Uterine Hemostasis is Achieved By Uterine Contraction

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UTERINE ATONY
Blood volume in pregnancy

50% Increase in Blood Volume in Pregnancy
Cardiac output in pregnancy

Cardiac output and placental blood flow

7.5 l/min

20% to the uterus

80% to the placenta
Potential for blood loss

- 7.5 l/min
- 20% to the uterus
- 80% to the placenta

1.2 l/min
Labor results in myocyte depolarization and GPCR activation

- Gq-mediated calcium production
- SR release
- Activation of voltage-gated calcium channels
Labor results in myocyte depolarization and GPCR activation
- Gq-mediated calcium production
- SR release
- Activation of voltage-gated calcium channels

Actin-myosin interactions
- prior to labor actin is in a globular form, unable to interact with myosin
- during labor, fibrillar actin is able to interact with myosin
  - myocyte contraction
Uterine atony risk factors

- Prolonged labor
- Prolonged oxytocin augmentation
- High-doses of oxytocin
- Uterine over-distension
  - macrosomia
  - multiple gestation
  - polyhydramnios
- Magnesium therapy
- Chorioamnionitis
- General anesthesia

OXTR Desensitization
OXTR desensitization – prolonged oxytocin infusions

• Paradoxically associated with
  – Dysfunctional labor…cesarean
  – Uterine atony…postpartum hemorrhage
Prolonged oxytocin infusions

- Crall, 1991
  - Measured uterine activity following prolonged, constant oxytocin infusions
  - After fixed dose rate of more than 80-90 minutes, uterine activity decreased
## Oxytocin exposure and atony

### Duke Data

<table>
<thead>
<tr>
<th></th>
<th>Uterine atony with PPH and Tx n=100</th>
<th>Control n=100</th>
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<tr>
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<td>27.6 ± 7.3</td>
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<td>EBL, ml</td>
<td>1579 ± 1205</td>
<td>517 ± 236</td>
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<td>10,054 ± 11,340</td>
<td>3762 ± 7092</td>
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<td>Oxt max dose, mU</td>
<td>23.6 ± 11.7</td>
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## Oxytocin exposure and atony

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\[
aOR = 1.58 \ (95\% CI 1.05, 2.57) \\
\text{AUC increase of 5000 mU} \\
\sim 4 \text{ hours at 20 mU/min}
\]
## Oxytocin exposure and atony

**MFMU Cesarean Registry**

<table>
<thead>
<tr>
<th></th>
<th>Uterine Atony n=2108</th>
<th>Control n=39,833</th>
<th>Adjusted OR* (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>27.3 ± 6.3</td>
<td>27.8 ± 6.3</td>
<td>0.99 (0.97, 1.01)</td>
<td>0.15</td>
</tr>
<tr>
<td>Hispanic Ethnicity, n (%)</td>
<td>691 (32.8)</td>
<td>9302 (23.3)</td>
<td>1.92 (1.47, 2.52)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>32.7 ± 6.7</td>
<td>32.2 ± 6.9</td>
<td>0.95 (0.87, 1.03)</td>
<td>0.16</td>
</tr>
<tr>
<td>Nulliparous, n (%)</td>
<td>1076 (51.0)</td>
<td>12,021 (30.2)</td>
<td>1.56 (1.14, 2.13)</td>
<td>0.005</td>
</tr>
<tr>
<td>Magnesium, n (%)</td>
<td>317 (15.0)</td>
<td>3139 (7.9)</td>
<td>1.76 (1.26, 2.46)</td>
<td>0.001</td>
</tr>
<tr>
<td>Chorioamnionitis, n (%)</td>
<td>439 (20.8)</td>
<td>3373 (8.5)</td>
<td>1.86 (1.33, 2.61)</td>
<td>0.001</td>
</tr>
<tr>
<td>Induction, n (%)</td>
<td>827 (39.2)</td>
<td>10,831 (27.2)</td>
<td>1.02 (0.74, 1.39)</td>
<td>0.92</td>
</tr>
<tr>
<td>Duration of oxytocin, hrs</td>
<td>10.5 (5.7, 15.8)</td>
<td>7.3 (3.8, 12.2)</td>
<td>1.05 (0.96, 1.14)</td>
<td>0.31</td>
</tr>
<tr>
<td>Maximal infusion &gt;20 mU/min, n (%)</td>
<td>532 (25.2)</td>
<td>3995 (10.0)</td>
<td>1.52 (1.15, 2.00)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
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*Adjusted OR for uterine atony while controlling for all listed variables and DM, HTN

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Grotegut et al. SMFM 2015
Prolonged or high-dose oxytocin exposure during labor

Decreases in uterine contractility postpartum

Uterine atony
Prolonged or high-dose oxytocin exposure during labor leads to oxytocin receptor desensitization as a mechanism for uterine atony.
Uterine contraction phenotype

- Phasic
- Tonic
Uterine contraction phenotype

• Labor characterized by *phasic contractions* – allows for placental gas exchange during uterine relaxation

• Postpartum state characterized by *tonic / tetanic contractions* – allows for closure of the uteroplacental arteries

• Molecular mechanisms regulating this transition are not known

• Failure of producing a tetanic contraction postpartum – Uterine atony and PPH
Uterine contraction phenotype
labor = phasic

Phasic contractions during labor:
- blood flow to the placental bed occurs between contractions
- utero-placental vessels become occluded during contractions
Uterine contraction phenotype postpartum = tonic / tetanic
Tetanic contraction PP closes the uteroplacental arteries

Intramyometrial Pressure = 10 mmHg

Mean Arterial Pressure = 85 mmHg

Veins

Intramyometrial Pressure = 120 mmHg

Mean Arterial Pressure = 90 mmHg

Veins
Tetanic contraction PP closes the uteroplacental arteries

Uterine Atony

Mean Arterial Pressure = 85 mmHg
Intramymetrial Pressure = 10 mmHg

Mean Arterial Pressure = 90 mmHg
Intramymetrial Pressure = 120 mmHg

Veins

1.2 l/min

Adapted from Brancazio LR
Prevention of Uterine Atony
Postpartum Oxytocin

- Oxytocin boluses
  - mainstay of uterine atony prevention
  - used prophylactically postpartum
  - dosing is 50-300 fold greater than augmentation dosing
    - results in tonic, rather than phasic contraction patterns

Oxytocin signaling

Oxytocin receptor = OXTR
- G protein-coupled receptor
- Activates Gq
- Increases intracellular calcium

Modified from Violin JD, Lefkowitz RJ, Tr Phar Sci 2007
OXTR desensitization

Modified from Violin JD, Lefkowitz RJ, Tr Phar Sci 2007
OXTR desensitization – contraction responses

Grotegut et al. Am J. Physiol Endocrinol Metab. 2011
OXTR desensitization – contraction responses

- **Wildtype**
- **Beta-arrestin 1 KO**
- **Beta-arrestin 2 KO**

![Graphs showing OXTR desensitization and contraction responses](image)

Grotegut et al. *Am J. Physiol Endocrinol Metab.* 2011
OXTR desensitization

Modified from Violin JD, Lefkowitz RJ, Tr Phar Sci 2007
OXTR desensitization – contraction responses

• OXTR desensitization
  – leads to decreases in uterine contractility
  – increases risk for uterine atony
• Absent OXTR desensitization
  – leads to increases in uterine contractility

• Are there individual variations in OXTR desensitization that could account for different contractile phenotypes?
Genetic predisposition to GPCR desensitization

ARTICLES

A GRK5 polymorphism that inhibits β-adrenergic receptor signaling is protective in heart failure

Enhanced β2-adrenergic desensitization = “genetic beta-blockade”

Genetic predisposition to GPCR desensitization

• Precedent exists in the β2AR-GRK5 system-
  enhanced GPCR desensitization

• Could genetic variation exist in the OXTR-
  GRK6 system which affects:
  – oxytocin dosing in labor (higher)
  – duration of labor (longer)
  – mode of delivery (failed labor)
  – uterine atony (PPH)

• Gene-association studies related to labor
  have largely focused on preterm delivery
Objective

• To determine if genetic variation in the OXTR or in GRK6 could explain variation in oxytocin dosing and labor outcomes among women being induced near term.
Study Design

- IRB-approved
- Duke *Healthy Pregnancy, Healthy Baby* Cohort
  - observational study of environmental exposure on pregnancy outcomes
- DNA obtained from 482 women undergoing induction of labor near term at Duke University Hospital
  - singleton gestation
  - non-anomalous
- Genotyped for haplotype tagging SNPs within the *OXTR* and *GRK6* genes
Study outcomes

• Primary study outcome:
  – maximal rate of oxytocin infusion

• Secondary outcomes:
  – total dose of oxytocin received in labor
  – duration of induced labor
  – cesarean delivery rate
  – cesarean rate for failed induction
  – uterine atony rate
SNP selection and genotyping cont.

- Genotyping performed by the Duke Molecular Physiology Institute’s Molecular Genotyping Core facility
  - Taqman SNP genotyping assays
  - Blinded duplicates and Centre d’Etude du Polymorphism Humain (CEPH) samples included as controls
  - Hardy-Weinberg Equilibrium (HWE) p-values as well as allele and genotype frequencies calculated by ethnicity
- PROC ALLELE SAS
Statistical analysis

• Linear regression tested association between SNPs and continuous outcome variables
• Logistic regression tested association between SNPs and categorical outcome variables
• Clinically important covariates selected \textit{a priori}
  – backwards selection used to choose covariates that independently correlated with each outcome
• Additive genetic model was employed
• Race/ethnicity included in all models
SNP selection and genotyping

• Haplotype tagging SNPs identified using LD Select from the Yoruban (YRI) and Caucasian (CEU) populations of the HapMap project
  – MAF of $\geq 10\%$

• All identified SNPs genotyped for subjects that self-reported as
  – non-Hispanic white
  – non-Hispanic black
  – Hispanic
  – non-Hispanic Asian
**OXTR SNP locations**

**OXTR gene: chromosome 3 (p25.3)**

OXTR: 18 haplotype tagging SNPs

- rs11131149
- rs237894
- rs237888
- rs237889
- rs9940864
- rs2268495
- rs237899
- rs2139184
- rs4686301
- rs237866
- rs9810278
- rs2254295
- rs237887
- rs2268490
- rs2324728
- rs9872310
- rs1042778

```plaintext
5'  ATG  TGA  3'
```

OXTR: 18 haplotype tagging SNPs
**GRK6 SNP locations**

*GRK6 gene: chromosome 5 (q35.3)*

![Diagram showing SNP locations on the GRK6 gene]

*GRK6: 7 haplotype tagging SNPs*
Subject characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value (n=482)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>26.9 ± 6.4</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>91 (18.9)</td>
</tr>
<tr>
<td>Non-Hispanic black</td>
<td>341 (70.7)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>24 (5.0)</td>
</tr>
<tr>
<td>Non-Hispanic Asian</td>
<td>26 (5.4)</td>
</tr>
<tr>
<td>Pre-pregnancy BMI, kg/m²</td>
<td>30.1 ± 9.4</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>230 (47.7)</td>
</tr>
<tr>
<td>Gestational age at delivery, weeks</td>
<td>38.9 ± 1.5</td>
</tr>
<tr>
<td>Birthweight, g</td>
<td>3203 ± 552</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>143 (30.0)</td>
</tr>
<tr>
<td>SNP (gene)</td>
<td>Genotype (n): Maximal oxytocin infusion rate (mU/min)</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------------------------------------------</td>
</tr>
<tr>
<td>rs1042778 (OXTR)</td>
<td>GG (n=91): 10.9 ± 6.6, GT (n=187): 13.8 ± 7.6, TT (n=140): 14.0 ± 7.6</td>
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$^1$While controlling for race/ethnicity, cervical dilation at start of induction, pre-pregnancy BMI, gestational age at delivery, chronic HTN, and magnesium therapy
## Maximal oxytocin infusion rate

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<th>p-value¹</th>
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<td>rs1042778 (OXTR)</td>
<td>GG (n=91): 10.9 ± 6.6 GT (n=187): 13.8 ± 7.6 TT (n=140): 14.0 ± 7.6</td>
<td>0.004</td>
</tr>
<tr>
<td>rs11706648 (OXTR)</td>
<td>AA (n=272): 12.7 ± 7.3 AC (n=132): 14.0 ± 7.5 CC (n=16): 16.4 ± 8.6</td>
<td>0.021</td>
</tr>
<tr>
<td>rs4686301 (OXTR)</td>
<td>CC (n=297): 12.7 ± 7.3 CT (n=111): 14.3 ± 7.6 TT (n=12): 17.6 ± 9.4</td>
<td>0.016</td>
</tr>
<tr>
<td>rs9810278 (OXTR)</td>
<td>CC (n=354): 12.9 ± 7.4 CT (n=64): 15.4 ± 7.7 TT (n=2): 11.0 ± 1.4</td>
<td>0.022</td>
</tr>
<tr>
<td>rs237895 (OXTR)</td>
<td>CC (n=270): 13.8 ± 7.6 CT (n=125): 12.0 ± 7.2 TT (n=24): 12.9 ± 7.6</td>
<td>0.027</td>
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¹While controlling for race/ethnicity, cervical dilation at start of induction, pre-pregnancy BMI, gestational age at delivery, chronic HTN, and magnesium therapy
## Total oxytocin dose

<table>
<thead>
<tr>
<th>SNP (gene)</th>
<th>Genotype (n): Total oxytocin dose (mU)</th>
<th>p-value&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1042778 (OXTR)</td>
<td>GG (n=94): 6,852 ± 7,871</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>GT (n=196): 10,159 ± 9,787</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT (n=143): 10,425 ± 10,658</td>
<td></td>
</tr>
<tr>
<td>rs4686301 (OXTR)</td>
<td>CC (n=308): 8,961 ± 9,377</td>
<td>0.034</td>
</tr>
<tr>
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<td>CT (n=114): 10,874 ± 10,682</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT (n=13): 11,426 ± 10,092</td>
<td></td>
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<sup>1</sup>While controlling for race/ethnicity, cervical dilation at start of induction, pre-pregnancy BMI, gestational age at delivery, chronic HTN, and magnesium therapy
# Duration of labor

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<th>SNP (gene)</th>
<th>Genotype (n): Duration of labor (hours)</th>
<th>p-value&lt;sup&gt;1&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>rs9810278 (OXTR)</td>
<td>CC (n=406): 20.2 ± 14.5, CT (n=68): 22.6 ± 16.9, TT (n=2): 14.4 ± 2.8</td>
<td>0.041</td>
</tr>
<tr>
<td>rs2731664 (GRK6)</td>
<td>AA (n=114): 17.7 ± 13.7, AC (n=223): 20.2 ± 14.3, CC (n=132): 23.5 ± 16.5</td>
<td>0.001</td>
</tr>
<tr>
<td>rs2287694 (GRK6)</td>
<td>CC (n=0): no subjects, CT (n=55): 26.2 ± 18.9, TT (n=421): 19.7 ± 14.1</td>
<td>0.009</td>
</tr>
</tbody>
</table>

<sup>1</sup>While controlling for race/ethnicity, nulliparity, cervical dilation at start of induction, pre-pregnancy BMI, gestational age at delivery, and diabetes
## Cesarean delivery rate

<table>
<thead>
<tr>
<th>SNP (gene)</th>
<th>Genotype (n): Cesarean delivery rate</th>
<th>p-value¹ (aOR, [95% CI])</th>
</tr>
</thead>
</table>
| rs2139184 (OXTR) | AA (n=6/16): 37.5%  
                | AC (n=35/110): 31.8%  
                | CC (n=101/355): 28.4%  | 0.023  
                | (aOR 0.55 [95% CI 0.33, 0.92]) |
| rs237888 (OXTR)  | CC (n=10/47): 21.3%  
                | CT (n=46/174): 26.4%  
                | TT (n=87/261): 33.3%  | 0.025  
                | (aOR 1.68 [95% CI 1.07, 2.66]) |
| rs2545796 (GRK6) | CC (n=19/54): 35.2%  
                | CT (n=57/203): 28.1%  
                | TT (n=66/224): 29.5%  | 0.032  
                | (aOR 0.64 [95% CI 0.43, 0.96]) |

¹While controlling for race/ethnicity, nulliparity, cervical dilation at start of induction, pre-pregnancy BMI, gestational age at delivery, chorioamnionitis, diabetes, and magnesium therapy
Genetic predisposition to GPCR desensitization

• Among women undergoing induction of labor near-term, OXTR and GRK6 genotype influence:
  – maximal oxytocin infusion rate
  – total dose of oxytocin received
  – duration of induced labor
  – cesarean delivery rate
• Too few cases of uterine atony in cohort
• Outcomes suggest that genetic predisposition affects contractile phenotypes
Genetic predisposition to GPCR desensitization

- Genetic variations in the OXTR-GRK6 system affect oxytocin dosing requirements and labor outcomes
- Identifying the functional significance of these variations may allow for personalization of labor management
- May explain racial/ethnic or individual risk variation seen for PPH
Uterine hemostasis is achieved by uterine contraction - Summary

• Transition of uterine contraction phenotype from phasic to tonic pattern is important to control PP blood loss

• Oxytocin is the mainstay for prevention of uterine atony
  – boluses of oxytocin produce a tonic contraction response

• OXTR desensitization contributes to risk for uterine atony
  – possible genetic predisposition
Questions?