aims to examine the role of FGFR3 in gonadal maturation and function by characterizing mice with a global inactivating mutation in the *FGFR3* gene. Pubertal onset was assessed by sex steroid-mediated events such as vaginal opening (VO) in females and balanopreputial separation (BPS) in males. Adult mice were also examined for estrous cyclicity in females and motile sperm concentrations in males. Female FGFR3 heterozygous global knockout mice ($FGFR3^{+/-}$) exhibited delayed VO and

abnormal estrous cyclicity with reduced time spent in diestrus. On the contrary, male *FGFR3*$^{+/-}$ mice exhibited normal timing of BPS and motile sperm concentration compared to WT mice. These results indicated that females were more adversely affected by FGFR3 deficiency than males. It is currently unclear if these female-specific adverse effects are exerted at the level of the hypothalamus, pituitary, or gonad. These results provide initial evidence that FGFR3 deficiency can disrupt female reproduction. Further, inactivating *FGFR3* mutations may contribute to human reproductive disorders such as hypogonadotropic hypogonadism.

**Introduction:**

Vertebrate reproduction requires proper development and function of the endocrine organs within the hypothalamic-pituitary-gonadal (HPG) axis. The cascade of hormones produced by this axis begins with the pulsatile release of gonadotropin-releasing hormone (GnRH) from the neuroendocrine hypothalamus. GnRH travels through the portal system where it acts on gonadotropes of the anterior pituitary and induces the release of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Finally, the gonadotropins travel through the general circulation and stimulate the gonads (ovaries and testes) to increase gametogenesis and the production of sex steroids. Effective communication within the HPG axis is essential to ensure adequate pubertal onset and fecundity.

Because GnRH is the most upstream activator of the HPG axis, defects in the GnRH system and HPG axis present a severe obstacle to fertility and reproduction. Therefore, it is of utmost importance to identify the factors needed for the development and maintenance of the GnRH system. There is growing evidence suggesting that the formation of the GnRH system is largely dependent on a class of signaling molecules, fibroblast growth factors (FGFs), and their receptors, fibroblast growth factor receptors (FGFRs), which collectively mediate the genesis, differentiation and migration of GnRH neurons (Chung et al. 2008). The FGF signaling family consists of 22 FGF ligands and 4 tyrosine kinase FGFRs. When an FGF binds the extracellular domain of the FGFR, the receptors dimerize, resulting in the cross phosphorylation of the intracellular domain. The cross phosphorylation induces a phosphorylation cascade, resulting in a variety of cellular effects.

FGF signaling molecules and FGFRs are present in the birthplace of GnRH neurons, the olfactory placode, at the time of neuron fate specification (Tsai et al. 2011). GnRH neurons emerge in the olfactory placode and migrate along the olfactory axons through the cribriform plate, where they reach their final destination, the preoptic area (POA) (Wierman et al. 2011). GnRH neuronal cell bodies remain in the POA while their axons extend to the median eminence. GnRH axon terminals release GnRH peptide into the hypophyseal portal system in a pulsatile fashion to induce the onset and maintenance of reproduction.

Although a critical relationship exists between the ligand FGF8 and receptor FGFR1 to allow proper GnRH neuron development, FGFR3, another FGF receptor present on GnRH neurons, has not been studied extensively (Chung et al. 2008). In humans, loss-of-function mutations in *FGFR1* are causally linked to hypogonadotropic hypogonadism (HH), a condition characterized by insufficient gonadotropins and therefore incomplete/absent puberty and infertility (Fraietta et al. 2013). However, a correlation between *FGFR3* mutations and HH has not been established in humans. There has been only one clinical report on a loss-of-function mutation in *FGFR3*, and no reproductive deficits were noted in the two male subjects studied (Makrythanasis et al. 2014). The correlation between FGFR3 and reproduction in rodents was similarly incomplete. One study suggested that FGFR3 did not affect the development of GnRH neurons but may be needed to maintain postnatal GnRH neurons, but it did not study the reproductive consequence of *FGFR3* deficiency (Chung et al. 2010).

The objectives of this study were to examine the timing of pubertal onset and adult reproductive function in *FGFR3* global knockout male and female mice. To assess the timing of pubertal onset, external indicators of puberty were examined: vaginal opening and first estrus in females and balanopreputial separation in males. Following pubertal onset, general reproductive functions including estrous cyclicity in females and motile sperm concentration in males were assessed by vaginal cytology and epididymal squash, respectively. These results should establish, for the first time, a direct correlation between *FGFR3* deficiency and reproduction that may be extrapolated to humans harboring inactivating *FGFR3* mutations.

## Materials and Methods:

### *Transgenic animals*

C57BL/6 mice with a global heterozygous deletion in the *FGFR3* gene were obtained from the Jackson Laboratories in Bar Harbor, ME and further propagated by breeding. ... Deletion of these components resulted in a truncated nonfunctional FGFR3.

[...]

### Phenotypic pubertal assessment

Male mice were checked for balanopreputial separation (BPS) by gently retracting the prepuce from the glans penis from PN20 until sufficient separation was apparent. ...

Female mice were examined for the age of vaginal opening (VO) by visual examination of the vulva from PN20 until a clear opening is present. ...

### Vaginal cytology

Once VO in female peripubertal mice (around PN30) had occurred, females were examined daily for vaginal cytology to determine their estrous cycle stages.

[...]

### Motile sperm concentration measurement by epididymal squash

At PN60, male mice were anaesthetized using isoflurane and rapidly decapitated. A V-shaped abdominal incision was made, and epididymal fat pads were gently pulled to expose the testes and epididymis. The epididymis was isolated from each testis and placed in a petri dish with 2 mL of prewarmed (37 °C) phosphate-buffered saline (PBS). Both epididymides were used for this procedure. The epididymal cauda was isolated from the rest of the epididymis and vas deferens, minced with two scalpel blades and incubated in 2 ml of PBS at 35 °C for 15 minutes with gentle agitation. Following, 500 µl of sperm-containing PBS was mixed with 500 µl of 3% NaCl to immobilize sperm for consistent counting. The solution containing immobilized sperm was then vortexed and a 10-µl aliquot was used for counting by a hemacytometer. Of the 25 grids, four corner grids and one center grid were counted for number of sperm. Sperm that were not fully enclosed in the grid were only counted if they passed over the top or left line of the grid. Average number of sperm contained in the 5 grids was calculated. The average number of sperm was multiplied by the dilution factor, the number of total grids, and the volume of the hemocytometer to result in a final concentration of sperm per mL.

### Statistical analysis

Homoscedasticity was observed for all data, thus differences between genotypes were determined by Students *t*-test. Differences were considered significant when $p<0.05$.

**Results:**

*Phenotypic pubertal assessment*

[...]

*First estrus*

[...]

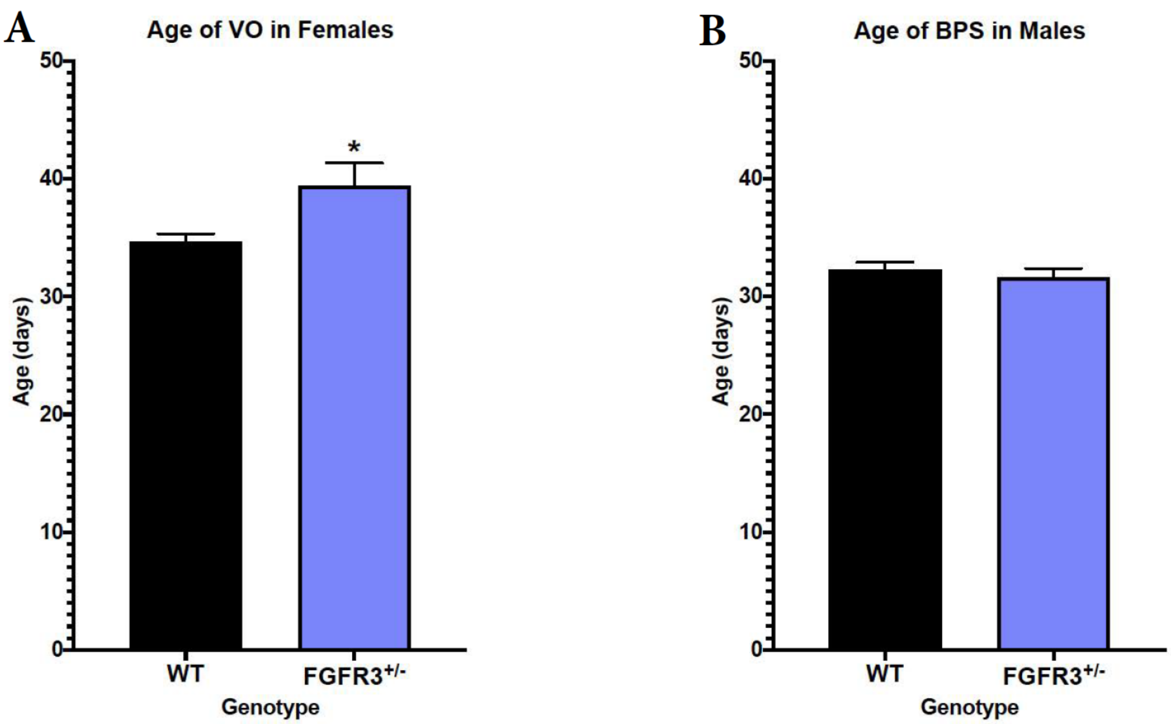


**Figure 1.** (A) Age of VO in *FGFR3*$^{+/-}$ females is significantly delayed compared to WT females (N=8-9). (B) Age of BPS is not significantly different between *FGFR3*$^{+/-}$ and WT males (N=4-5). All data represent mean ± SE.

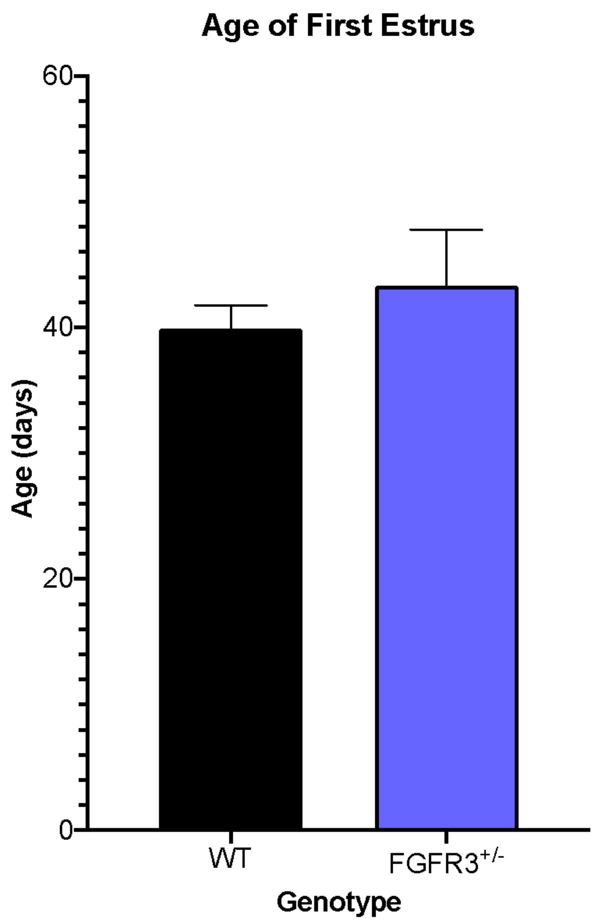


**Figure 2.** Age of first estrus is not significantly different between WT and *FGFR3*$^{+/-}$ females (N=6-9). All data represent mean ± SE.

Days in estrus

[...]

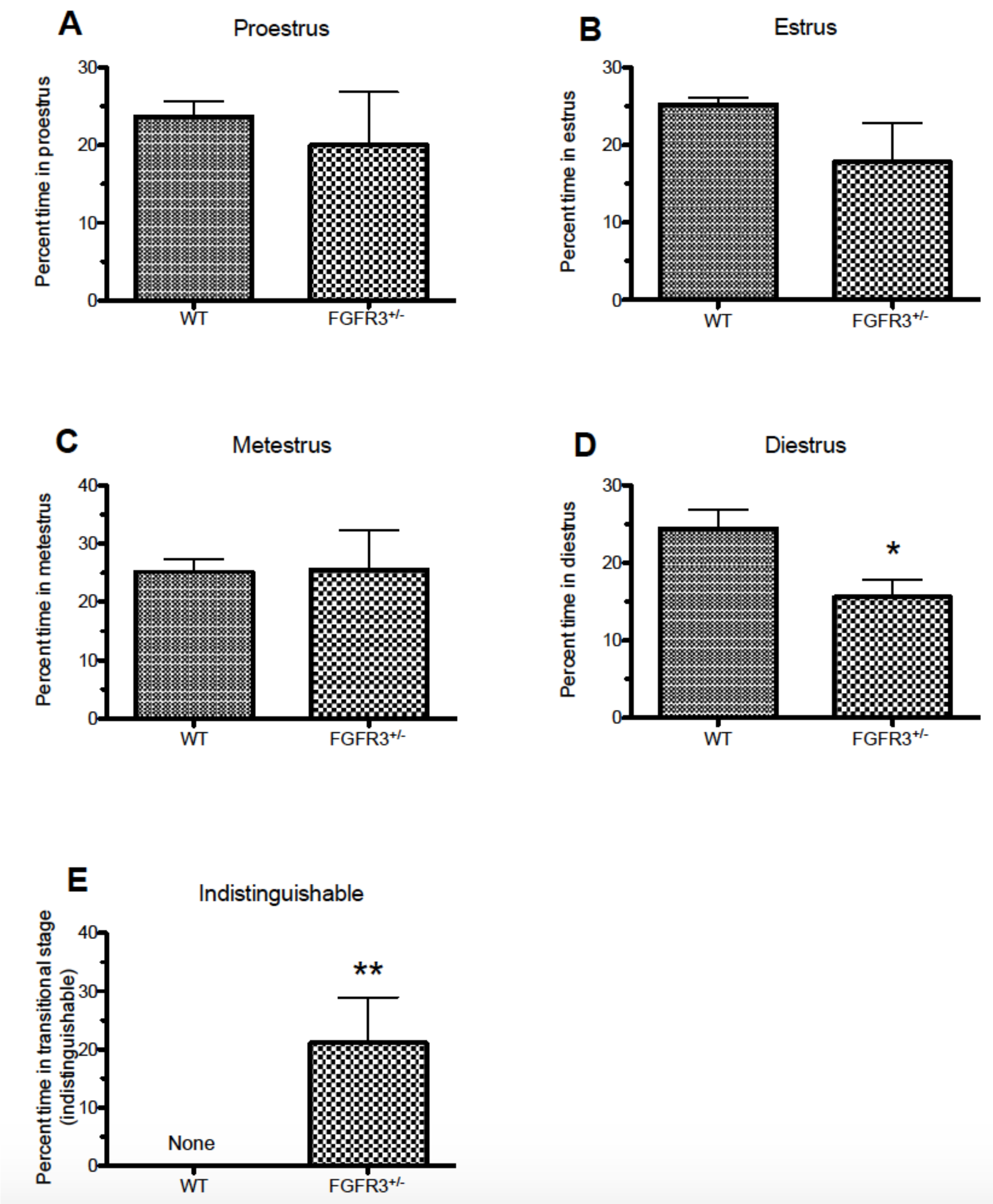


Figure 3. (A) Percent of time in proestrus over 15 days is not significantly different between WT and *FGFR3*$^{+/-}$ mice (N=9, N=5). (B) Percent of time in estrus over 15 days is not significantly different between WT and *FGFR3*$^{+/-}$ mice (N=9, N=5). (C) Percent of time in metestrus over 15 days is not significantly different between WT and *FGFR3*$^{+/-}$ mice (N=9, N=5). (D) Percent of time in diestrus over 15 days is significantly different between WT and *FGFR3*$^{+/-}$ mice (N=9, N=5) ($p<0.05$). (E) Percent of time in an indistinguishable stage over 15 days is significantly different between WT and *FGFR3*$^{+/-}$ mice (N=9, N=5) ($p<0.05$). All data represent the mean ± SE.

Motile Sperm Concentration

[...]

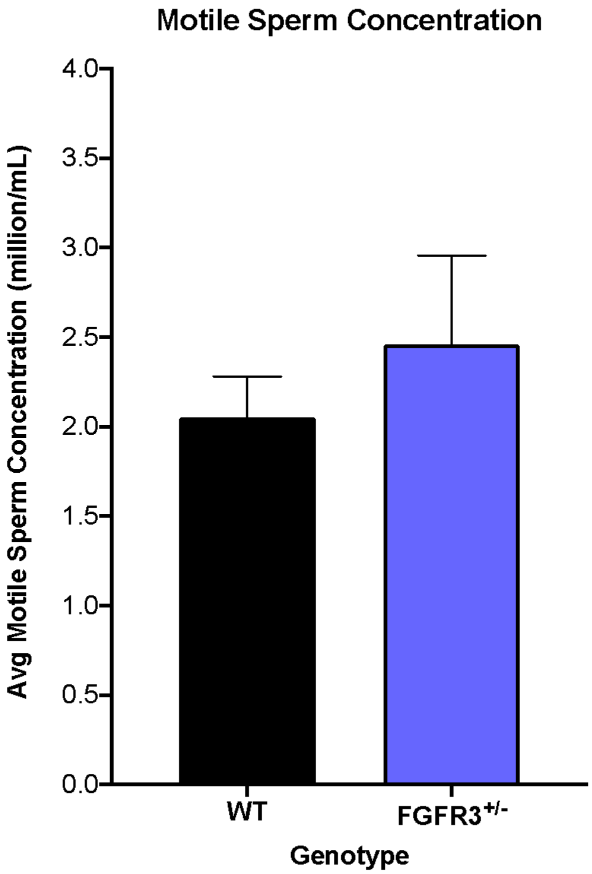


**Figure 4.** Motile sperm concentration obtained by epididymal squash of the cauda epididymis was not significantly different between PN60 WT and *FGFR3*$^{+/-}$ males (N=3-5 respectively). All data represent mean ± SE.

**Discussion:**

Characterization of the reproductive function is fundamental to understanding the effects of *FGFR3* deficiency on the HPG axis. Previously studies showed that *FGFR3*$^{+/-}$ mice were born with a normal set of GnRH neurons but later exhibited a postnatal loss (PN60) of these neurons (Chung et al. 2010). However, the reproductive consequence of this neuronal loss was not examined. In addition, *FGFR3* is also expressed in the pituitary and gonad (Sharma et al. 2019, Kaminskas et al. 2019), suggesting it may directly mediate the functions of these two downstream organs. This study aimed to examine the onset of puberty in young FGFR3-deficient mice as well as the maintenance of reproduction in older adults.

External pubertal markers for both female and male mice were assessed. BPS is an androgen-dependent event that coincides with the maturation of the testes and is an external indicator of puberty (Korenbrot et al. 1977). Our results showed that WT and *FGFR3*$^{+/-}$ males attained BPS at the same time (Figure 1B), suggesting their circulating androgen levels were likely to be similar. These results suggest that the postnatal loss of GnRH neurons in *FGFR3*$^{+/-}$ males may not have yet occurred at the time of BPS (around PN30).

Another indication of puberty, VO, was determined after weaning (PN20) of female mice. Vaginal opening is initiated by a surge of estrogen occurring around the time of puberty in female mice (Ojeda et al. 1994). Although their male counterparts achieved puberty at a normal age, *FGFR3*$^{+/-}$ females exhibited delayed VO compared to WT (Figure 1A). These results suggest a potential lack of adequate estrogen production to induce apoptosis of lower vaginal mucosa (Rodriguez et al. 1997), leading to delayed VO. Decreased estrogen levels could result from decreased GnRH neurons found in *FGFR3*$^{+/-}$ mice at PN60. Interestingly, *FGFR3*$^{-/-}$ mice (N=3) never achieved VO even 72 days after birth (data not shown). These mice died at various ages with the oldest being PN72, yet never underwent VO at the time of death. Absence of VO in these mice could be due to a complete absence of functional *FGFR3* allele, resulting in absent or insufficient GnRH neurons and therefore no pubertal onset. That being said, *FGFR3*$^{-/-}$ mice suffer significant musculoskeletal deformities and may experience issues unrelated to primary GnRH deficiency, such as reduced feeding or enhanced stress, that could suppress the HPG axis (Rivier et al. 1991).

Evaluating the age of first estrus is particularly relevant to fertility in females because it indicates not just an estrogen surge sufficient to induce VO, but the beginning of ovarian cyclicity. Despite a delayed VO, *FGFR3*$^{+/-}$ and WT females attained the first estrus at the same time. These results suggest that like humans, puberty in mice consists of many stages, and FGFR3 deficiency may influence the earlier (VO) but not the later (first estrus) stages.

Over a 15-day period, females were examined for estrous cyclicity, including the number of days spent in each stage. WT and *FGFR3*$^{+/-}$ mice spent the same percentage of time in proestrus, estrus, and metestrus (Figure 3A, 3B, 3C). Interestingly, WT spent 8.88% more time in diestrus compared to *FGFR3*$^{+/-}$ mice (Figure 3D). Additionally, *FGFR3*$^{+/-}$ mice spent 21.11% of their cycle in an indistinguishable phase consisting of little to no cells and severely crenated cells (Figure 3E). Abnormal GnRH numbers could disrupt HPG axis signaling and interfere with normal estrous cyclicity. Lack of gonadotropins and sex steroids due to a GnRH deficiency could produce the indistinguishable estrous phase seen in these mice. Ovarian analyses should be performed to examine the morphology of a follicle at this indistinguishable phase. Additionally, levels of gonadotropins and sex steroids should be examined for a potential discrepancy. Abnormal estrous cyclicity can be seen in *FGFR3*$^{+/-}$ mice and may cause a decrease in fertility as compared to WT mice.

Male mice were evaluated for fertility by examining motile sperm concentration in adulthood (PN60). Despite the previously shown reduction in GnRH neurons in PN60 *FGFR3*$^{+/-}$ males (Chung et al. 2010), motile sperm concentration at PN60 was not significantly different between WT and *FGFR3*$^{+/-}$ males (Figure 4). Overall, male mice appear to be less affected by *FGFR3* deficiency than female mice. The cause of this sex difference is unknown. Multiple studies have reported that females are, in general, more vulnerable to HPG axis disruption due to a need for robust hormonal changes to drive the female cycle. The precise locus along the HPG axis leading to this sex difference remains to be investigated.

Our results showed that FGFR3 deficiency did not impact male puberty and reproductive function, but significantly disrupted female pubertal onset and cyclicity. These results are novel and suggest FGFR3 inactivating mutations in humans may similarly impact certain aspects of women's reproduction. Future studies should examine tissue-specific *FGFR3*$^{+/-}$ knockout mice to ensure reproductive defects are due to a suboptimal HPG axis and not secondarily to defects in musculoskeletal functions (Kubota et al. 2020). Additionally, the precise extra-hypothalamic loci of the HPG axis impacted by *FGFR3* deficiency, such as the pituitary and the gonad, should be investigated.

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# Effects of Cross-Sex Hormone Replacement Therapy on Fertility in Transgender Patients: A Systematic Review

Arin Barth

The following is an excerpt from a longer piece. For full text, please visit https://scholar.colorado.edu/concern/undergraduate_honors_theses/2n49t2555

## Lay Summary

Although 62% of transgender patients want to have biological kids, 83% of their healthcare providers have never discussed the possible effects of hormone replacement therapy (HRT) on their fertility. Transgender people are often prescribed hormones as a part of gender affirming treatment to change traits that are typically perceived as gendered. Testosterone and estrogen regulate our reproductive systems and impact secondary sex characteristics, such as facial hair and breast development. These hormones can affect one's ability to have kids; however, the extent of that effect is not well researched.

In this paper, I compiled data from 39 studies on the effects of HRT on transgender patients' reproductive systems. I compared and contrasted findings to identify trends and performed statistical analysis to determine the extent of the impact of HRT on the reproductive system. I found that in patients with ovaries, testosterone did not significantly affect the release of egg cells, and only moderately affected the inner layer of the uterus. Meanwhile, estrogen negatively impacted semen quality and therefore fertility. The effect on the reproductive system varied widely from person to person. If HRT was stopped, fertility would generally return to what it was before HRT, but research on long-term effects is especially limited.

Currently, trans people's reproductive choices are limited by lack of research and uninformed healthcare providers, as well as other systemic barriers. This paper provides a comprehensive resource describing our current understanding of this topic. Prioritizing further research in this area will ensure that transgender individuals are empowered to make informed decisions about their reproductive futures.

## Abstract

A growing area of research among the scientific community is the methods and effects of medical transition for transgender patients. One common component of medical transition is the administration of exogenous cross-sex hormones to patients to obtain a masculinized or feminized phenotype, referred to as hormone replacement therapy (HRT). There is limited research on much of these effects, including the effects of HRT on patients' fertility. In this study, I conducted a systematic review of research on the impacts of HRT on fertility, including calculating risk ratios and etiologic fractions. Thirty-nine papers were examined both qualitatively and quantitatively to assess the impacts of HRT on semen quality, ovulation, polycystic ovary morphology, and endometrial function. This study found that HRT reduces fertility in transgender patients, but not universally or completely; there is still evidence of fertility during HRT administration in many patients. Following discontinuation of HRT, reduced fertility will return to its pre-HRT status in most patients. This research will help clinicians counsel their transgender patients on fertility before and after their medical transition.

## Terminology and Abbreviations List

**Assigned female at birth (AFAB):** Describes an individual who was assigned a female sex based on their external genitalia at birth.

**Assigned male at birth (AMAB):** Describes an individual who was assigned a male sex based on their external genitalia at birth.

**Cisgender:** Describes an individual whose gender identity aligns with their assigned sex.

**Estrogen (E):** The primary hormone involved in hormone replacement therapy (HRT) for AMAB individuals. Administration results in a more feminine appearing phenotype for these individuals.

**Gender dysphoria:** An experience of distress or discomfort brought on by a conflict between one's gender identity and sex assigned at birth. Gender dysphoria is experienced by many but not all transgender individuals.

**Gender-nonconforming:** Describes an individual whose behavior or appearance does not conform to prevailing cultural and social expectations about what is appropriate for their gender. These individuals may or may not identify as transgender.

**Hormone replacement therapy (HRT):** The process of administering one or more exogenous hormones to transgender patients to achieve a physical appearance more congruent with their gender identity. One component of medical transition.

**Intersex:** Describes numerous conditions in which sex development does not progress in a manner consistent with typical pathways of "male" or "female." Intersex infants are often coercively assigned a sex at birth, sometimes along with surgical intervention to mimic typical genitalia.

**Medical transition:** The process of undergoing medical treatments to change one's body to better align with one's gender identity. This could include hormone replacement therapy (HRT), voice therapy, laser hair removal, or surgeries such as mastectomy, genital reconstruction surgery, or facial feminization surgery. Many but not all transgender individuals undergo some form of medical transition.

**Nonbinary/genderqueer:** Umbrella terms for individuals whose gender identity falls outside of or is not confined to the established gender binary. These terms fall under the "transgender" umbrella, but there are nuanced social, political, and cultural differences between them, and they are not interchangeable.

**Testosterone (T):** The primary hormone involved in hormone replacement therapy (HRT) for AFAB individuals. Administration results in a more masculine appearing phenotype for these individuals.

**Transgender:** Describes an individual whose gender identity differs from their assigned sex.

**Transgender men:** Individuals whose gender identity is male and who were assigned female at birth (AFAB).

**Transgender women:** Individuals whose gender identity is female and who were assigned male at birth (AMAB).

## Introduction

Medical care for transgender individuals is a growing field in health care as broad cultural awareness and acceptance of diverse gender identity increases.... One common component of medical transition is hormone replacement therapy (HRT), where one or more exogenous sex hormones are administered to change a patient's hormone profile and therefore, the phenotype of some secondary sex characteristics.

[...]

Many of the full-body effects of HRT are moderately well-researched and understood; however, research on the effects of HRT on fertility is lacking.

[...]

In AMAB individuals taking HRT, high serum estrogen could result in reduced nutrients, growth factors, and proteins needed for proper sperm development and impaired release of mature spermatids (Figure 1c).

[...]

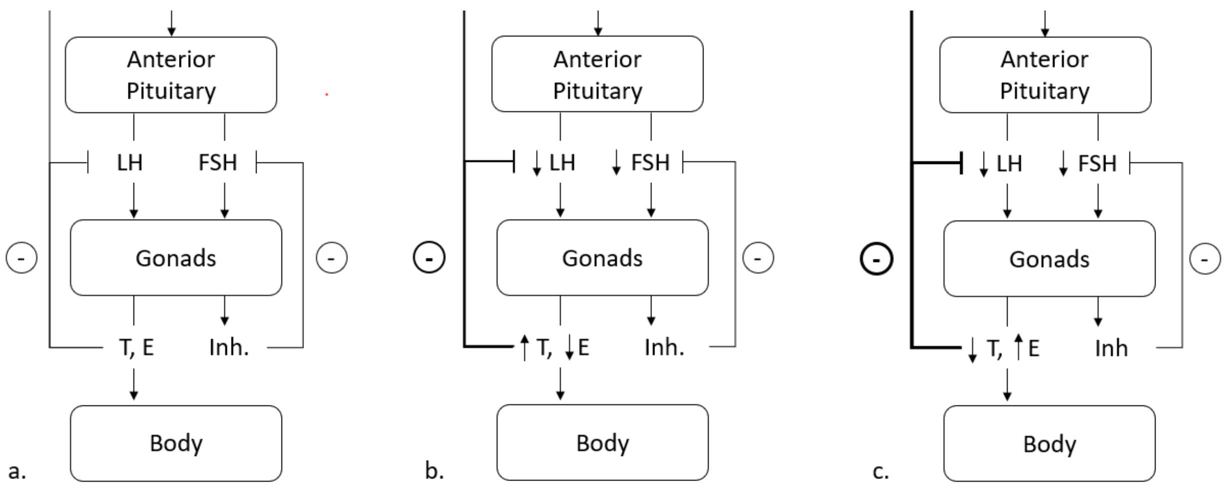


Figure 1: Diagram of the typical HPG axis (a), an HPG axis with interference by supraphysiological exogenous testosterone (b), and an HPG axis with interference by supraphysiological exogenous estrogen (c). Arrows between boxes indication stimulation and (-) indicates feedback regulation. Small arrows indicate increased or decreased hormone levels relative to baseline. Bold lines indicate increased strength of negative feedback. Conversely, high serum testosterone in AFAB patients is less likely to disrupt the hormone cycles necessary for ovulation because of the intricacies of the feedback mechanisms (Figure 1b).

[...]

Overall, I hypothesize measures of fertility in transgender patients on HRT to be significantly reduced relative to cisgender controls, but not indicating a complete absence of fertility among the population.

**Methods**

*Literature Search*

A literature review of the biomedical database PubMed was performed from August 28, 2019 to October 17, 2019.

[...]

Data Analysis

Risk Ratios (RR) and confidence intervals (CI) were calculated for eight studies: six in AFAB subjects, and two in AMAB subjects.

[...]

Standard errors (SE) were calculated from CIs ....

The etiologic fraction (EF) indicates the proportion of the changes to a specific outcome that can be attributed to the treatment applied, which is HRT for the purposes of this study.

[...]

For outcomes which had a RR less than one, the EF calculated by this method is not meaningful, so an alternative etiologic fraction was (AEF) was calculated.

[...]

Prevalence rates were calculated for various outcomes in all patients taking HRT in all studies: presence of a corpus lutea or corpus albicantia, endometrial activity, presence of endometrial polyps, polycystic ovary morphology, and spermatogenesis.

[...]

**Results**

*Literature Search Results*

During the literature search, 567 articles were screened for relevancy. Two hundred and twenty studies in non-humans were excluded and 347 human studies were screened for relevancy. Of these, 39 articles were determined to meet the criteria and were relevant to the research.

[...]

Results in AMAB Patients

Twenty relevant articles in AMAB patients were examined for this analysis (Table 1). Risk ratios, 95% confidence intervals, and standard errors were calculated for two papers in transgender women (Figure 3, Table 2). Data in this section is listed as "RR (Lower CI-Upper CI, SE)."

All of the RRs I calculated were greater than 1, which indicated increased risk of sperm abnormalities in transgender patients relative to cisgender controls.

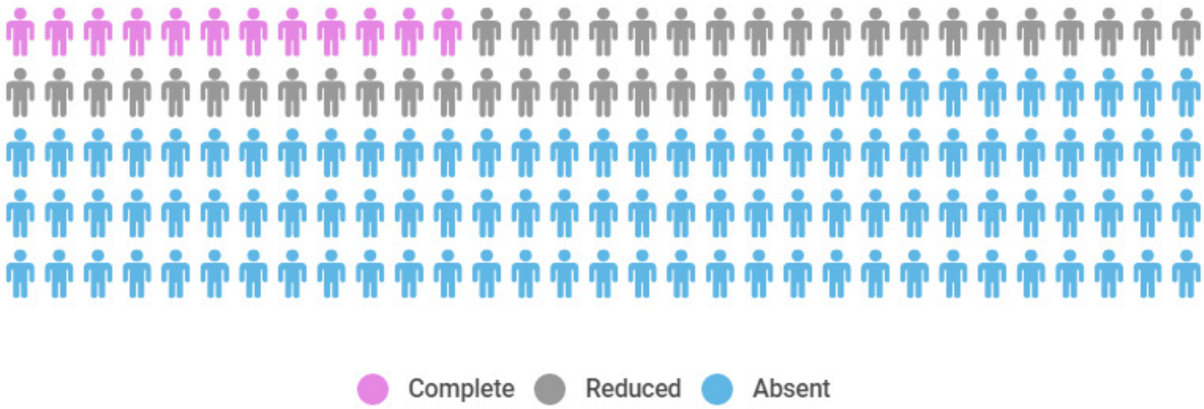


Figure 4: Calculated prevalence rates of observed complete, reduced, or absent spermatogenesis in the testes of AMAB transgender people taking HRT.

[...]

### Results in AFAB Patients

Nineteen articles in AFAB patients were examined for this analysis. Risk ratios, 95% confidence intervals, and standard errors were calculated for six papers in AFAB patients (Figure 5, Tables 4 and 5).

Several outcomes resulted in RRs greater than 1, indicating increased risk of the relevant outcome in transgender patients relative to cisgender controls.

[...]

Several other outcomes had RRs less than or equal to 1, including several studies noting the presence of a corpus lutea and/or albicantia.

[...]

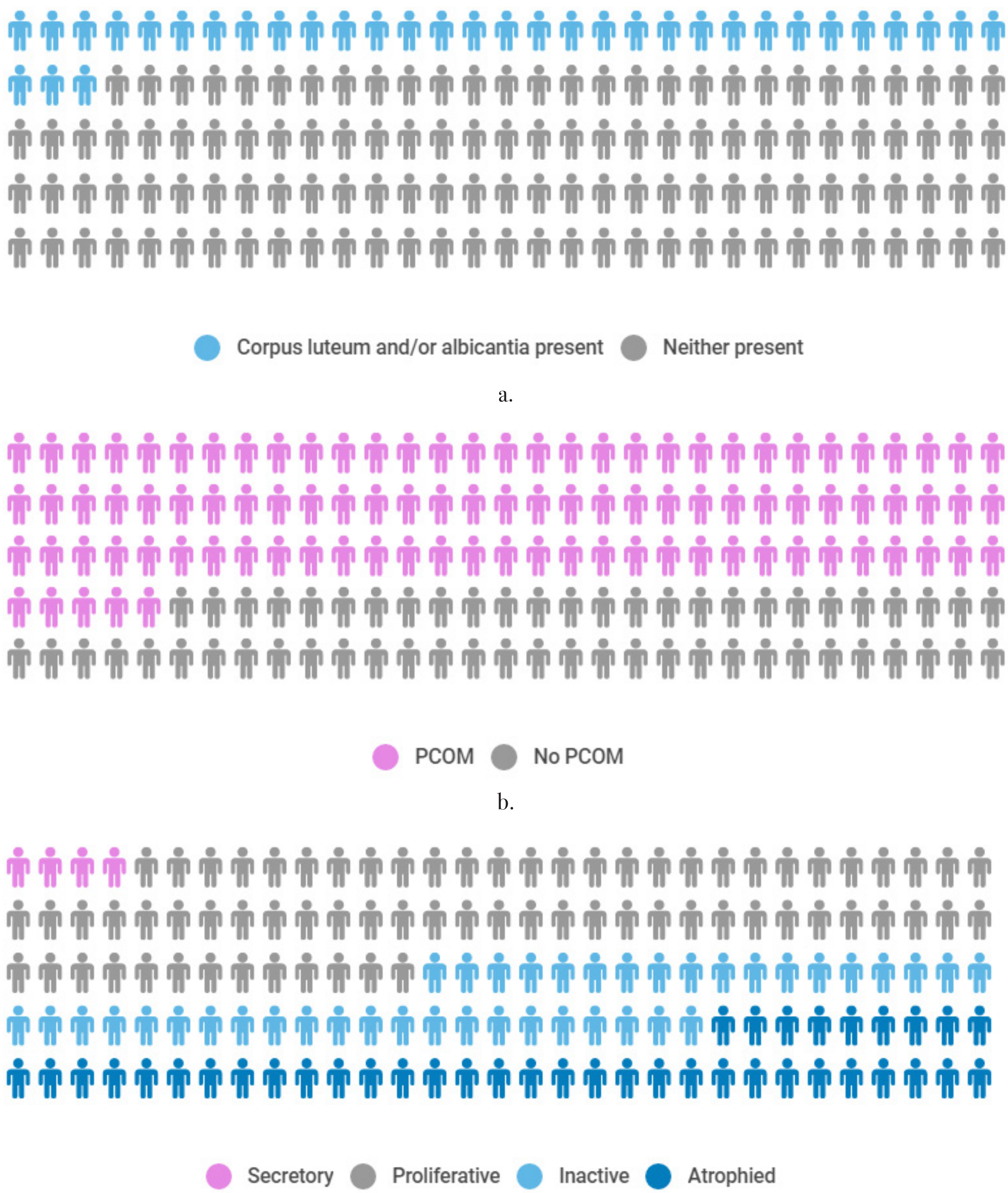


Figure 6: Calculated prevalence rates of evidence of ovulation (a), PCOM (b), and endometrial states (c) observed AFAB transgender people taking HRT.

## Discussion

### *Effects in AMAB Patients*

Although there is some disagreement between studies, semen quality in AMAB transgender patients currently using HRT appears to be significantly lower than in cisgender controls. Transgender patients were shown to have a statistically significant increased risk of oligospermia (decreased sperm concentration) and of teratospermia (abnormal sperm morphology) compared to cisgender controls.

[...]

### Effects in AFAB Patients

One of the most important factors in whether an AFAB individual can conceive is the presence or absence of ovulation. Evidence of ovulation in AFAB transgender individuals taking hormone replacement therapy were not necessarily significantly reduced.... Taken together, these data indicate that HRT does not necessarily negatively impact ovulation in AFAB patients.

[...]

Hormone replacement therapy did not appear to result in endometrial atrophy in most AFAB patients.

[...]

### Effects Following HRT Discontinuation

Following discontinuation of HRT in AMAB patients, sperm quality seems to largely return to pre-HRT levels. ... These data indicate that semen quality return to baseline levels according to most measures in as little as 6 weeks post discontinuation of HRT in AMAB patients.

There is limited data on the fertility of AFAB patients following discontinuation of HRT. ... Hormone replacement therapy in AFAB patients does not result in long-term infertility.

[...]

### Non-hormonal Factors

It is important to consider factors besides HRT that could result in reduced fertility in transgender patients. It was not possible for this analysis to control for possible confounding variables, but differences in fertility before initiation of HRT are important to consider. Many studies recorded baseline measures for their subjects, otherwise assessed pre-treatment parameters, or studied patients prior to initiating HRT.

[...]

### Strengths and Limitations

This is the first broad systematic review of the effects of HRT on fertility in both AFAB and AMAB subjects. However, this study was limited in several ways. First, I was unable to perform meta-analysis. Relatively few studies met all the criteria for this type of analysis, because of failure to use controls, fully discuss the results of subjects in control groups, or because the sample sizes were too small to provide adequate data for a defined risk ratio. If a meta-analysis had been possible, it would have lent another dimension of statistical analysis to the data, improving the overall understanding of the overall findings of the research available.

[...]

This review of existing research makes the scientific understanding of fertility on HRT more accessible to health care providers, so that they can accurately counsel their patients on how HRT may affect their fertility, so that patients can more easily make informed decisions about their bodies.

Additionally, this is the only study that I know of that focuses on the effects of HRT, or any other aspect of medical transition, on transgender patients, that was authored by a transgender researcher.

[...]

Research into transgender bodies is not complete without an understanding of transgender communities....

### Conclusions

There is a shortage of data on the effects of HRT on the fertility of transgender individuals, and what data does exist is often conflicting or inconclusive. The data we have indicates that fertility may be somewhat, but not completely reduced in transgender patients. Effects of HRT do not appear to increase past initial changes to cause more significant changes with increased duration, and HRT does not result in long term loss of fertility; following discontinuation of the medication normal fertility should return in most patients. However, there is some possibility that reduction in semen quality in AMAB transgender patients could be present prior to HRT, and therefore related to other factors such as stress and anxiety. Transgender patients hoping to avoid pregnancy should use birth control methods and not consider their HRT regimen to be a substitute for birth control. Transgender patients hoping to conceive should be able to do so but may wish to consider discontinuing HRT to raise their chances. Transgender patients who were assigned female at birth will have to discontinue HRT use to sustain a healthy pregnancy.

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[…]

# Little Ambergris Cay, a Case Study for Ooid Rich Island Development on the Turks and Caicos Carbonate Platform

Drew G. Brown

The following is an excerpt from a longer piece. For full text, please visit https://scholar.colorado.edu/concern/undergraduate_honors_theses/8910jv661

**Lay Summary:**
This study proposes a hypothesis for the formation of an island located within the Turks and Caicos carbonate platform, named Little Ambergris Cay. Little Ambergris Cay is unusual in that it formed during a period of sea level rise within the Holocene. This anomaly coincided with unique sediment characteristics, as the island is dominantly composed of ooids. Ooids are concentrically coated calcium carbonate sand grains. In this circumstance, the rapid accumulation of ooids resulted in island formation. This coupling of high ooid concentrations alongside sea level rise provided an opportunity to build a new model for modern carbonate island growth. This study used data from the loose sediments of Little Ambergris Cay, such as grain type, grain size, biological composition, and roundness of grains, compared with different carbonate islands data, allowing us to track changes in environment over time. By performing this comparison, we can see how Little Ambergris Cay formed within the previous five thousand years. Through interpreting the changes in environment over this time, we draw conclusions about formation direction and the possible reasons for accumulation during sea level rise. This study provides a unique model for carbonate island formation that has relevance in the carbonate sedimentology field.

**Abstract:**
Little Ambergris Cay (LAC) within the Turks and Caicos is a useful field site to examine the accumulation of a carbonate island, within a typical carbonate platform environment, because it does not follow the typically accepted modes of island accretion. There are several methods used to describe carbonate island formation after Schlager (2003), who described carbonate "factories" in reference to the modern analogues of Florida and the Bahamas. The Turks and Caicos, and specifically Little Ambergris Cay, offers a field side that differs from the comparison analogues in sediment composition, energy flux, and Holocene development. Little Ambergris Cay is unusual in that it formed during a period of sea level rise (Figure 2, Toscano & Macintyre, 2003), and thus presents an opportunity for research on how modern carbonate platform environments accumulate through time. This paper presents an argument for eastward direction of island accumulation of Little Ambergris Cay. Based on the research of Trower et al. (2018), Dravis & Wanless (2008), and Schlager (2003), I interpret that Little Ambergris Cay formed via eastward accumulation based on the influence of wind-wave current energy as means of supplying ample sediment to a zone of accumulation; in other words, in this system, sediment supply outpaces accommodation space. To support this interpretation, I will present several sets of data that were derived from unlithified sediment cores collected from the interior of LAC as means to characterize and compare against representative environments. First, I performed thin section point-count analysis on loose

sediments from a variety of depths in a representative collected core to identify how grain composition varied with depth. I then compared this point count data with a range of grain characteristics such as grain size, sorting, sphericity, etc., determined via Camsizer analysis, in order to determine the parameters that best described the changes in grain concentration as seen in the point count. Finally, by integrating plots of the grain size parameters determined in this paper vs. depth, stereoscope microscopy, and radiocarbon ages of core sediments, I compared representative facies of modern carbonate environments to determine a facies development through time of Little Ambergris Cay. I concluded that Little Ambergris Cay underwent a series of high to low energy facies changes, where early lithification on the south-western edge of the modern LAC allowed successive accumulation of incoming easterly ooids due to sediment entrapment, possibly due to microbial mat and bedrock influence. The incoming sediment grains from the east onto an early lithified fragment to the west allowed eastward accumulation, where facies changed from an ancient shoal to a modern island. This paper furthers the research of modern carbonate analogues and their interpreted growth patterns. LAC offers a unique environment that does not follow typical carbonate island development, and by providing a case study of wind-wave influenced island accretion, sectors of geologic interest have the opportunity to interpret unique climatic and chemical factors. The petroleum unconventional subsurface carbonate play search is greatly related to LAC, as grainstones offer potential reservoirs (Dravis & Wanless, 2008). The geochemical field may have interest in the accumulation of LAC, as specific seawater concentrations play a role in the uniquely high concentrations of ooids found on the Caicos Platform. The geobiological field may be interested in LAC, as the relatively low abundance of microbes and biotic influence differs greatly from the other modern analogues for carbonate platform development.

**Introduction:**

Little Ambergris Cay (LAC, Figure 1) within the Turks and Caicos is known to be composed of dominantly Holocene aged carbonate sediments (Orzechowski et al., 2016). Toscano & Macintyre (2003) recognized that a period of sea level rise occurred in the Atlantic Holocene. Generally speaking, using the Bahamas and Florida as a modern analogue for carbonate platform development, the most common way for carbonate accumulation to outpace or remain at increasing sea level is through a process of "backstepping," or retrogradation of the platform that is primarily influenced by reef growth (Schlager, 2003). Reefs are rare on the Caicos platform, so considering that we instead primarily observe Holocene ooids (Dravis and Wanless, 1989), and recognizing that LAC accreted past sea level during a period of increasing accommodation space, it then becomes clear that LAC does not follow the most obvious modern models of island formation in the Holocene. This anomaly offers an opportunity to better understand how and why formation of this carbonate platform island occurred in such conditions.

[...]

Before research was conducted, I developed hypotheses regarding the question of how an island forms during a period of sea level rise. I hypothesized that, in order for an island to form, sediment supply must have outpaced sea level rise (accommodation space). Trower et al. (2018), proposed a westward growth hypothesis of LAC, which I decided to test through facies development and stratigraphy. It is known that current and wind influence westward migration of sediment (Trower et al., 2018; Dravis and Wanless, 2008), and Big Ambergris Cay lies to the east of LAC and is


Figure 1:
A: A map of Little Ambergris Cay with associated core locations.

older in age. Given this observation, I hypothesize that sediments from BAC are migrating to LAC where early lithification of ooid grainstones could have trapped incoming sediments. I therefore also developed an alternative hypothesis that Little Ambergris Cay accreted eastward, towards BAC, as a function of incoming sediment supply onto a pre-existing bedrock.

Three steps will be taken in testing these hypotheses (westward vs eastward accretion), by analyzing sediment sample characteristics from cores collected on the interior of LAC. Using point counting data (Figure 3), I am able to determine sediment composition within the VC03 core. Secondly, I used Matlab to analyze Camsizer data, and was able to determine characteristic grain size (D10, D50, D90), mean roundness, and sorting for the other cores (VC01-VC08). By comparing the point counting data to the Camsizer data for VC03, I determined that the roundness, grain size and sorting parameters correlated to the interpreted grain composition.... The third and final step was to use both the point counting and Camsizer data, compared against a set of thin sections and Camsizer data, for samples that represented modern depositional environments to determine facies and environmental change over time on LAC.

Based on the conclusions that I draw in these three steps, using the parameters described above, I will test that the westward growth hypothesis of Little Ambergris Cay (Trower et al., 2018) is not supported by the data analyzed in this paper. Rather, based on the facies development of the VC cores and the comparison facies deposits of Table 1 & 2, I will attempt to support an eastward growth hypothesis of Little Ambergris Cay.

## Materials and Methods:

[...]

### Sediment Core and Sample Collection

I began work on a set of sediment samples collected from a variety of depths in eight sediment cores previously collected in 2017 and 2018 via vibracoring.

[...]

### Point Count Analysis Data

The first step was to conduct a point count for sediment composition using JMicrovision, an image analysis toolbox. Analyzing 11 thin sections of the loose sediment in VC03, at each successive depth (up to 220cm), I performed a 400-sample point count for each increment depth.

[...]

### Grain Size and Shape Data

The next step was to analyze grain size and shape data, which had previously been measured via a Retsch Camsizer P4, from all the cores through a Matlab code that was able to process the $D_{10}$, $D_{50}$, $D_{90}$, mean roundness, mean sphericity, mean aspect ratio, roundness, and sorting.

[...]

### Stereoscope Microscopy

Stereoscope microscopy was performed on the four samples.... This imaging allowed me to qualitatively assess the abundance of ooids and skeletal grains, and confirm the Camsizer sorting, roundness, and diameter data.

### Radiocarbon Data

Radiocarbon dating was performed on 11 samples (Figure 2 & FA-2-A and FA-4-A) via Accelerator Mass Spectrometer (AMS) at the Keck-Carbon Cycle AMS facility at UC Irvine and the NOSAMS facility at WHOI.

[...]

### Facies Correlations to Modern Depositional Environments

...By collecting the sorting and roundness data for the representative environments, I was able to compare the previously processed sorting and roundness data of the VC cores and correlate each depth's values to the associated representative environments. This allowed me, along with the radiocarbon age of the given cores, to formulate an idea of how facies changed over time.

**Results:**

The ages of the three cores (ranging from youngest to oldest) display similar trends vs. depth between each core. CC-J18 and VC03 are in similar positions on the northern side of LAC, while FML-J18A is located on the eastern side of LAC (Figure 1.A) Corrected radiocarbon age for exposed bedrock samples FA-2-A & FA-4-A (Figure 1.C) show ages of 5946BP & 5220BP. The bedrock has been interpreted as a cross-stratified eolianite ooid grainstone (Orzechowski et al. 2016).

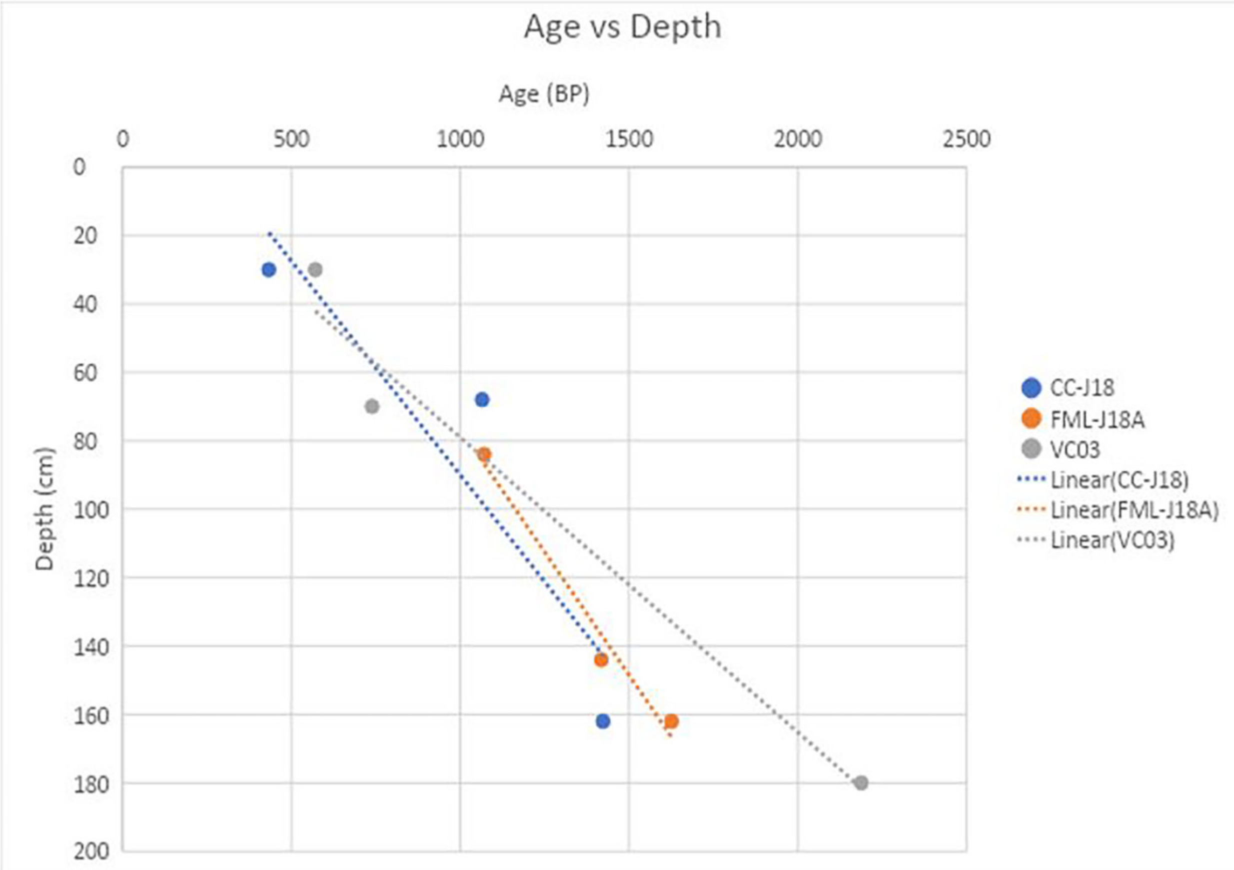


Figure 2: Corrected radiocarbon data for three cores collected on northern LAC.

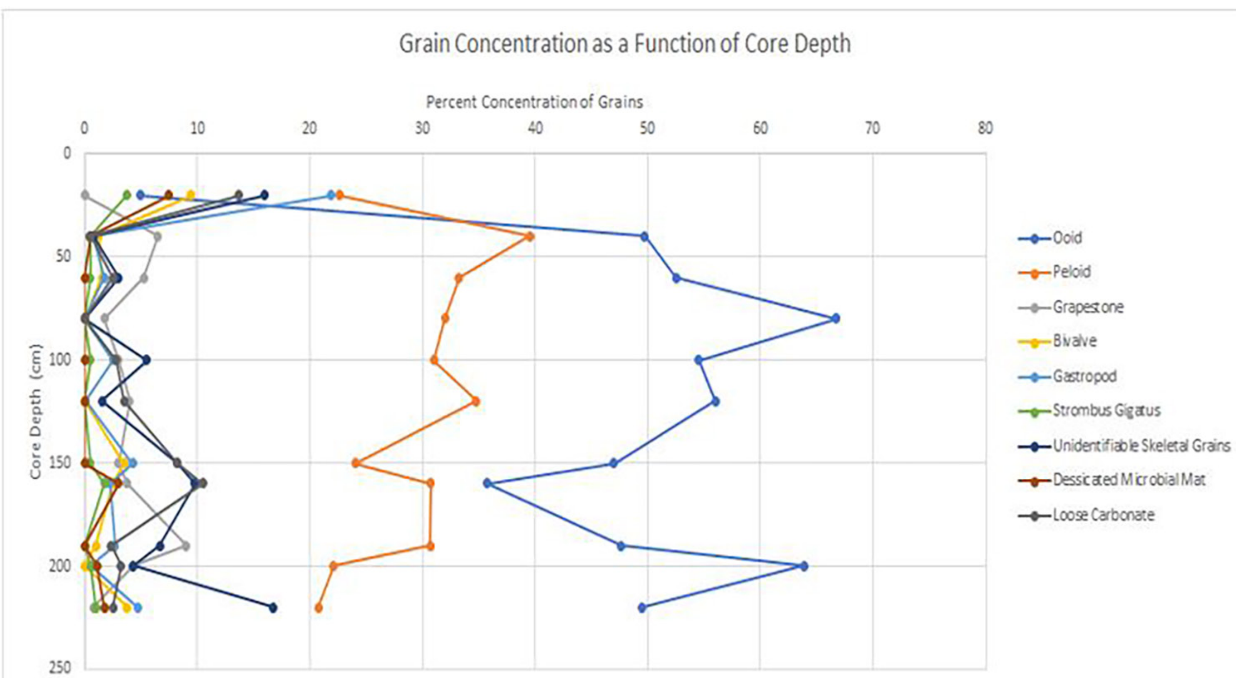


Figure 3: The scatter plot above shows sediment compositions a function of core depth.

The data was determined through a 400-sample point count for each depth in VC03. Trends imply that skeletal grains were abundant in the shallower and deeper sections of the core, and ooid abundance increased in the mid-core (60cm+) and decreased in the late-core (~150cm), followed by another period of increase (~180cm). Sediment composition provides key information into depositional environment and will be discussed in more specific analysis further in the discussion.

Each depth displayed offers three general trends: percent changes in ooid, peloid, and skeletal grain abundance. I determined that roundness, median diameter (D50), and sorting, as provided through Camsizer analysis, best correlated with the changes in concentration of ooid and skeletal presence described in Figure 3, and should thus be interpreted for other cores of interest as described in Figures 4, 5 and 6.

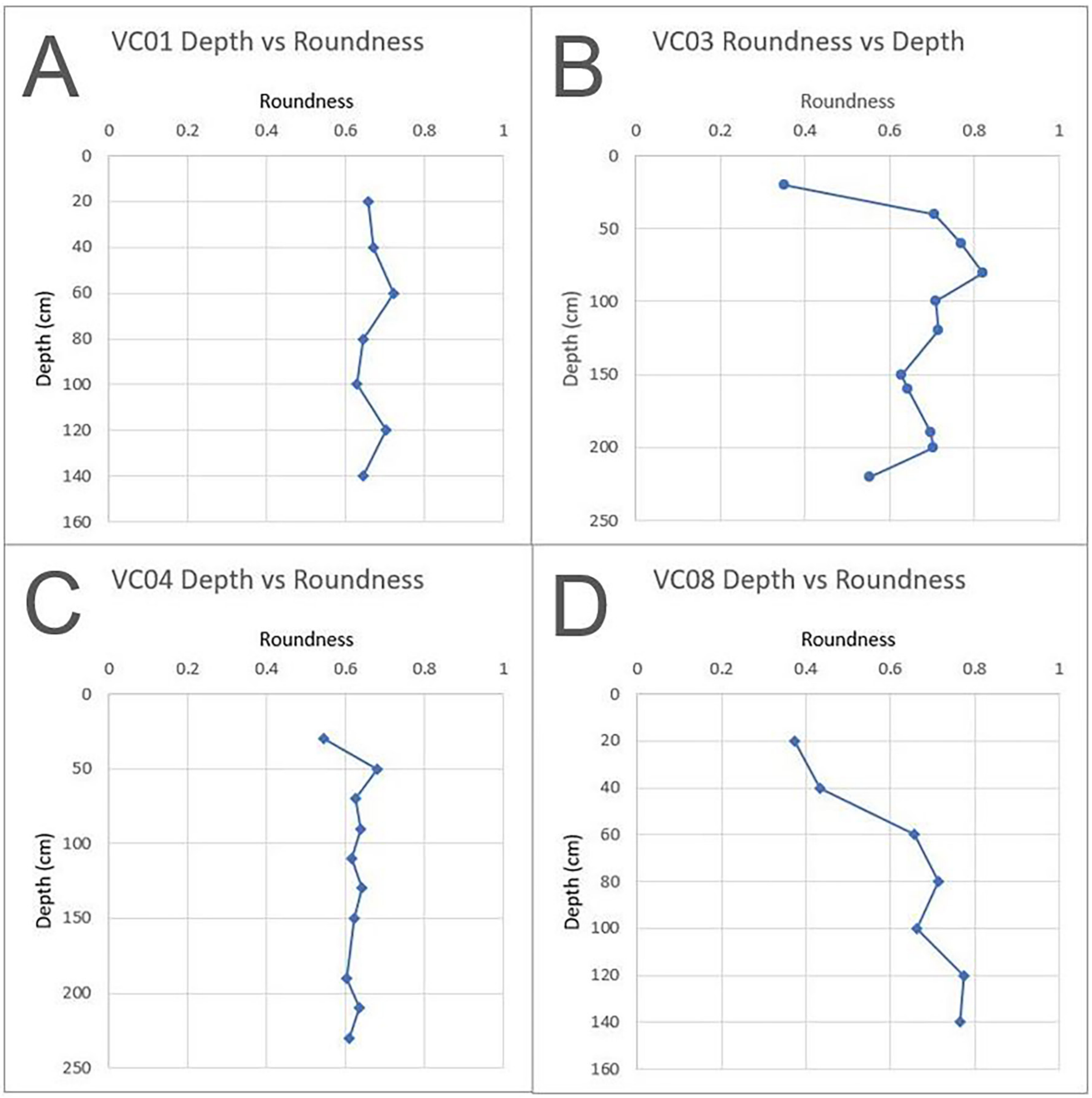


Figure 4: The photos above represent each core's depth compared against roundness. The higher the values, the higher the roundness.

Four cores were chosen to compare roundness, D50, and sorting versus depth to serve as a proxy for ooid concentration. Each core is located within different cardinal direction points around LAC (Figure 1.A) to give an interpretation of ooid concentrations based on differing locations.

The maximum roundness described in Figure 4.B is 0.81 in VC03, while the minimum value is 0.38 in VC08. Each core shows different patterns vs. depth, where VC08 increases with depth, and VC03 initially increases, but gradually decreases following 100cm. VC04 and VC01 remain relatively constant throughout their entire section.

The maximum median diameter within the described cores is just <1200m in VC03. The minimum median diameter is ~300m in VC08. Once again, VC04 and VC01 display relatively consistent values throughout their section. Median diameter gives an interpretation of overall grain size, where consistent values of D50 represent consistent grain composition

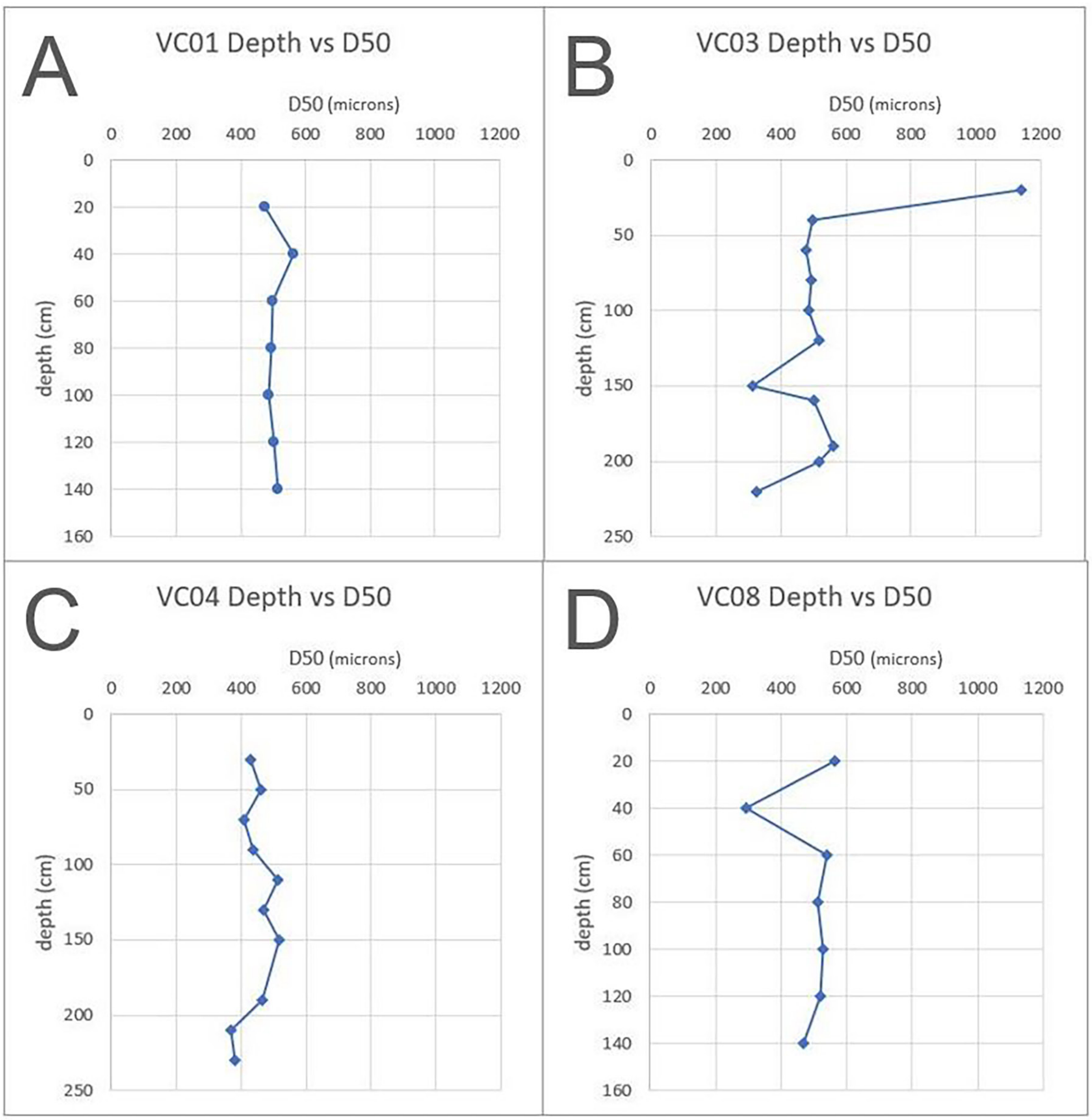


Figure 5: The photos above represent each core's depth compared against D50, or the median diameter. Units of D50 are in microns.

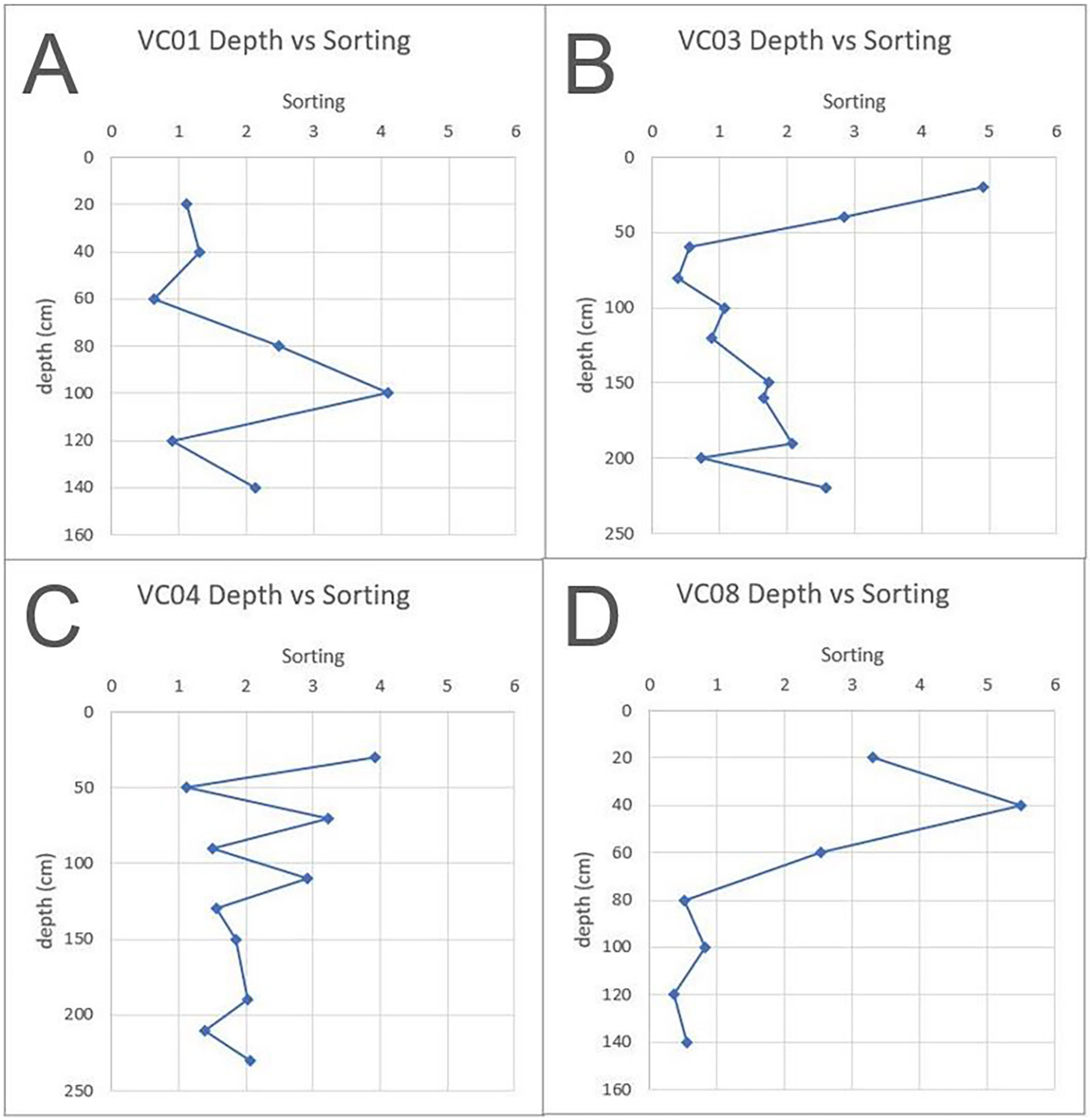


Figure 6: The figures above represent each core's depth compared against sorting. Higher numbers reflect poorer sorting.

The lowest degree of sorting is described in VC08 at ~5.5. The best degree of sorting is also within VC08 at 0.35 at 80cm depth. VC03 also exhibits a high degree of sorting at 0.38 at 60cm depth. Sorting provides an interpretation of grain presence, where the best degrees of sorting for LAC are consisting of dominantly ooids, and the lower degrees are a large mix of skeletal grains with ooids..

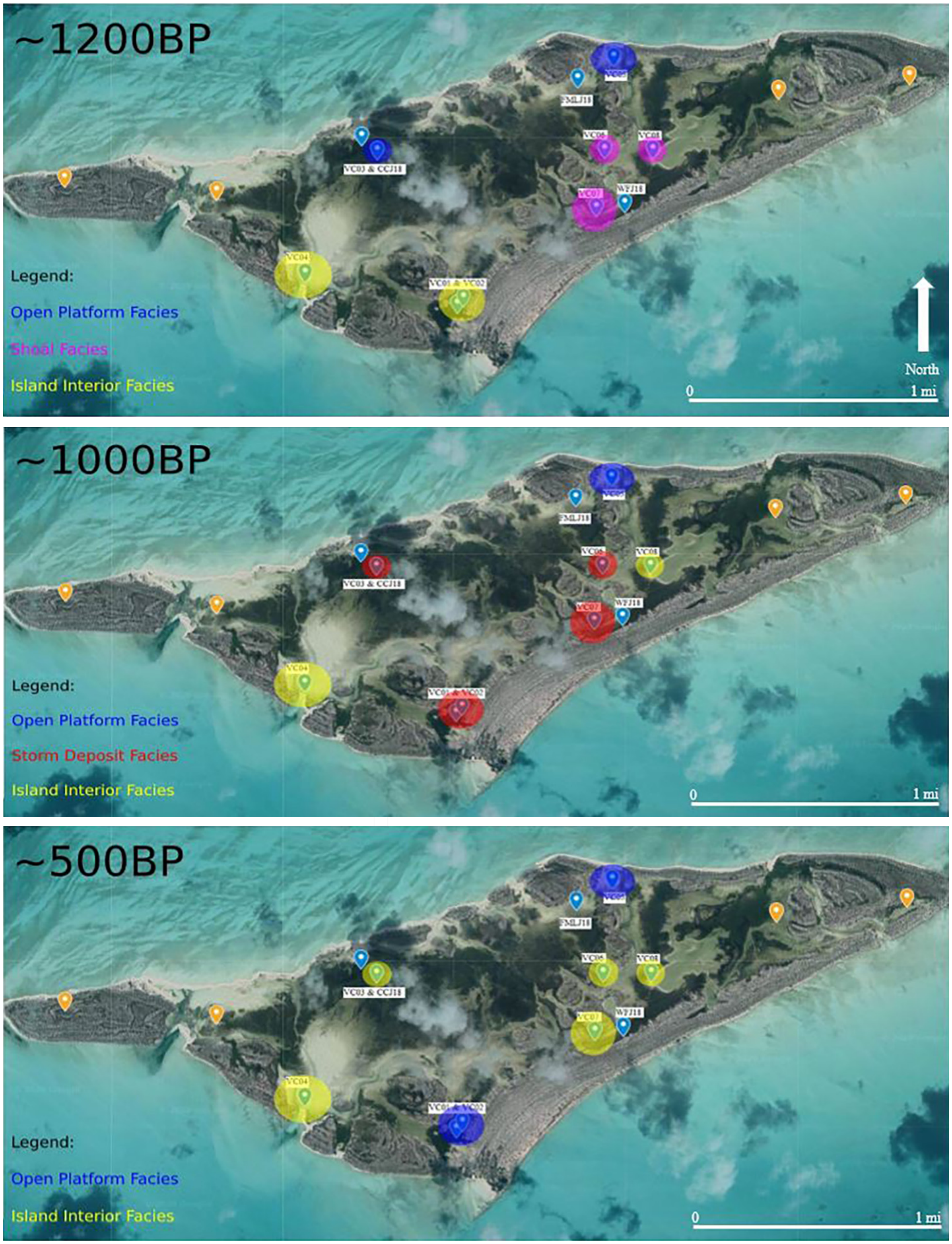


Figure 7:
The three photographs above show the interpretation of the succession of depositional facies through time based on the radiocarbon ooid data collected (Figure 2).

[...]

**Discussion:**

[...]

Regarding the question of why LAC accumulates in the position it does (~3 miles off of BAC), instead of accreting off the western edge of BAC, I have formulated three hypotheses that could explain such formation.

1. BAC poses as a large geographic "block" for current and wind-wave influence coming in from the east. This "block" forces wave refraction off the northern and southern edges of BAC. It is possible that the two refractions combine at the ~3 miles mark where the initial bedrock could have lithified, thus allowing sediment supply to be particularly higher at this specific location, and in turn allowing eolianite processes or cement compaction processes to allow an early lithification.

2. Patch reefs are visible and have been observed around the entirety of the Caicos Platform (Dravis and Wanless, 2008). It is possible that a singular patch reef was/is in-situ at the ~3-mile point west of BAC. It is possible that the westward migrating ooids were able to be trapped in this patch reef, and in turn allow sediment accumulation to form an early lithification to surpass sea level rise.

3. Knowing that ooids are increasing in size as they transport west (Trower et al., 2018), it could be possible that the Intra-Cay shoal grew westward enough to sufficiently surpass water depth. In other words, it could be possible that the westward growth of ooids along the BAC shoal grew to high enough size in high enough concentrations that they were able to succeed sea level, and thus allow an early lithification along with eolianite processes.

i. Microbial mat influence has the potential to affect each of the above listed scenarios. While in-situ biology has not proved to be a leading cause of LAC island accumulation based on the research provided in this paper, it should be noted that microbial mats greatly increase the ability for sediment entrapment, and even minor presence of microbial mats could help support accumulation of incoming ooid sediments.

### Future Directions

...However, I propose that research be conducted on a series of processes within the Intra-Cay shoal and LAC. Based on the above hypotheses, the direction and energy relationship of refraction off BAC should be considered in how exactly the wave energy may influence the combination or direction of ooid sediment transport.

[...]

**Conclusion:**

*Building the Overall Picture of Little Ambergris Cay Formation*

1. The Ambergris Shoal (Intra-Cay Shoal), extending a total of ~2.2km west (reaching the modern LAC shoreline) of BAC, of age greater than 6000BP, develops due to easterly wind-wave action and the prior abundance of ooid grains.

2. An abundant supply of ooids are continuously transported from BAC to the west, accumulating and extending along the Intra-Cay shoal.

3. Sediment supply outpaces accommodation space following the eolianite influence of LAC ooid bedrock lithification ~5946BP. Possible reasons for sediment supply to accumulate past the subsurface and lithify could be due to wave refraction induced

sediment accumulation, patch reef presence and subsequent sediment accumulation, or transport induced ooid growth resulting in carbonate sediment growth surpassing sea level rise.

4. Westward migration of ooids became trapped onto a newly existing surface area of bedrock <6000BP, where possible microbial mat development could have aided in incoming sediment entrapment and further cementing lithification, allowing sediment accumulation to succeed sea level rise in the early Holocene.

5. Island growth occurs as per Figure 7.

[...]

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