The Influence of Maternal Body Mass Index on Myometrial Oxytocin Receptor Expression in Pregnancy

Reproductive Sciences
20(12) 1471-1477
© The Author(s) 2013
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1933719113488446
rs.sagepub.com

Chad A. Grotegut, MD, MHS¹, Ravindu P. Gunatilake, MD², Liping Feng, MD¹, R. Phillips Heine, MD¹, and Amy P. Murtha, MD¹

Abstract

Obese pregnant women have higher rates of dysfunctional labor patterns, need for oxytocin augmentation, labor induction, postdates pregnancy, and cesarean delivery compared to normal weight pregnant women. We tested the hypothesis that myometrial oxytocin receptor (*OXTR*) gene and protein expression are affected by obesity in pregnancy. Myometrial samples were obtained at the time of cesarean delivery from the upper aspect of the uterine hysterotomy incision and processed for real-time quantitative polymerase chain reaction and Western blot. There were 63 myometrial samples available for analysis. The median body mass index (BMI) at delivery was 31.0 kg/m² (interquartile range, 26.0, 40.0 kg/m²), and the median gestational age at delivery was 38.0 weeks (interquartile range, 33.0, 39.1 weeks). The *OXTR* gene expression did not correlate with maternal BMI at delivery by linear regression, and the median *OXTR* gene expression did not differ between women with a BMI $\leq$ 30 kg/m² and those with a BMI $\geq$ 40 kg/m². The *OXTR* protein expression was also not affected by maternal BMI. Myometrial *OXTR* gene expression appears to be independent of BMI at the time of delivery. Dysfunctional labor patterns and increased oxytocin utilization seen in obese women may not be due to differences in *OXTR* expression, though functional studies are required.

Keywords

obesity, oxytocin, oxytocin receptor, pregnancy

Introduction

Obesity is a significant public health issue in the United States and is a source of substantial morbidity and mortality. In 2009 to 2010, 35.8% of the adult US women were found to be obese, and the number of pregnant women who are obese is increasing.¹⁻³ In addition to being at risk of medical complications in pregnancy such as diabetes, hypertension, and preeclampsia, obese women are at significant risk of labor abnormalities and cesarean delivery.^{4,5} Labor abnormalities common among obese women include dysfunctional labor patterns, prolonged latent and active phases of the first stage of labor, greater need of oxytocin augmentation, postterm pregnancy, and cesarean delivery.⁶⁻¹¹ Labor abnormalities seen in obese women may be due to inadequate myometrial contraction patterns or decreased responsiveness to oxytocin.

The assessment of uterine contractility among obese women has demonstrated conflicting results. Zhang et al tested the strength and frequency of uterine contraction in myometrial strips isolated from obese and nonobese women undergoing planned elective cesarean delivery. They found that myometrial strips from obese women contracted less frequently and with less force compared to myometrial strips obtained from nonobese women.¹² In contrast, another in vitro study comparing contraction strength and frequency of myometrial tissue

strips from obese women compared to nonobese women demonstrated no differences between the 2 groups.¹³ Similarly, in an in vivo study Chin et al found that though obese women had prolonged labor compared to nonobese women, obese women generated similar strength of uterine contraction during labor, measured by intrauterine pressure assessment (Montevideo units), when compared to nonobese women.⁹

Oxytocin signaling is an important mediator of uterine contractility in labor.¹⁴ Altered expression of myometrial oxytocin receptor (*OXTR*) could potentially contribute to dysfunctional labor patterns and greater need for oxytocin augmentation in obese women. Therefore, our objective was to determine whether myometrial *OXTR* gene and protein expression are affected by maternal obesity and thus a function of maternal body mass index (BMI) at delivery.

¹ Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Duke University School of Medicine, Durham, NC, USA

² University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Corresponding Author:

Chad A. Grotegut, Division of Maternal-Fetal Medicine, Duke University School of Medicine, DUMC Box 3967, Durham, NC 27710, USA.

Email: chad.grotegut@duke.edu

Materials and Methods

Tissue Collection and Quantitative Real-Time PCR

To determine whether myometrial *OXTR* gene expression correlated with maternal BMI, quantitative real-time polymerase chain reaction (PCR) was performed. Myometrial samples had been previously collected at the time of cesarean delivery and stored as part of the Duke University institutional review board-approved protocol, and Duke Pregnancy Outcomes Blood and Tissue Repository were identified. The myometrial samples were pulverized into a powder while keeping the tissue frozen in liquid nitrogen. The homogenized tissue was then collected in TRIzol reagent (Invitrogen, Carlsbad, California), total RNA was extracted using an RNeasy Mini kit (Qiagen, Valencia, California), and RNA concentrations were calculated using the Nanodrop ND-1000 spectrophotometer. A reverse transcription reaction was performed to create complementary DNA (cDNA) using the Superscript III (Invitrogen) first strand synthesis system. Reverse transcription quantitative real-time PCR (RT-PCR) was performed using a Bio-Rad iCycler (Bio-Rad, Hercules, California) and the Taqman Gene Expression Assay (Applied Biosystems, Carlsbad, California), with probes directed against *OXTR* and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a housekeeping gene used for normalization of samples (Assay ID Hs00168573_m1 for the *OXTR* and Assay ID Hs00266705_g1 for GAPDH; Applied Biosystems, Carlsbad, California).

Protein Analysis

To determine whether myometrial *OXTR* protein expression correlated with maternal BMI, 12 myometrial samples from term, elective cesarean deliveries without labor were randomly selected from the Duke Pregnancy Outcomes Blood and Tissue Repository, 6 from patients with a BMI < 30 kg/m² and 6 from patients with a BMI > 40 kg/m². Myometrial tissue was homogenized in cell lysis buffer (Cell Signaling Technologies, Danvers, Massachusetts) and processed for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) electrophoresis using standard protocols. Western blots were performed using antibodies directed against human *OXTR* (product number sc-33209; Santa Cruz Biotechnology, Inc, Santa Cruz, CA), after which the blots were stripped and probed for GAPDH (Cell Signaling Technology) as a protein loading control.

Oxytocin binds the *OXTR* at the plasma membrane, after which, a member of the G protein-coupled receptor kinase family phosphorylates an internal domain of the receptor, allowing for beta-arrestin recruitment to the receptor and receptor internalization and desensitization.¹⁵ The receptor then recycles back to the plasma membrane, where it is then again available for signaling and uterine contraction.¹⁶ Only receptor that is at the plasma membrane is available for oxytocin binding and *OXTR*-mediated uterine contractility. Furthermore, desensitization of the *OXTR* leads to decreases in uterine contractility that are mediated by beta-arrestin.¹⁷ To determine whether there were differences in the amount of plasma membrane-associated *OXTR* protein

Table 1. Demographic Characteristics.

Characteristics	N = 63
BMI, kg/m ²	31.0 (26.0, 40.0)
Race/ethnicity	
Caucasian	29 (46)
Noncaucasian	34 (54)
Gestational age, weeks	38.0 (33.0, 39.1)
Term delivery ≥ 37 weeks, n (%)	38 (60.3)
Labored, n (%)	21 (33.3)
Prior cesarean delivery, n (%)	25 (39.7)

Abbreviation: BMI, body mass index.

by maternal BMI, plasma membrane preparations (preps) were prepared from the same 12 myometrial samples utilized for the assessment of *OXTR* protein expression in whole cell lysate. All myometrial samples were from term, unlabored myometrium, 6 from participants with a BMI < 30 kg/m² and 6 from participants with a BMI > 40 kg/m². Myometrial samples were homogenized in osmotic lysis buffer (25 mM Tris HCl [pH 7.4], 5 mM EDTA, proteinase inhibitor cocktail [product number P2714, Sigma Aldrich, St. Louis, Missouri in water]) on ice. The samples were then centrifuged at low speed in 4°C to pellet the nuclear fraction and cellular debris. The supernatant was then collected and spun at 30 000g for 60 minutes at 4°C. The pellet that contained the membrane fraction was resuspended in cell lysis buffer and processed for SDS–PAGE using standard protocols. Western blots were performed using antibodies directed against human *OXTR* (product number sc-33209; Santa Cruz Biotechnology, Inc.), after which the blots were stripped and probed for caveolin (BD Biosciences, San Jose, California) as a protein loading control.¹⁸

Statistical Analysis

The RT-PCR was performed from myometrial cDNA obtained for each patient and run in duplicate using TaqMan Gene Expression Probes (Applied Biosystems). For each patient, the mean cycle threshold (CT) values for both *OXTR* and *GAPDH* gene expression were obtained. Normalized *OXTR* gene expression was then reported for each sample using the equation, $2^{(OXTR\ CT - GAPDH\ CT)}$. Linear regression was used to correlate normalized *OXTR* gene expression with maternal BMI at delivery. Next, geometric mean and median normalized *OXTR* gene expression were calculated for women with BMI ≤ 30 kg/m² and women with BMI > 40 kg/m² and the median values compared with a nonparametric *t* test (Mann-Whitney test).

To compare the relative *OXTR* protein expression by Western blot, densitometry was performed by measuring the density of the *OXTR* and the *GAPDH* bands (or the *OXTR* and the caveolin bands for the membrane prep experiments) for each sample, and the ratio of the relative density of the *OXTR* band to the *GAPDH* (or caveolin) band was calculated. The mean ratios for each of the 2 BMI groups were compared using a nonparametric *t* test (Mann-Whitney test). All analyses were performed using GraphPad Prism for Macintosh (GraphPad Prism version 5.0, GraphPad Software, San Diego, California) and JMP for Macintosh (JMP

Figure 1. Correlation of myometrial oxytocin receptor (*OXTR*) gene expression with maternal body mass index (BMI) at delivery. To determine whether myometrial *OXTR* gene expression correlated with maternal BMI at delivery, myometrial samples were obtained from the upper aspect of the hysterotomy incision at the time of cesarean delivery and processed for real-time quantitative polymerase chain reaction (PCR). The *OXTR* gene cycle threshold (CT) values were calculated and normalized to the CT value obtained from the housekeeping gene, *GAPDH*, using the equation $2^{(OXTR\ CT - GAPDH\ CT)}$. Plots of normalized *OXTR* gene expression $\times 10\,000$ (log₂ scale) by BMI (linear scale) were constructed. Linear regression was used to compare the normalized *OXTR* gene expression to maternal BMI at delivery. A, Among all the 63 patients, the *OXTR* gene expression did not correlate with maternal BMI at delivery ($r^2 = .023$, $P = .250$). B, The median myometrial *OXTR* gene expression at the time of delivery did not differ between nonobese women (BMI ≤ 30 kg/m², $n = 27$) and obese women (BMI ≥ 40 kg/m², $n = 14$), $P = .151$ for difference in mean *OXTR* gene expression by Mann-Whitney test (bars represent geometric mean with 95% confidence interval). The y-axis for each graph represents normalized *OXTR* gene expression as $2^{(OXTR\ CT - GAPDH\ CT)} \times 10\,000$ (log₂ scale).

version 10.0, SAS Institute Inc, Cary, North Carolina). A P value of $<.05$ was considered significant for all analyses.

Results

Myometrial samples were available, having been previously collected as part of the Duke Pregnancy Outcomes Blood and Tissue Repository, for 63 women. The median (quartile) BMI at delivery was 31.0 (26.0, 40.0) kg/m². In all, 46% of the patients were caucasian, the median (quartile) gestational age of delivery was 38.0 (33.0, 39.1) weeks, 60.3% of the patients delivered at greater than or equal to 37 weeks, and 33.3% of the patients labored prior to delivery. All women delivered via cesarean, and 39.7% of the patients had a prior cesarean delivery (Table 1).

Linear regression demonstrated that myometrial normalized *OXTR* gene expression obtained from myometrial samples at delivery did not correlate with maternal BMI (Figure 1A; $r^2 = .023$, $P = .250$). The linear regression analysis was repeated while controlling for labor status and gestational age at delivery. While controlling for labor status and gestational age at delivery, the normalized myometrial *OXTR* gene expression did not correlate with the maternal BMI at delivery (adjusted analysis, $r^2 = .058$, $P = .227$). Also, the median normalized myometrial *OXTR* gene expression did not differ between women with a BMI of less than 30 kg/m² and women with a BMI of greater than 40 kg/m² ($P = .151$; Figure 1B).

Myometrial samples were processed for Western blot to determine whether *OXTR* protein expression varied by maternal BMI at delivery. Twelve randomly selected myometrial samples were processed for Western blot and probed for *OXTR* (Figure 2A). Densitometry demonstrated that the mean relative

OXTR protein expression, when normalized to *GAPDH* as a protein-loading control did not differ between women with a BMI less than 30 kg/m² compared to women with a BMI greater than 40 kg/m² ($P = .938$ for difference in mean; Figure 2B).

To determine whether there were differences in the amount of *OXTR* protein in the myometrial plasma membrane by maternal BMI, plasma membranes were isolated from myometrial samples obtained from 12 patients who delivered at term without labor (6 from women with BMI < 30 kg/m² and 6 from women with BMI > 40 kg/m²), processed for Western blot and probed for *OXTR* (Figure 2C). Densitometry demonstrated that the mean relative *OXTR* protein expression, when normalized to caveolin as a protein-loading control, did not differ between women with a BMI < 30 kg/m² and women with a BMI > 40 kg/m² ($P = .537$ for difference in mean; Figure 2D).

Because myometrial *OXTR* gene expression may be affected by the presence of a uterine scar at the site of repeat hysterotomy, the data were analyzed in women having their first cesarean delivery (Figure 3A). There were myometrial samples available from 38 women. Among women having their first cesarean delivery, there was no correlation of normalized *OXTR* gene expression with maternal BMI ($r^2 = .013$, $P = .450$). The median normalized myometrial *OXTR* gene expression also did not differ between women having a primary cesarean delivery with a BMI of less than 30 kg/m² compared to women with a BMI of greater than 40 kg/m² ($P = .301$; Figure 3B).

Discussion

Obese pregnant women have higher rates of postterm pregnancy, dysfunctional labor patterns, greater need for oxytocin

Figure 2. Myometrial oxytocin receptor (OXTR) protein expression at delivery by BMI. To determine whether myometrial OXTR protein expression at delivery was influenced by maternal body mass index (BMI), the myometrial samples were obtained from the upper aspect of the hysterotomy incision during cesarean delivery, processed for Western blot, and probed with antibodies directed against the OXTR and GAPDH as a protein-loading control. A, Representative Western blot of myometrial samples obtained from 6 women with BMI < 30 kg/m² and from 6 women with BMI > 40 kg/m². B, Densitometry was used to compare the relative OXTR protein expression between the 2 groups. The relative density of the OXTR band compared to the relative density of the protein-loading control, GAPDH, band was determined for each patient. The mean ratio of OXTR–GAPDH was compared between normal weight and obese groups using the nonparametric Mann-Whitney test. There was no difference in the mean OXTR/GAPDH protein levels between nonobese and obese groups ($P = .938$, Mann-Whitney nonparametric t test). To determine whether myometrial plasma membrane OXTR protein expression at delivery was influenced by maternal BMI, myometrial samples were obtained from the upper aspect of the hysterotomy incision during cesarean delivery, membrane fractions were isolated, processed for Western blot, and probed with antibodies directed against the OXTR and caveolin as a protein-loading control. C, Representative Western blot of myometrial membrane prep samples obtained from 6 women with BMI < 30 kg/m² and from 6 women with BMI > 40 kg/m². D, Densitometry was used to compare the relative OXTR membrane protein expression between the 2 groups. The relative density of the OXTR band compared to the relative density of the protein-loading control, caveolin, band was determined for each patient. The mean ratio of OXTR–caveolin was compared between normal weight and obese groups using the nonparametric Mann-Whitney test. There was no difference in the mean OXTR/caveolin protein levels between normal weight and obese groups ($P = .537$, Mann-Whitney nonparametric t test).

augmentation, longer labor, and higher rates of cesarean delivery compared to nonobese women.^{6,8,10,12,19,20} Inadequate uterine contractility may be a key contributor to these adverse outcomes. Oxytocin receptor signaling is an important component of uterine contractility in labor¹⁴; thus, we tested the hypothesis that maternal obesity affects *OXTR* gene expression, which may explain these adverse events. Contrary to our hypothesis, we demonstrate that myometrial *OXTR* gene and protein expression determined at the time of delivery does not correlate with maternal BMI.

In vitro myometrial contractility experiments examining the effect of obesity on uterine contraction have produced conflicting results. Zhang et al tested myometrial contraction strength and frequency in myometrial samples obtained from women undergoing term elective cesarean delivery. They found that myometrial strips from obese women contracted less forcefully

and less frequently compared to strips obtained from nonobese women.¹² In contrast, Higgins et al, also utilizing myometrial strips obtained from women at the time of cesarean delivery, demonstrated no differences in spontaneous myometrial contraction strength or frequency, or oxytocin-induced contraction strength or frequency, in samples obtained from obese compared to nonobese women.¹³ Neither group measured myometrial OXTR mRNA or protein expression; therefore we do not know whether variations in *OXTR* expression accounted for their findings. Clinically, not all obese women, ultimately requiring cesarean delivery for failed labor, have dysfunctional labor patterns. The mechanism affecting myometrial contractility in obesity may only affect a subset of obese women rather than all. This variation in contractile phenotype may be an explanation for the differences in contractile properties seen in these 2 studies.^{12,13}

Figure 3. Correlation of myometrial oxytocin receptor (*OXTR*) gene expression with maternal body mass index (BMI) at delivery among women having a primary cesarean delivery. Because *OXTR* gene expression may be affected by the presence of uterine scar in women with prior cesarean delivery, we correlated normalized *OXTR* gene expression with maternal BMI only among those women undergoing a primary cesarean delivery. A, The *OXTR* gene expression data were available from 38 women who had a primary cesarean delivery. Among these women, the normalized *OXTR* gene expression did not correlate with maternal BMI at delivery ($r^2 = .013$, $P = .450$). B, Among women having a primary cesarean delivery, the median myometrial *OXTR* gene expression at the time of delivery did not differ between normal weight women (BMI ≤ 30 kg/m 2 , $n = 18$) and obese women (BMI ≥ 40 kg/m 2 , $n = 11$), $P = .301$ for difference in mean *OXTR* gene expression by Mann-Whitney test (bars represent geometric mean with 95% confidence interval). The y-axis for each graph represents normalized *OXTR* gene expression as $2^{(OXTR_{CT} - GAPDH_{CT})} \times 10\,000$ (log $_2$ scale).

Abnormal myometrial contractility in obese patients might be due to altered *OXTR* signaling or associated cofactors instead of differential expression of myometrial *OXTR*. Leptin and cholesterol have been shown to affect uterine contractility, both of which are commonly elevated in obesity. Furthermore, obese pregnant women have higher levels of serum leptin compared to nonobese pregnant women.²¹ In vitro studies demonstrate that both leptin and cholesterol independently led to dose-dependent decreases in spontaneous and oxytocin-induced uterine contractions, though the mechanism by which these findings occur in vitro is unknown.^{22,23} Cholesterol is an important component of the cell membrane and has been shown to stabilize the *OXTR* within the plasma membrane.²⁴ Increases in membrane cholesterol levels can affect oxytocin binding to the *OXTR* and receptor conformation.^{25,26} Furthermore, in other G protein-coupled receptor (GPCR) systems, increases in cell membrane cholesterol content leads to enhanced beta-arrestin-mediated GPCR desensitization.²⁷ Variations in cell membrane cholesterol levels may affect *OXTR* binding, signaling, and enhance desensitization, all of which could affect myometrial contractility. Despite the important role of cholesterol in *OXTR* function, it is unknown whether obesity or dietary cholesterol affects myometrial cell membrane cholesterol levels, which could accordingly alter *OXTR* signaling.

Myometrial contractile responsiveness to oxytocin correlates with myometrial *OXTR* expression.²⁸ Multiple studies have looked at the effect of labor and gestational age on myometrial *OXTR* gene expression with conflicting results. Some studies report no change in myometrial *OXTR* expression with labor and gestational age, while others demonstrate increases in *OXTR* expression with labor and/or gestational age.²⁹⁻³³ Steroid hormones and uterine stretch are also important mediators

of myometrial *OXTR* gene expression.^{30,34,35} We are not aware of any studies attempting to link maternal BMI with myometrial *OXTR* gene expression, especially in an attempt at explaining why obese women are prone to dysfunctional labor patterns. It is possible that myometrial sensitivity to oxytocin may be more important for uterine contractility than absolute myometrial *OXTR* quantity.^{28,29} Finally, although we did not appreciate differences in absolute *OXTR* protein expression by maternal BMI in myometrial membrane prep samples, we did not assess *OXTR* binding. We are unaware of any studies demonstrating variations in GPCR binding by obesity, but future studies are needed to determine whether oxytocin binding to the *OXTR* is affected by maternal obesity.

We obtained myometrial samples from the lower uterine segment. The *OXTR* gene expression varies through different regions of the pregnant uterus, where the fundus expresses greater amounts of *OXTR* compared to the lower uterine segment and cervix.³⁶⁻³⁸ Variations in *OXTR* density throughout the myometrium may be important in the coordination of contractions from fundus toward cervix.³⁶ It is possible that fundal expression of *OXTR* varies with maternal obesity, while lower uterine segment levels remain unaffected by maternal obesity. As our samples were all obtained from the lower uterine segment, we would be unable to detect these differences, if they indeed exist.

In summary, dysfunctional labor patterns manifesting as postterm pregnancy, prolonged labor, increased need for induction or oxytocin augmentation, and increased rates of cesarean delivery are common among obese pregnant women. Adequate uterine contractility is an important aspect of normal labor, though it is unknown whether differences in uterine contractility fully explain dysfunctional labor patterns seen in obesity.

Here, we demonstrate that lower uterine segment myometrial OXTR expression does not seem to be a function of maternal BMI. Further molecular studies are needed to understand the mechanism by which some obese women have dysfunctional labor patterns including potential differences in myometrial OXTR cycling and signaling among obese and nonobese pregnant women.

Authors' note

This study was presented in part at the 31st Annual Meeting, Society for Maternal-Fetal Medicine, San Francisco, CA; February 10, 2011.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Eunice Kennedy Shriver National Institute of Child Health & Human Development under award number K12-HD-043446 (to CAG, BIRCWH Scholar) and by the Duke Perinatal Research Initiative, supported by the Krzyzewski Family (to APM).

References

- Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA*. 2012;307(5):491-497.
- Kim SY, Dietz PM, England L, Morrow B, Callaghan WM. Trends in pre-pregnancy obesity in nine states, 1993-2003. *Obesity (Silver Spring)*. 2007;15(4):986-993.
- Mokdad AH, Serdula MK, Dietz WH, Bowman BA, Marks JS, Koplan JP. The spread of the obesity epidemic in the United States, 1991-1998. *JAMA*. 1999;282(16):1519-1522.
- Baeten JM, Bukusi EA, Lambe M. Pregnancy complications and outcomes among overweight and obese nulliparous women. *Am J Public Health*. 2001;91(3):436-440.
- Ovesen P, Rasmussen S, Kesmodel U. Effect of prepregnancy maternal overweight and obesity on pregnancy outcome. *Obstet Gynecol*. 2011;118(2 pt 1):305-312.
- Roos N, Sahlin L, Ekman-Ordeberg G, Kieler H, Stephansson O. Maternal risk factors for postterm pregnancy and cesarean delivery following labor induction. *Acta Obstet Gynecol Scand*. 2010;89(8):1003-1010.
- Verdiales M, Pacheco C, Cohen WR. The effect of maternal obesity on the course of labor. *J Perinat Med*. 2009;37(6):651-655.
- Pevzner L, Powers BL, Rayburn WF, Rumney P, Wing DA. Effects of maternal obesity on duration and outcomes of prostaglandin cervical ripening and labor induction. *Obstet Gynecol*. 2009;114(6):1315-1321.
- Chin JR, Henry E, Holmgren CM, Varner MW, Branch DW. Maternal obesity and contraction strength in the first stage of labor. *Am J Obstet Gynecol*. 2012;207(2):129. e1-e6.
- Halloran DR, Cheng YW, Wall TC, Macones GA, Caughey AB. Effect of maternal weight on postterm delivery. *J Perinatol*. 2012;32(2):85-90.
- Caughey AB, Stotland NE, Washington AE, Escobar GJ. Who is at risk for prolonged and postterm pregnancy? *Am J Obstet Gynecol*. 2009;200(6):683. e1-e5.
- Zhang J, Bricker L, Wray S, Quenby S. Poor uterine contractility in obese women. *BJOG*. 2007;114(3):343-348.
- Higgins CA, Martin W, Anderson L, et al. Maternal obesity and its relationship with spontaneous and oxytocin-induced contractility of human myometrium in vitro. *Reprod Sci*. 2010;17(2):177-185.
- Vrachnis N, Malamas FM, Sifakis S, Deligeoroglou E, Iliodromiti Z. The oxytocin-oxytocin receptor system and its antagonists as tocolytic agents. *Int J Endocrinol*. 2011;2011:350546.
- Oakley RH, Laporte SA, Holt JA, Barak LS, Caron MG. Molecular determinants underlying the formation of stable intracellular G protein-coupled receptor-beta-arrestin complexes after receptor endocytosis*. *J Biol Chem*. 2001;276(22):19452-19460.
- Conti F, Sertic S, Reversi A, Chini B. Intracellular trafficking of the human oxytocin receptor: evidence of receptor recycling via a Rab4/Rab5 "short cycle". *Am J Physiol Endocrinol Metab*. 2009;296(3):E532-E542.
- Grotegut CA, Feng L, Mao L, Heine RP, Murtha AP, Rockman HA. β -Arrestin mediates oxytocin receptor signaling, which regulates uterine contractility and cellular migration. *Am J Physiol Endocrinol Metab*. 2011;300(3):E468-E477.
- Ku CY, Murtazina DA, Kim YS, Garfield RE, Sanborn BM. Changes in rat myometrial plasma membrane protein kinase A are confined to parturition. *Reprod Sci*. 2010;17(7):696-704.
- Gilead R, Yaniv Salem S, Sergienko R, Sheiner E. Maternal "isolated" obesity and obstetric complications. *J Matern Fetal Neonat*. 2012;25(12):2579-2582.
- Arrowsmith S, Wray S, Quenby S. Maternal obesity and labour complications following induction of labour in prolonged pregnancy. *BJOG*. 2011;118(5):578-588.
- Misra VK, Trudeau S. The influence of overweight and obesity on longitudinal trends in maternal serum leptin levels during pregnancy. *Obesity (Silver Spring)*. 2011;19(2):416-421.
- Moynihan AT, Hehir MP, Glavey SV, Smith TJ, Morrison JJ. Inhibitory effect of leptin on human uterine contractility in vitro. *Am J Obstet Gynecol*. 2006;195(2):504-509.
- Jie Z, Kendrick A, Quenby S, Wray S. Contractility and calcium signaling of human myometrium are profoundly affected by cholesterol manipulation: implications for labor? *Reprod Sci*. 2007;14(5):456-466.
- Gimpl G, Fahrenholz F. Cholesterol as stabilizer of the oxytocin receptor. *Biochim Biophys Acta*. 2002;1564(2):384-392.
- Wiegand V, Gimpl G. Specification of the cholesterol interaction with the oxytocin receptor using a chimeric receptor approach. *Eur J Pharmacol*. 2012;676(1-3):12-19.
- Gimpl G, Reitz J, Brauer S, Trossen C. Oxytocin receptors: ligand binding, signalling and cholesterol dependence. *Prog Brain Res*. 2008;170:193-204.
- Qiu Y, Wang Y, Law PY, Chen HZ, Loh HH. Cholesterol regulates micro-opioid receptor-induced beta-arrestin 2 translocation to membrane lipid rafts. *Mol Pharmacol*. 2011;80(1):210-218.
- Arthur P, Taggart MJ, Zielnik B, Wong S, Mitchell BF. Relationship between gene expression and function of uterotonic systems

- in the rat during gestation, uterine activation and both term and preterm labour. *J Physiol*. 2008;586(pt 24):6063-6076.
29. Tattersall M, Engineer N, Khanjani S, et al. Pro-labour myometrial gene expression: are preterm labour and term labour the same? *Reproduction*. 2008;135(4):569-579.
30. Mesiano S, Chan EC, Fitter JT, Kwek K, Yeo G, Smith R. Progesterone withdrawal and estrogen activation in human parturition are coordinated by progesterone receptor A expression in the myometrium. *J Clin Endocrinol Metab*. 2002;87(6):2924-2930.
31. Condon JC, Jeyasuria P, Faust JM, Wilson JW, Mendelson CR. A decline in the levels of progesterone receptor coactivators in the pregnant uterus at term may antagonize progesterone receptor function and contribute to the initiation of parturition. *Proc Natl Acad Sci U S A*. 2003;100(16):9518-9523.
32. Wathes DC, Borwick SC, Timmons PM, Leung ST, Thornton S. Oxytocin receptor expression in human term and preterm gestational tissues prior to and following the onset of labour. *J Endocrinol*. 1999;161(1):143-151.
33. Helguera G, Eghbali M, Sforza D, Minosyan TY, Toro L, Stefani E. Changes in global gene expression in rat myometrium in transition from late pregnancy to parturition. *Physiol Genomics*. 2009;36(2):89-97.
34. Terzidou V, Sooranna SR, Kim LU, Thornton S, Bennett PR, Johnson MR. Mechanical stretch up-regulates the human oxytocin receptor in primary human uterine myocytes. *J Clin Endocrinol Metab*. 2005;90(1):237-246.
35. Ou CW, Chen ZQ, Qi S, Lye SJ. Increased expression of the rat myometrial oxytocin receptor messenger ribonucleic acid during labor requires both mechanical and hormonal signals. *Biol Reprod*. 1998;59(5):1055-1061.
36. Havelock JC, Keller P, Muleba N, et al. Human myometrial gene expression before and during parturition. *Biol Reprod*. 2005;72(3):707-719.
37. Fuchs AR, Fuchs F, Husslein P, Soloff MS. Oxytocin receptors in the human uterus during pregnancy and parturition. *Am J Obstet Gynecol*. 1984;150(6):734-741.
38. Blanks AM, Vatish M, Allen MJ, et al. Paracrine oxytocin and estradiol demonstrate a spatial increase in human intrauterine tissues with labor. *J Clin Endocrinol Metab*. 2003;88(7):3392-3400.