Uterine Hemostasis is Achieved By Uterine Contraction

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DukeHealth

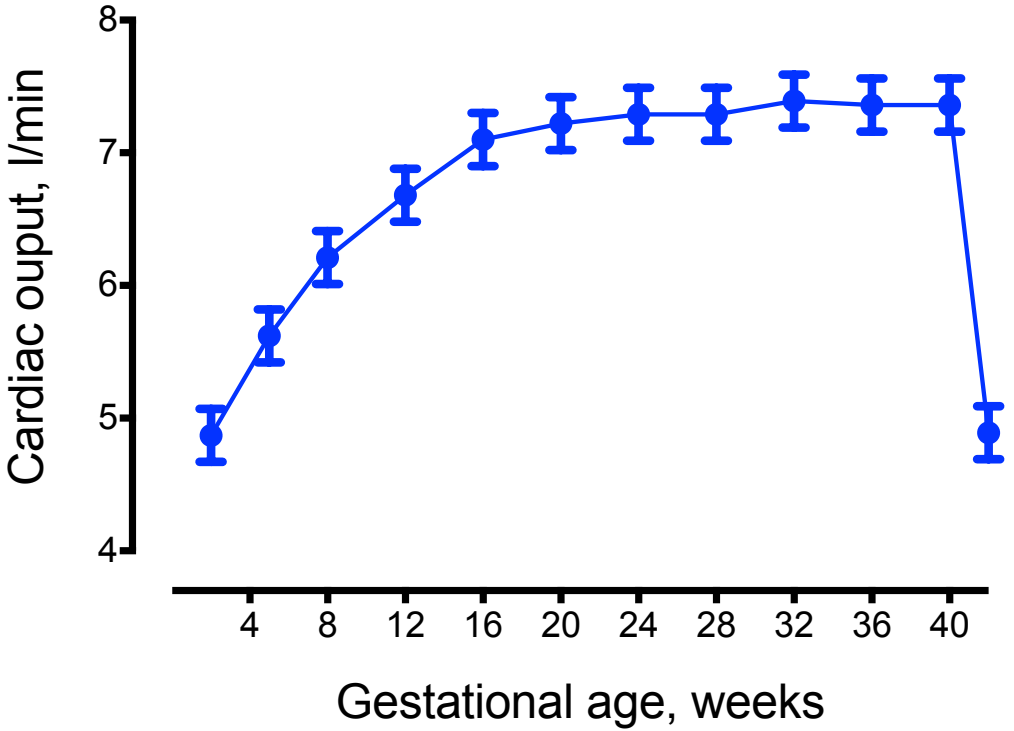
FOUNDATION FOR Women & Girls with Blood Disorders

UTERINE ATONY

Blood volume in pregnancy

50% Increase in Blood Volume in Pregnancy

Cardiac output in pregnancy



Adapted from Hunter S, et al. *Br Heart J*. 1992

Cardiac output and placental blood flow

7.5 l/min

20% to the uterus

80% to the placenta

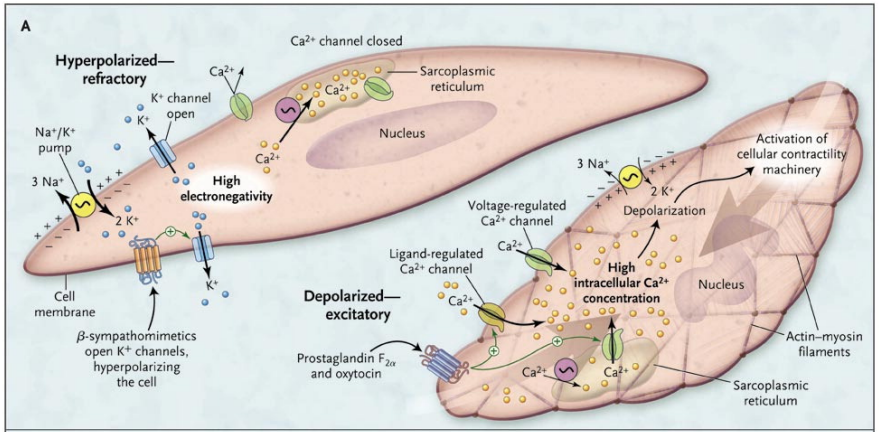
Potential for blood loss

7.5 l/min

20% to the uterus

1.2 l/min

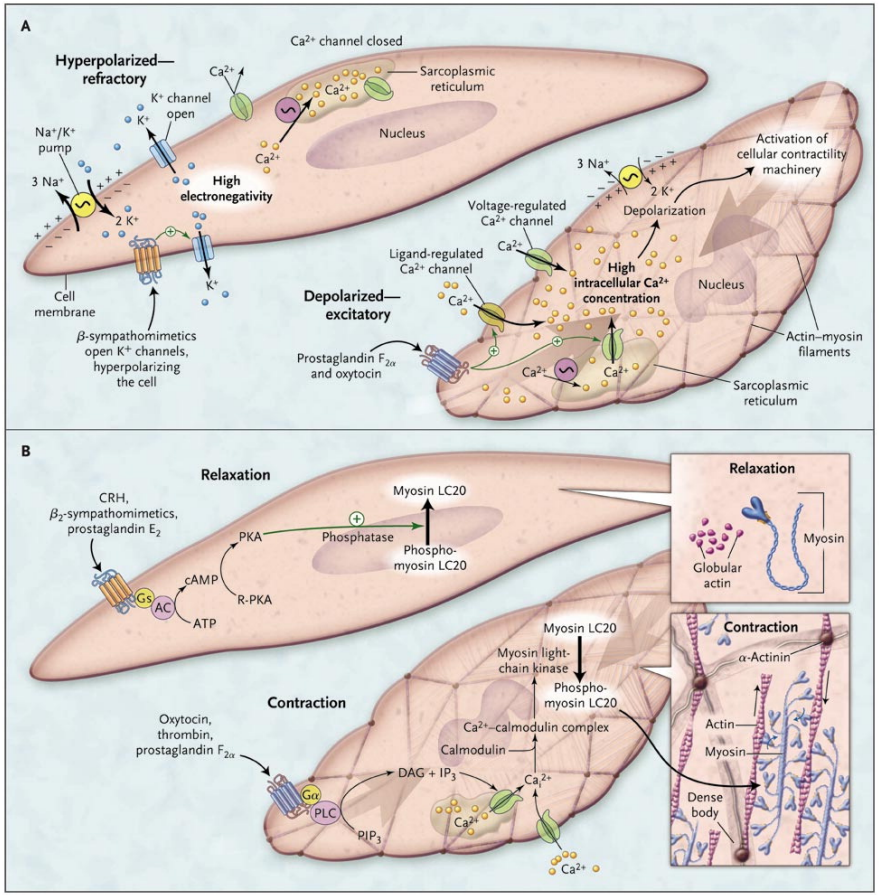
80% to the placenta



Labor results in myocyte depolarization and GPCR activation

- Gq-mediated calcium production
- SR release
- Activation of voltage-gated calcium channels

Smith R. *N Engl J Med*. 2007



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

Smith R. *N Engl J Med*. 2007

Uterine atony risk factors

- Prolonged labor
- Prolonged oxytocin augmentation
- High-doses of oxytocin

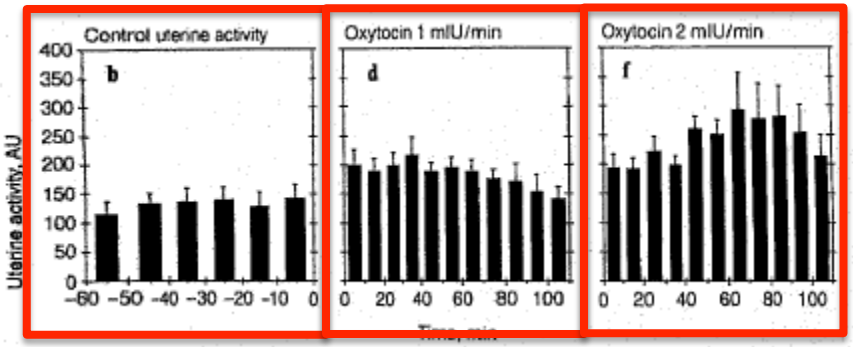
OXTR Desensitization

- Uterine over-distension
  - macrosomia
  - multiple gestation
  - polyhydramnios
- Magnesium therapy
- Chorioamnionitis
- General anesthesia

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

Crall, et al. *Gynecol Obstet Invest.* 1991

Oxytocin exposure and atony

Duke Data

	Uterine atony with PPH and Tx n=100	Control n=100	p-value

Grotegut, et al. *Am J Obstet Gynecol*, 2011

Oxytocin exposure and atony

Duke Data

	Uterine atony with PPH and Tx n=100	Control n=100	p-value
Age, yrs	27.6 ± 7.3	27.2 ± 7.3	0.783
EBL, ml	1579 ± 1205	517 ± 236	<0.001
Oxt AUC, mU	10,054 ± 11,340	3762 ± 7092	<0.001
Oxt max dose, mU	23.6 ± 11.7	18.9 ± 9.8	0.107
Oxt total time, mins	892 ± 478	793 ± 437	0.431

aOR = 1.58 (95% CI 1.05, 2.57)
AUC increase of 5000 mU
~ 4 hours at 20 mU/min

Grotegut et al. *Am J Obstet Gynecol*, 2011

Oxytocin exposure and atony

MFMU Cesarean Registry

	Uterine Atony n=2108	Control n=39,833	Adjusted OR* (95% CI)	p-value
Age, yrs	27.3 ± 6.3	27.8 ± 6.3	0.99 (0.97, 1.01)	0.15
Hispanic Ethnicity, n (%)	691 (32.8)	9302 (23.3)	1.92 (1.47, 2.52)	<0.0001
BMI, kg/m^2	32.7 ± 6.7	32.2 ± 6.9	0.95 (0.87, 1.03)	0.16
Nulliparous, n (%)	1076 (51.0)	12,021 (30.2)	1.56 (1.14, 2.13)	0.005
Magnesium, n (%)	317 (15.0)	3139 (7.9)	1.76 (1.26, 2.46)	0.001
Chorioamnionitis, n (%)	439 (20.8)	3373 (8.5)	1.86 (1.33, 2.61)	0.001
Induction, n (%)	827 (39.2)	10,831 (27.2)	1.02 (0.74, 1.39)	0.92
Duration of oxytocin, hrs	10.5 (5.7, 15.8)	7.3 (3.8, 12.2)	1.05 (0.96, 1.14)	0.31
Maximal infusion >20 mU/ min, n (%)	532 (25.2)	3995 (10.0)	1.52 (1.15, 2.00)	<0.0001

*Adjusted OR for uterine atony while controlling for all listed variables and DM, HTN

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

↓

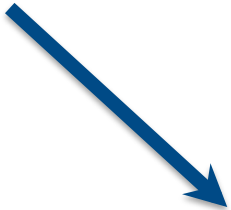
Oxytocin receptor desensitization as a mechanism

↓

Uterine atony

Uterine contraction phenotype

Phasic

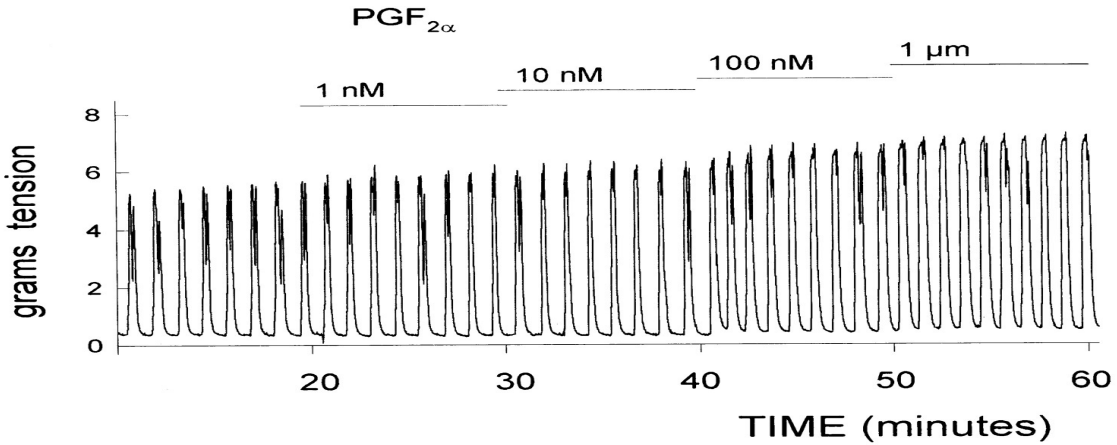


Tonic

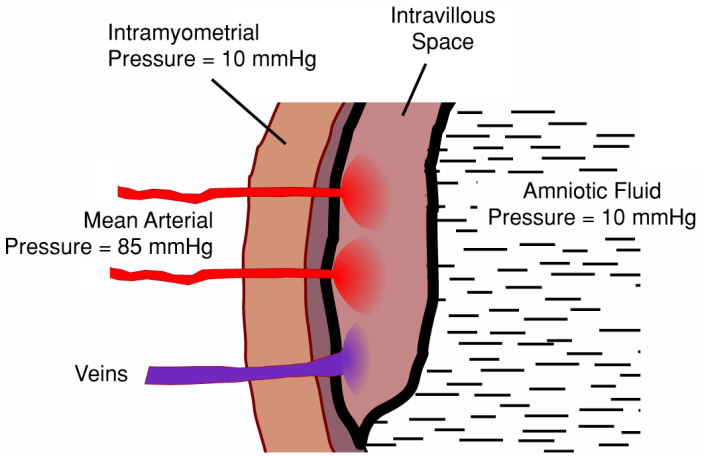
Uterine contraction phenotype

- 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

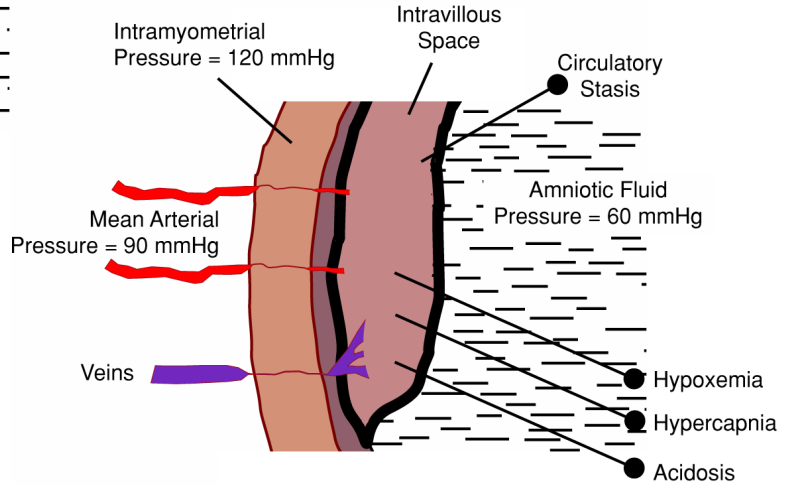


Modified from Phillippe M, et al. *Am J Physiol Endocrinol Metab*. 1997



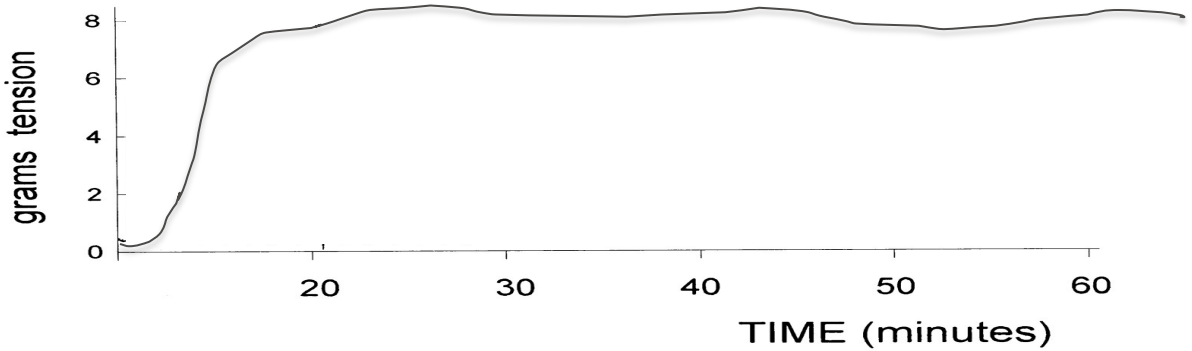
Phasic contractions during labor:

- blood flow to the placental bed occurs between contractions
- utero-placental vessels become occluded during contractions

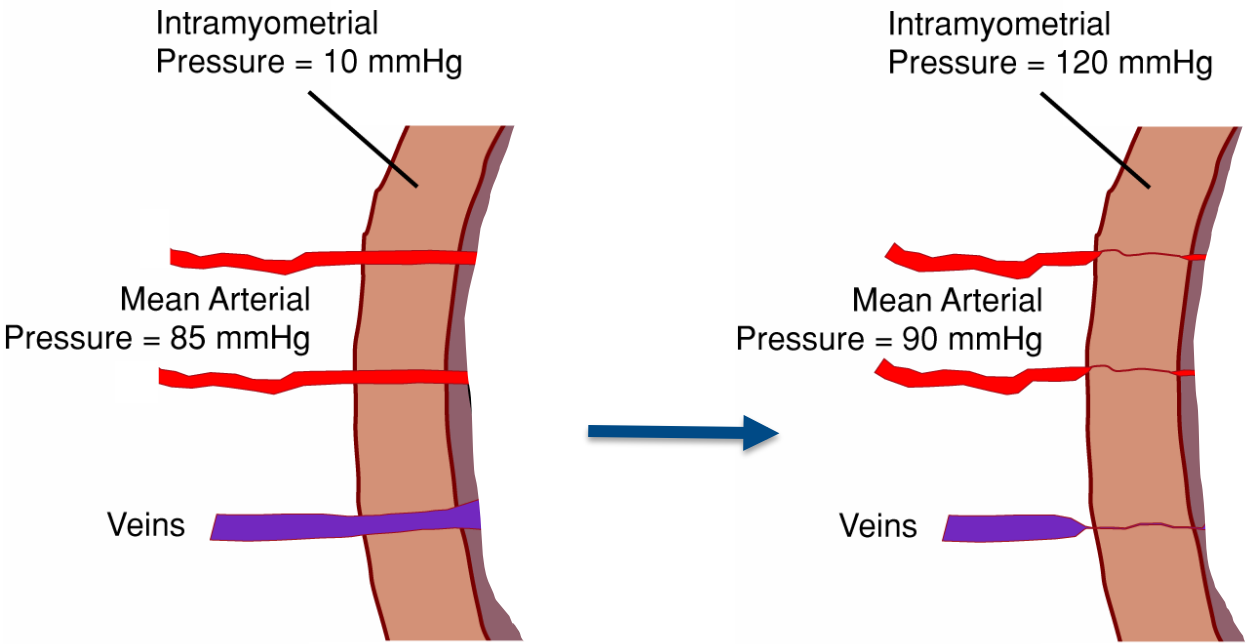


Provided by Brancazio LR

Uterine contraction phenotype
postpartum = tonic / tetanic

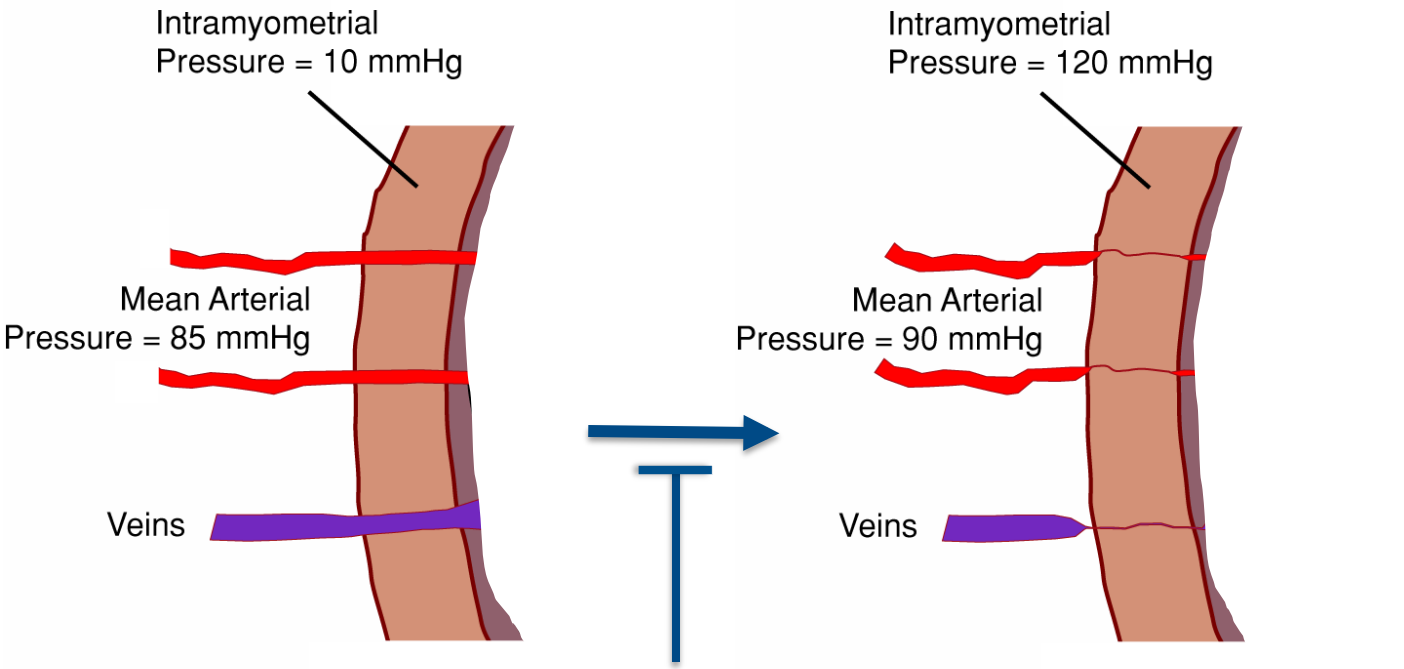


Tetanic contraction PP closes the uteroplacental arteries



Adapted from Brancazio LR

Tetanic contraction PP closes the uteroplacental arteries



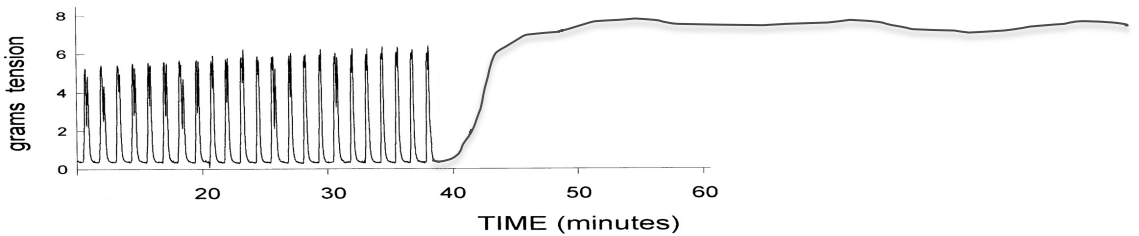
Uterine Atony → 1.2 l/min

Adapted from Brancazio LR

Prevention of Uterine Atony

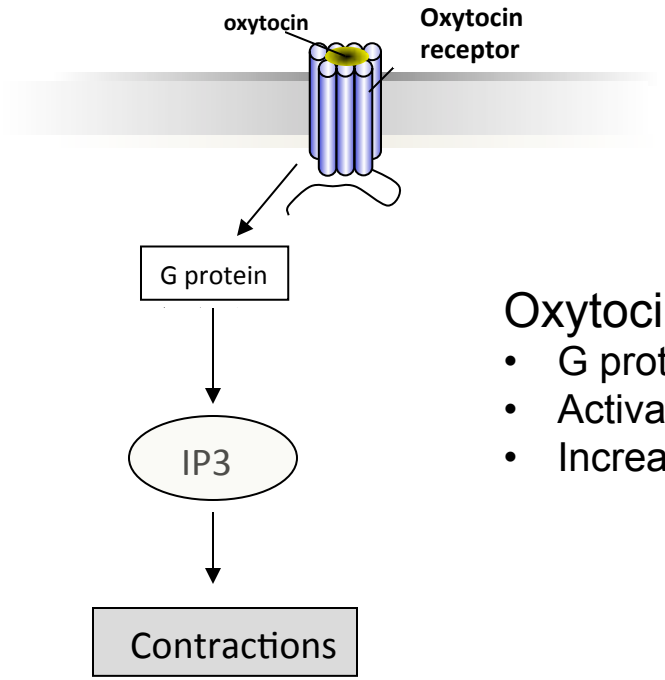
Postpartum Oxytocin

- 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



Modified from Phillippe M et al. *Am J Physiol Endocrinol Metab*. 1997

Oxytocin signaling

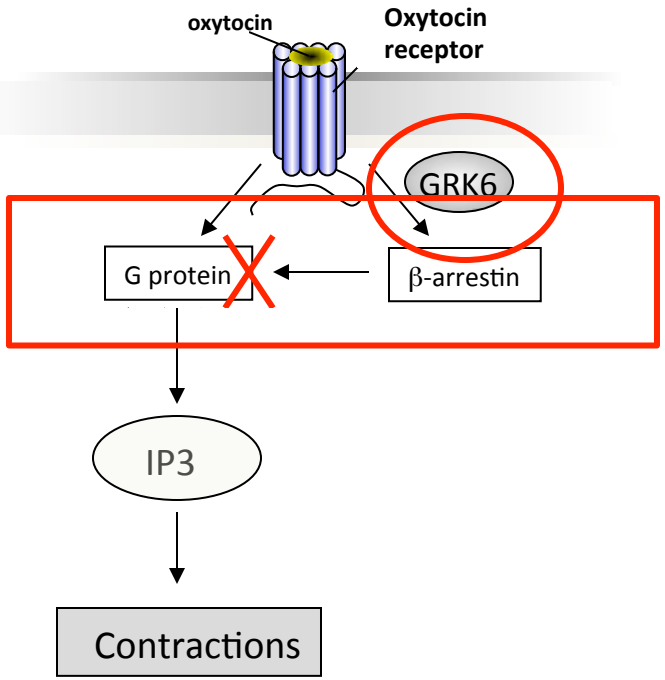


Oxytocin receptor = OXTR

- G protein-coupled receptor
- Activates Gq
- Increases intracellular calcium

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

OXTR desensitization



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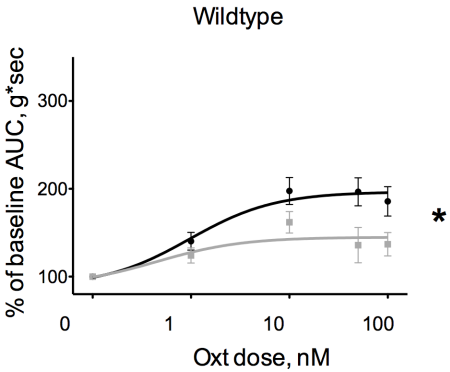
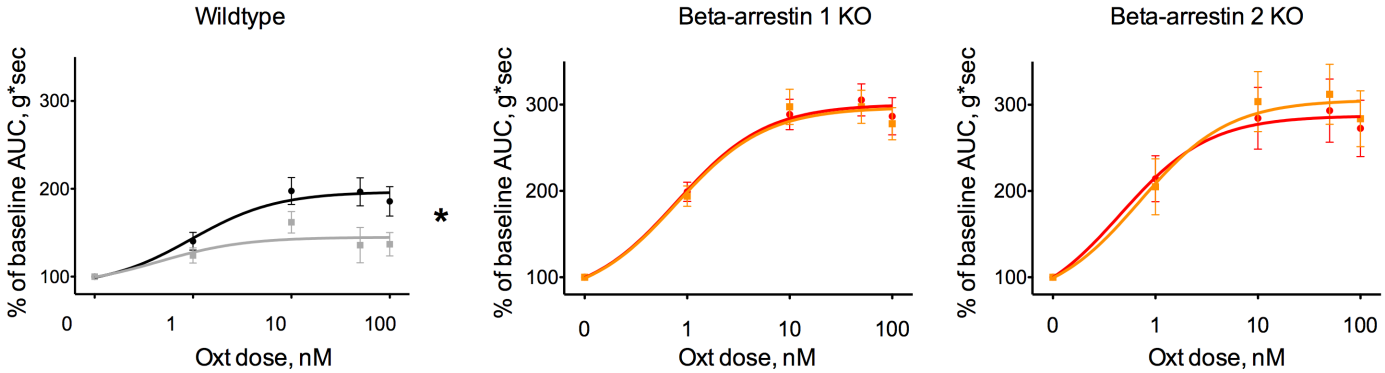
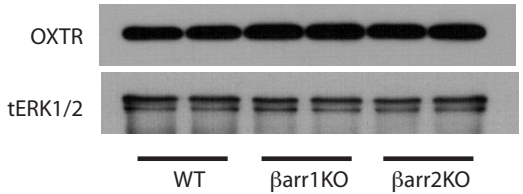
OXTR desensitization – contraction responses



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

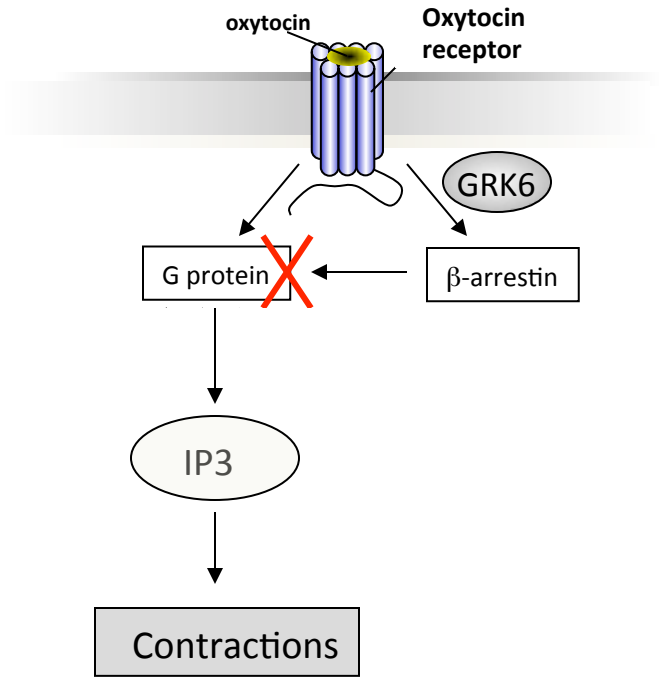
OXTR desensitization – contraction responses





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

OXTR desensitization



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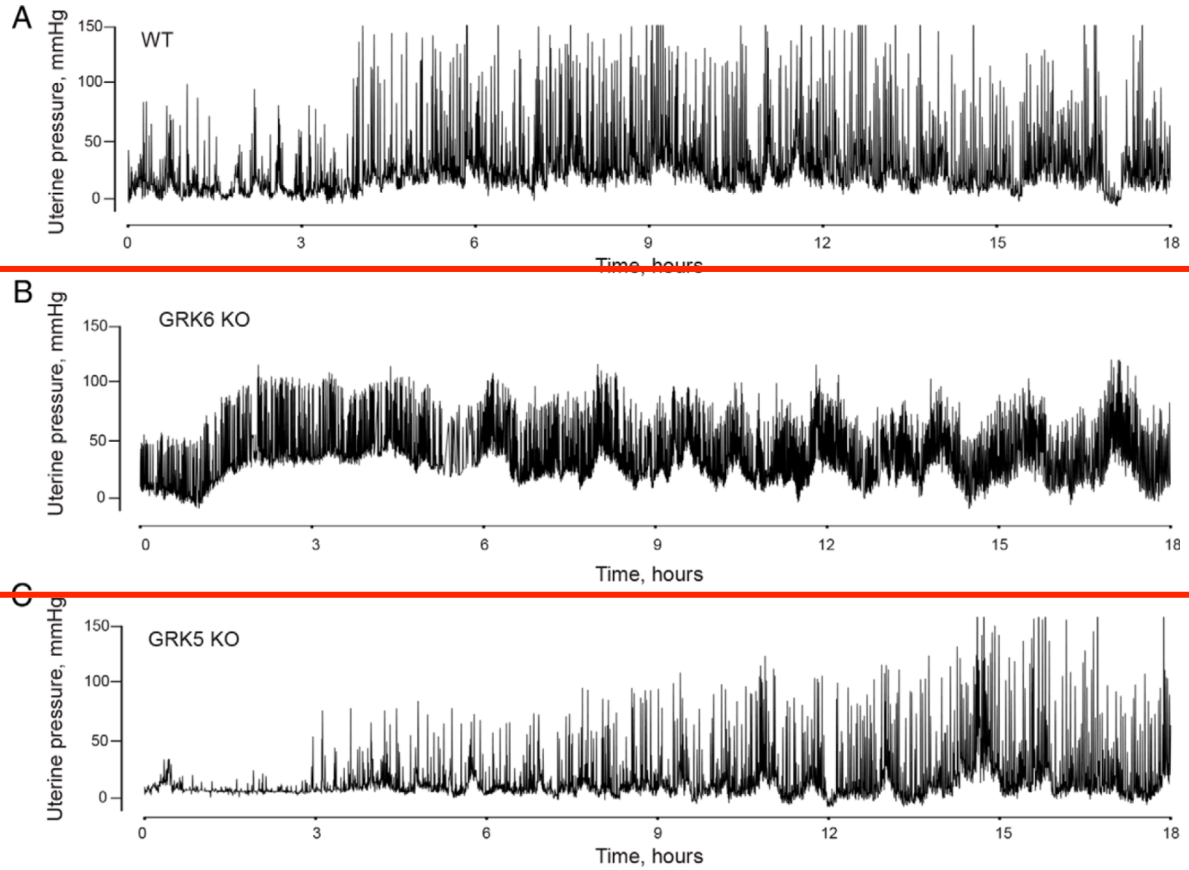


Grotegut, et al. *Mol Endocrinol.* 2016

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

nature medicine

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

Stephen B Liggett[1,6,7], Sharon Cresci[2,7], Reagan J Kelly[3,7], Faisal M Syed[1], Scot J Matkovich[2], Harvey S Hahn[1], Abhinav Diwan[1], Jeffrey S Martini[4], Li Sparks[1], Rohan R Parekh[1], John A Spertus[5], Walter J Koch[4], Sharon L R Kardia[3] & Gerald W Dorn II[1,2]

Enhanced β2-adrenergic desensitization = "genetic beta-blockade"

Liggett et al. *Nat Med*, 2008

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

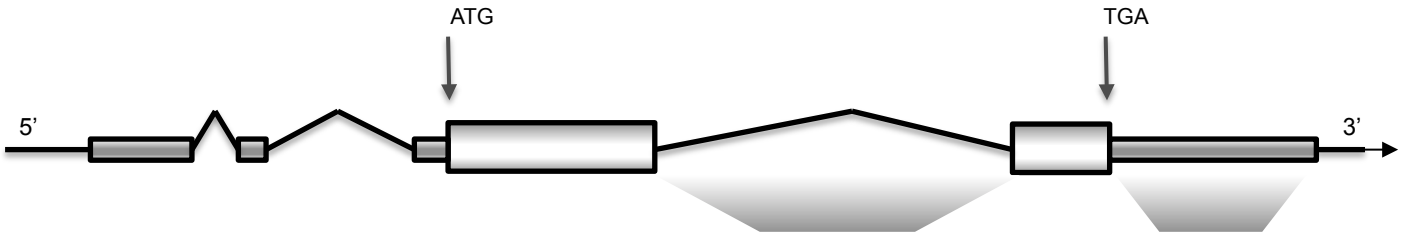
- Linear regression tested association between SNPs and continuous outcome variables
- Logistic regression tested association between SNPs and categorical outcome variables
- Clinically important covariates selected *a priori*
  - backwards selection used to choose covariates that independently correlated 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 ≥ 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)

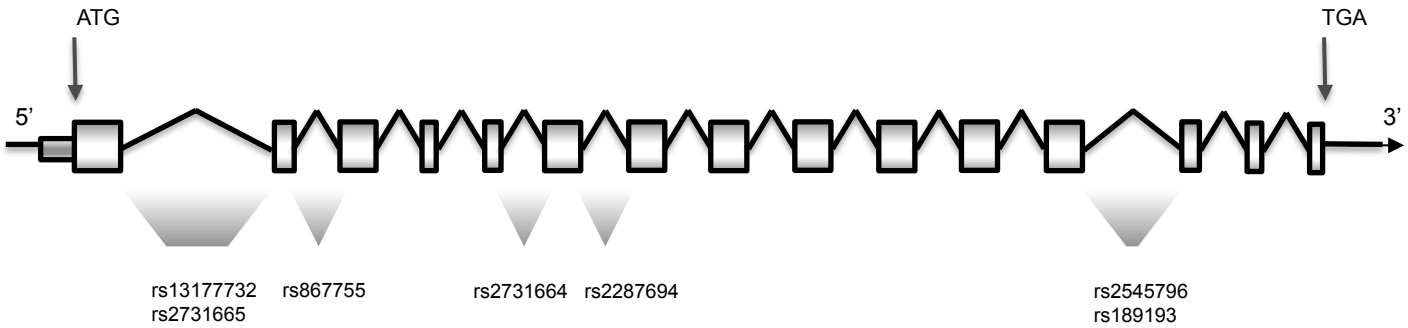


rs11131149	rs237888	rs2139184	rs2324728
rs237894	rs4686301	rs237886	rs9872310
rs237895	rs9840864	rs11706648	rs1042778
rs2268495	rs9810278	rs237887	
rs237899	rs2254295	rs2268490	

OXTR: 18 haplotype tagging SNPs

GRK6 SNP locations

GRK6 gene: chromosome 5 (q35.3)



GRK6: 7 haplotype tagging SNPs

Subject characteristics

Characteristic	Value (n=482)
Age, years	26.9 ± 6.4
Race/ethnicity	
Non-Hispanic white	91 (18.9)
Non-Hispanic black	341 (70.7)
Hispanic	24 (5.0)
Non-Hispanic Asian	26 (5.4)
Pre-pregnancy BMI, kg/m^2	30.1 ± 9.4
Nulliparous	230 (47.7)
Gestational age at delivery, weeks	38.9 ± 1.5
Birthweight, g	3203 ± 552
Cesarean delivery	143 (30.0)

Maximal oxytocin infusion rate

SNP (gene)	Genotype (n): Maximal oxytocin infusion rate (mU/min)	p-value[1]
rs1042778 (*OXTR*)	GG (n=91): 10.9 ± 6.6 GT (n=187): 13.8 ± 7.6 TT (n=140): 14.0 ± 7.6	0.004

[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

SNP (gene)	Genotype (n): Maximal oxytocin infusion rate (mU/min)	p-value[1]
rs1042778 (*OXTR*)	GG (n=91): 10.9 ± 6.6 GT (n=187): 13.8 ± 7.6 TT (n=140): 14.0 ± 7.6	0.004
rs11706648 (*OXTR*)	AA (n=272): 12.7 ± 7.3 AC (n=132): 14.0 ± 7.5 CC (n=16): 16.4 ± 8.6	0.021
rs4686301 (*OXTR*)	CC (n=297): 12.7 ± 7.3 CT (n=111): 14.3 ± 7.6 TT (n=12): 17.6 ± 9.4	0.016
rs9810278 (*OXTR*)	CC (n=354): 12.9 ± 7.4 CT (n=64): 15.4 ± 7.7 TT (n=2): 11.0 ± 1.4	0.022
rs237895 (*OXTR*)	CC (n=270): 13.8 ± 7.6 CT (n=125): 12.0 ± 7.2 TT (n=24): 12.9 ± 7.6	0.027

[1]While 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

SNP (gene)	Genotype (n): Total oxytocin dose (mU)	p-value[1]
rs1042778 (*OXTR*)	GG (n=94): 6,852 ± 7,871 GT (n=196): 10,159 ± 9,787 TT (n=143): 10,425 ± 10,658	0.015
rs4686301 (*OXTR*)	CC (n=308): 8,961 ± 9,377 CT (n-114): 10,874 ± 10,682 TT (n=13): 11,426 ± 10,092	0.034

[1]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

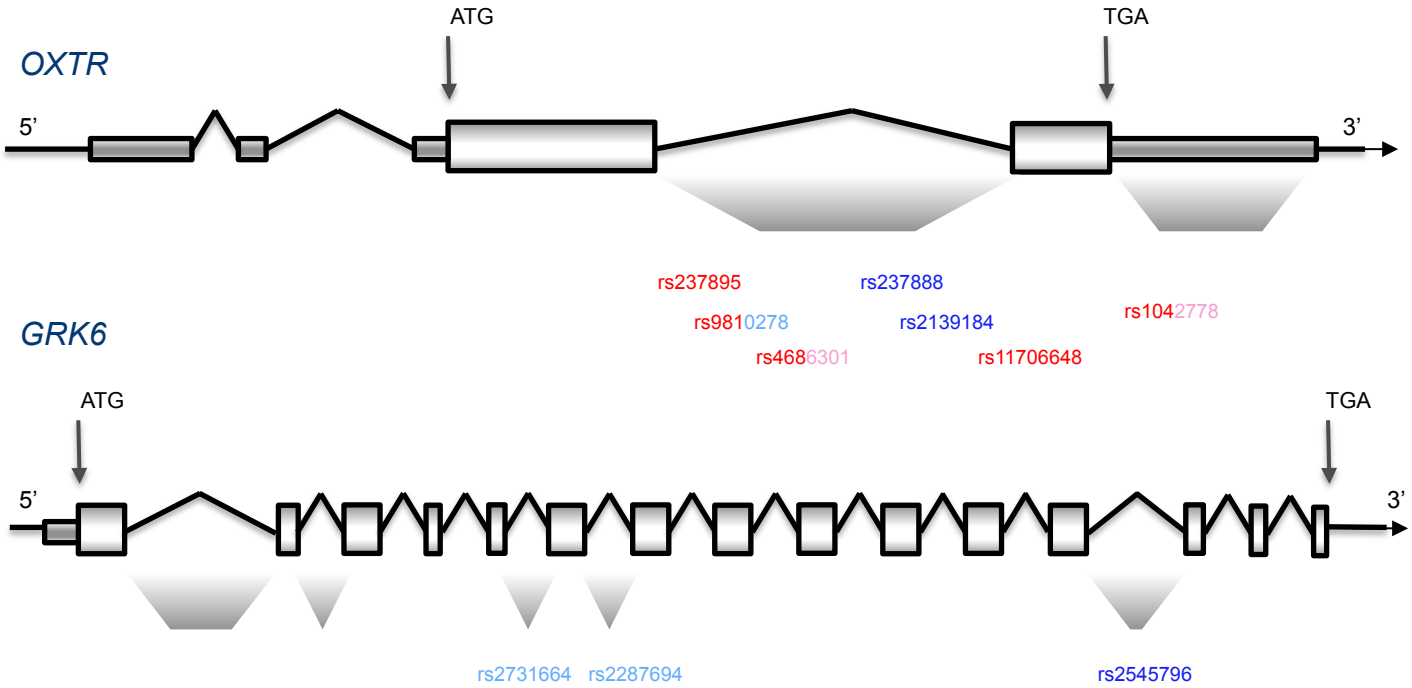
SNP (gene)	Genotype (n): Duration of labor (hours)	p-value[1]
rs9810278 (*OXTR*)	CC (n=406): 20.2 ± 14.5 CT (n=68): 22.6 ± 16.9 TT (n=2): 14.4 ± 2.8	0.041
rs2731664 (*GRK6*)	AA (n=114): 17.7 ± 13.7 AC (n=223): 20.2 ± 14.3 CC (n=132): 23.5 ± 16.5	0.001
rs2287694 (*GRK6*)	CC (n=0): no subjects CT (n=55): 26.2 ± 18.9 TT (n=421): 19.7 ± 14.1	0.009

[1]While controlling for race/ethnicity, nulliparity, cervical dilation at start of induction, pre-pregnancy BMI, gestational age at delivery, and diabetes

Cesarean delivery rate

SNP (gene)	Genotype (n): Cesarean delivery rate	p-value[1] (aOR, [95% CI])
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])

[1]While controlling for race/ethnicity, nulliparity, cervical dilation at start of induction, pre-pregnancy BMI, gestational age at delivery, chorioamnionitis, diabetes, and magnesium therapy


OXTR
ATG
TGA
5'
3'
rs237895
rs237888
rs9810278
rs2139184
rs1042778
rs4686301
rs11706648
GRK6
ATG
TGA
5'
3'
rs2731664
rs2287694
rs2545796
Oxytocin maximal infusion rate
Duration of induced labor
Total oxytocin dose
Cesarean delivery

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?