

centers. Given the current opioid crisis, these data provide support for the development of standardized urologic opioid prescribing guidelines for postoperative analgesia.

Disclosure: Work supported by industry: no.

## 064

**"WE CAN SET YOU UP FOR AN ABORTION": THE IMPORTANCE OF REPRODUCTIVE HEALTH TO YOUNG WOMEN WITH SPINA BIFIDA AND THE LACK OF SUPPORT FROM THEIR DOCTORS**

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**Background:** Research suggests that the reproductive health (RH) of women with spina bifida (SB) is not addressed adequately in usual care which puts them at risk for unplanned pregnancies and unnecessary stress when considering their reproductive plans.

**Objective:** We sought to understand what women with SB know about their RH, experiences with pregnancies, and communication about RH with their physicians.

**Methods:** In this exploratory qualitative study, interviews with women with SB who were 16+ years old, were transcribed verbatim, and analyzed using Grounded Theory. Three investigators coded the interviews and reached consensus on overarching themes.

**Results:** Twenty-five women with SB participated (mean age 27.1 years, range 16-52). Only seven (29.2%) reported speaking to a doctor about SB-related RH, of those, 2 after the woman became pregnant and 2 because the women prompted the conversation. Of the 6 women who delivered at least one baby, 4 were unplanned; 3 of these women had been told by doctors they could not get pregnant and 1 assumed she could not get pregnant due to her SB, 4 were encouraged to terminate their pregnancy. All women delivered healthy babies. The following themes emerged: SB women's 1) evolving understanding of their reproductive capacity, 2) attitude towards having children or not, 3) coping with physicians' ignorance about their ability to carry and deliver a child, 4) going into pregnancy blind with physicians not understanding their pregnancy and delivery needs, 5) experiencing and overcoming the stigma surrounding their reproductive health. **Conclusions:** Women with SB have a poor understanding of their reproductive potential and enter pregnancy anxious about possible SB-related problems. Women with children or interested in having children had discouraging interactions with one or more providers, some of whom



provided misinformation. All participants wished to have had information about RH including the impact of SB on pregnancy and delivery, from their providers. Provider education about the RH capacity and needs of women with SB can help these women feel better supported and make informed RH decisions.

Disclosure: Work supported by industry: no.

## 065

**ABSENT DIURNAL VARIATION IN SERUM TESTOSTERONE OBSERVED IN MEN WITH BASELINE LOW TESTOSTERONE**

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**Introduction:** Young healthy men demonstrate a diurnal pattern of serum testosterone (T), with highest levels in the morning, then decreasing in the afternoon and evening. For this reason, clinical guidelines recommend obtaining serum specimens for T testing in the early morning, often before 10am. However, diurnal variation is blunted in men over 40y. It is unknown whether T deficiency itself may be associated with blunted diurnal variation.

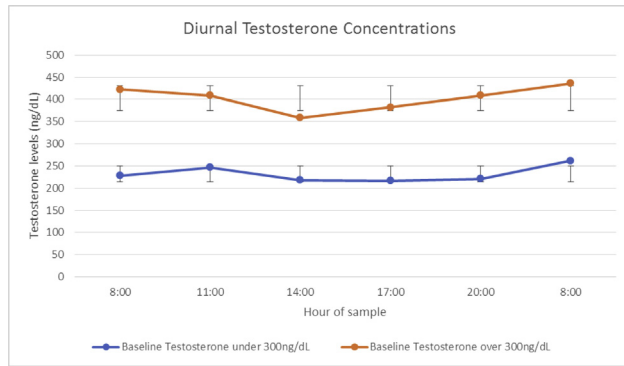
**Objective:** The primary objective of this study was to assess diurnal variation of serum T in men with normal and abnormally low serum T.

**Methods:** This was a single-center case-control study in healthy men restricted to a younger age group of 46 years or less. Blood samples were obtained from subjects at 6 time points over a 24-hour period at 3h intervals, at 8am, 11am, 2pm, 5pm, 8pm, and then at 8am the following morning. Men were categorized as having normal T if 8am serum T was  $\geq 300$ ng/dl, and low T if  $< 300$ ng/dl. Statistical analysis comparing differences between groups was performed using the paired two sample t-test. Signed informed consent was obtained from all subjects. Human subject approval was obtained from New England Independent Review Board.

**Results:** The study population consisted of twenty one subjects with mean age of  $32y \pm 7.9$ , including ten men with low T and eleven men with normal T. The highest T concentration was observed at 8am, with a mean serum T of the entire study population of 325ng/dL. The lowest T concentration was observed at 2pm, with mean T of 287ng/dL. Mean T levels at 8am were 423ng/dl for the normal group and 228ng/dl for the low group ( $p=0.008$ ). At 2pm these values were 358ng/mL and 218ng/mL, respectively ( $p=0.019$ ). Serum T concentrations declined by 64ng/dl (15%) from 8am to 2pm in the normal group and by 10ng/dl (4%) for the low group. This decline was significantly greater for the normal group ( $p=0.0003$ ). The

**Table.**

Theme	Quote
Evolving understanding of their reproductive capacity	"I'm honestly not positive if I would be able to get pregnant and then if the child would have spina bifida..." "...I don't know the answer [of if I can get pregnant] still, even with all my googling."
Attitude towards having children or not	"I've always, always wanted to be a mother ever since I was little." "...it got to the point where... [my reproductive] organs were more problematic than anything. So, it was just time to have them removed... I had my hysterectomy and 6 months later my sister got pregnant... it was kind of emotionally traumatic for me because then it hit me..."
Coping with physician's ignorance	"...medical doctors... always told me I would never bear a child, ever." (unintended pregnancy at age 18) "...once I did get pregnant, I... felt like a little discouraged... from the doctors because they made me feel like it was just the worst thing that could happen to me."
Going into pregnancy blind	"[My doctors] didn't have a clue what to do with me being pregnant, and that was the scariest part..." "... you go through this absolute sheer terror of I don't know if I can handle my body not being able to get the child here, and losing it."
Experiencing and overcoming stigma	"...[my gynecologist] told me that he wanted nowhere near me if I ever decided that I was going to have kids because he said it would be extremely selfish for me to even think about it..."



decline for the low group was not statistically significant ( $p=0.54$ ). Mean luteinizing hormone concentration at baseline was  $5 \pm 2.39$  (1.8–8.5) for the entire group. Hypothesis testing led us to reject the null and conclude there is significant diurnal variation in T concentrations for men with normal levels, but not for men who are T-deficient.

**Conclusion:** Diurnal variation was not observed in testosterone-deficient men, suggesting an abnormality in the hypothalamic-pituitary-gonadal axis. This finding has implications for understanding the underlying pathophysiology of testosterone deficiency, and may also have clinical implications for recommendations regarding the diagnosis of testosterone deficiency (hypogonadism).

**Disclosure:** Work supported by industry: yes, by Beckman coulter.

## 066

### HIGH TESTOSTERONE LEVELS CAUSE CHONDROCYTE METAPLASIA AND REDUCED SMOOTH MUSCLE CONTENT IN THE MOUSE PENIS

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**Introduction:** The luteinizing hormone receptor (LHCGR) is a member of the G-protein-coupled receptor family and has an important role in male and female fertility. Activating mutations in LHCGR in humans cause familial male-limited precocious puberty in 2–4-year-old boys which is characterized by Leydig cell hyperplasia, rapid virilization and high testosterone levels with low levels of luteinizing hormone. Previously, we have created a knock-in-mouse model (KiLHR<sup>D582G</sup>) with a D582G mutation in mouse LHCGR, which is a phenocopy of the D578G activating mutation in humans. Male KiLHR<sup>D582G</sup> mice exhibit precocious puberty, Leydig cell hyperplasia and high testosterone levels. We observed that KiLHR<sup>D582G</sup> male mice become progressively infertile due to sexual dysfunction. Histological analysis of the penile body of KiLHR<sup>D582G</sup> male mice showed chondrocyte metaplasia as early as 7–8 weeks of age. Compared to wild type (WT) mice, KiLHR<sup>D582G</sup> male mice showed a decrease in smooth muscle content but similar collagen levels in the penile body at 24 weeks of age.

**Objective:** The objective of this study was to test the hypothesis that high levels of testosterone cause chondrocyte metaplasia, reduced smooth muscle content in the corpus cavernosa and reduced fertility.

**Methods:** WT mice were implanted with empty (control) or testosterone filled silastic capsules at the prepubertal age of 2 or post-pubertal age of 5 weeks to determine if there was a critical time period when testosterone treatment could cause morphological changes in penile body. Mice were re-implanted with control or testosterone filled capsules at 12 or 14 weeks of age. Mice were dissected at 24 weeks of age to harvest penile bodies for histological analysis and serum was collected. Serum levels of testosterone were determined by using a commercially available ELISA kit. Penile bodies were fixed in 10% neutral buffered formalin overnight, embedded in paraffin

and 5  $\mu$ m sections were used to perform immunohistochemistry for the chondrocyte specific marker SOX9 and for alpha smooth muscle actin ( $\alpha$ -SMA). Quantification of  $\alpha$ -SMA staining was performed by image J analysis. For mating studies, female WT mice were superovulated with PMSG and hCG injections and paired with WT male mice that had been implanted with control or testosterone capsules at 2 weeks. Success of mating was determined by the presence of copulatory plugs and sperm in the female reproductive tract.

**Results:** Serum testosterone levels were higher in 24-week-old WT mice implanted with testosterone capsules at both 2 and 5 weeks of age compared to mice implanted with control capsules indicating that the testosterone capsules were functional. Immunohistochemical staining of penile bodies for SOX9 showed the presence of chondrocytes in mice treated with testosterone at 2 and 5 weeks of age. Immunohistochemical quantification of  $\alpha$ -SMA in penile body showed reduced smooth muscle content in WT mice implanted with testosterone at 2 weeks of age. Mating studies indicated that testosterone treatment reduced fertility to 57%.

**Conclusions:** These studies support our hypothesis and demonstrate that elevated testosterone levels at either pre or post-pubertal ages cause histological changes in the penile body and a reduction in fertility.

**Disclosure:** Work supported by industry: no.

## 067

### RAPAMYCIN SUPPRESSES NADPH OXIDASE-MEDIATED REACTIVE OXYGEN SPECIES PRODUCTION AND REVERSES ERECTILE DYSFUNCTION IN WESTERN DIET-FED MICE

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**Introduction:** The mammalian target of rapamycin (mTOR) is a nutrient-sensitive cellular signaling kinase that has been shown to be activated by high-fat and/or high-sugar feeding in highly metabolic tissues such as adipose tissue, liver, and skeletal muscle. mTOR activation has been implicated in the excess production of reactive oxygen species (ROS) resulting from overnutrition, which has been associated with activation of NADPH oxidase (Nox) as a source of excess ROS production. A Western style high-fat, high-sugar diet has recently been used to induce erectile dysfunction (ED) in rodents, in which elevated Nox has been implicated in ED pathogenesis.

**Objective:** The objective of this study was to determine if mTOR is an upstream activator of Nox in the penis in response to the Western diet (WD) and to determine if this pathway is relevant in WD-induced ED.

**Methods:** Young male C57Bl/6J mice ( $n = 90$ ) were fed a control diet (CD) or WD *ad libitum* for 12 weeks. For the final four weeks of the dietary intervention, mice were intraperitoneally injected with either vehicle or the mTOR inhibitor rapamycin (Group 1: 1 mg/kg; Group 2: 2 mg/kg) three days/week. Following the intervention, erectile function was assessed by measuring intracavernosal pressure (ICP) and mean arterial pressure (MAP) during cavernous nerve stimulation. In separate mice following the same intervention, *in vivo* ROS production was measured in the penis utilizing a microdialysis approach. Microdialysis probes were inserted into the penis of anesthetized mice and perfused with saline containing 100  $\mu$ M Amplex Ultrared, 1 U/ml horseradish peroxidase, and 10 U/ml superoxide dismutase. ROS convert the reagents to a fluorescent byproduct resorufin, which was measured in the outflowing dialysate. Three replicate samples were collected, after which 300  $\mu$ M apocynin, a Nox inhibitor, was added to the perfusate, and additional samples were collected. Nox-mediated ROS were determined by calculation of the ROS that was inhibited by apocynin. Significant differences between groups were determined by two-way ANOVA with Tukey's multiple comparisons post-hoc analysis, with an alpha level of 0.05.

**Results:** Erectile function was significantly depressed in mice fed the WD in the vehicle condition (CD+Veh:  $8.65 \pm 1.1$  vs. WD+Veh:  $3.58 \pm 0.7$  ICP area/MAP;  $p < 0.01$ ). Rapamycin restored erectile function in the WD