

PROTOCOL TITLE: Effect of Adiponectin and TNF α on uterine contractility in GDM and obese pregnant patients

PROTOCOL DATE: 02/26/2018

Protocol Title	Effect of Adiponectin and TNF α on uterine contractility in GDM and obese pregnant patients
Principal Investigator/Faculty Advisor	Name: Katherine Jacobs
	Department: Assistant Professor, Maternal-Fetal Medicine
	Telephone Number: 612-301-3410
	Institutional Email Address: gran0254@umn.edu
Co-Investigator	Name: Hiba Mustafa
	Department: Fellow, Maternal-Fetal Medicine
	Telephone Number: (832)475-4061
	Institutional Email Address: musta062@umn.edu
Co-Investigator	Name: Lisa Gill
	Department: Fellow, Maternal-Fetal Medicine
	Telephone Number: 612-301-3408
	Institutional Email Address: lgill@umn.edu
Co-Investigator	Name: Paul Iaizzo
	Department: Professor, Surgery; Integrative Biology and Physiology
	Institutional Email Address: iaizz001@umn.edu
Scientific Assessment	HRPP facilitated scientific assessment
IND/IDE # (if applicable)	N/A.
IND/IDE Holder	N/A.
Investigational Drug Services # (if applicable)	N/A.
Version Number/Date:	1/02-14-2018

PROTOCOL TITLE: Effect of Adiponectin and TNFa on uterine contractility in GDM and obese pregnant patients

PROTOCOL DATE: 02/26/2018

REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Change?

Table of Contents

1.0	Objectives	5
2.0	Background.....	5
3.0	Study Endpoints/Events/Outcomes	7
4.0	Study Intervention(s)/Investigational Agent(s).....	7
5.0	Procedures Involved	7
6.0	Data and Specimen Banking	11
7.0	Sharing of Results with Participants: results will not be shared with participants.....	12
8.0	Vulnerable Populations	12
9.0	Local Number of Participants:	13
10.0	Withdrawal of Participants	13
11.0	Risks to Participants	13
12.0	Potential Benefits to Participants	13
13.0	Statistical Considerations	13
14.0	Confidentiality:.....	14
15.0	Provisions to Protect the Privacy Interests of Participants	14
16.0	Compensation for Research-Related Injury	14
17.0	Consent Process.....	14
18.0	Setting.....	15
19.0	Multi-Site Research: N/A	16
20.0	References	16

PROTOCOL TITLE: Effect of Adiponectin and TNFa on uterine contractility in GDM and obese pregnant patients

PROTOCOL DATE: 02/26/2018

ABBREVIATIONS/DEFINITIONS

GDM Gestational Diabetes Mellitus

DM Diabetes Mellitus

OGTT Oral glucose tolerance test

CS Cesarean section

OR Odds Ratio

P4 Progesterone

HPC 17a-Hydroxyprogesterone

PROTOCOL TITLE: Effect of Adiponectin and TNF α on uterine contractility in GDM and obese pregnant patients

PROTOCOL DATE: 02/26/2018

1.0 Objectives

Primary objective: Determine the effect of adipokines adiponectin and TNF α on the force of myometrial contractility in diabetic, non-obese pregnant women and obese, non-diabetic term pregnant women at the time of scheduled cesarean delivery

Secondary objectives:

1. Compare the force of myometrial contractility in study groups compared to control group (non-DM, non-obese term pregnant patients)
2. Investigate the anti-inflammatory properties of Progesterone (P4) and 17 α -Hydroxyprogesterone (HPC) on Adiponectin induced myometrial contractility in control group.

2.0 Background

Gestational diabetes mellitus (GDM) and obesity are two of the most important global health issues that are associated with significant fetal and maternal morbidity and mortality [1-4].

There is a consensus among researchers that the Caesarean section (CS) rate is higher in women with diabetes and obese women [5-7], with reported rates of up to 67.4% in these populations [8].

Some authors suggest that the increased CS rate is due to the confounding factor of obesity [9,10]; however, other analyses have found diabetes mellitus to be an independent risk for CS [11-13].

The literature reports that at least half of indicated cesarean deliveries in a diabetic population are due to labor dystocia or failed induction of labor. This is true even when controlling for infant size. Post-partum hemorrhage is also six times more common in diabetic women, raising suspicion that myometrial contractility may be impaired in this population [14,15].

Therefore, poor myometrial contractility may be an important factor contributing to the increased CS rates. Further evidence to implicate poor myometrial contractility is that post-partum hemorrhage is six times more common in diabetic women [15].

Obesity may alter the pregnant uterine contractility as well, and hence make the gestation period shorter or longer [16,17]

Obesity was found to be an independent risk factor for emergency cesarean section (CS) delivery [18-23].

PROTOCOL TITLE: Effect of Adiponectin and TNF α on uterine contractility in GDM and obese pregnant patients
PROTOCOL DATE: 02/26/2018

When stratified by BMI, the indication for cesarean section was significantly more likely to be arrest of labor in the first stage at higher BMI categories [24].

A subsequent analysis of more than 230,000 Swedish births concluded that labor dystocia is a key factor leading to the increased rate of CS among obese women, and found no evidence that obstruction was a cause after control for birth weight. As progress in labor is critically dependent on myometrial contractions, these data suggest that myometrial contractility is adversely influenced by obesity. As in diabetic women, obese women delivering vaginally have excessive blood loss which is also consistent with poor myometrial contractility [25]. This has subsequently been validated using in vitro evaluation of myometrium from obese women obtained at the time of scheduled cesarean delivery (Zhang et. al).

The mechanism of this change in myometrial contractility is not completely elucidated, however the increased adiposity in obese women has yielded a hypothesis. Adipokines are defined as cell signaling mediators secreted by the adipose tissue [26]. The adipose tissue is a multifunctional organ which secretes a large spectrum of adipokines. These substances may act in a pro- or anti-inflammatory manner. In the inflammatory states of diabetes and obesity, pro-inflammatory adipokines such as leptin, TNF- α , visfatin, and resistin are elevated, while the anti-inflammatory adipokine adiponectin is decreased in these populations. This is true both in and outside of pregnancy. The same adipokine profile is found in gestational diabetics [28-30].

Smooth muscle in the vasculature is known to contain receptors for adipokines, and these substances have been shown to modulate smooth muscle contraction in vascular smooth muscle. Likewise, adipokine receptors are found in human myometrium, yielding an interesting potential mechanism for differences in myometrial contractility in an obese or diabetic population. Myometrial contractility in vitro has been shown to be modulated by adipokines. Pro-inflammatory adipokines, such as leptin and visfatin, have been shown to decrease myometrial contractility in vitro. Unpublished data from our group shows that adiponectin may act to stimulate myometrial contraction in vitro in an otherwise healthy population (manuscript submitted for publication currently) If adiponectin increases myometrial contractility in a normal population, we hypothesize that this effect may be enhanced in a population known to be deficient in this substance.

3.0 Study Endpoints/Events/Outcomes

Eligible individuals will be recruited during their pre-natal clinic visits after they had been scheduled for a CS delivery. They will be presented with the consent form, and HIPPA form for their review. Eligible subjects will be recruited by one of the co-investigators. Those who agree to participate will be consented for the study upon their presentation for their scheduled CS delivery.

Uterine muscle biopsies will be obtained from the upper aspect of a low transverse hysterotomy during the CS procedure after delivery of the fetus and placenta and prior to uterine incision closure. The sample will be placed in physiologic buffer by the study staff. The sample will be given a number by Bionet staff for tracking purposes and the sample will be transported to the Visible Heart Lab (PI: Dr. Paul Iaizzo) in the Mayo Building, University of Minnesota.

4.0 Study Intervention(s)/Investigational Agent(s)

No intervention will be used directly on participants, however obtained myometrial biopsy will be tested for contractility in the presence of human adiponectin or TNF- α . Chemicals will be purchased through the University

5.0 Procedures Involved

5.1 Study Design: Prospective observational cohort study

5.2 Study groups:

Control group: healthy, term, non-obese (BMI < 30) pregnant women with a singleton gestation scheduled for CS delivery at 37-41 weeks of gestation.

Study group 1: term pregnant, non-obese (BMI <30), diagnosed with gestational diabetes, scheduled for CS delivery between 37-41 weeks of gestation.

Study group 2: term pregnant, obese (BMI >30), non-diabetic and scheduled for CS delivery between 37-41 weeks of gestation.

5.3 Study Procedures:

Inclusion criteria: the inclusion criteria for all groups:

- 1) Pregnant patients scheduled for a CS at 37-41 weeks of gestation at M Health Birth Place
- 2) Participants age ≥ 18 .
- 3) Full informed consent able to be provided by the participant.

Exclusion criteria

- 1) Patients undergoing general anesthesia for their CS.
- 2) Pre-gestational DM, and DM diagnosed < 24 weeks gestation.
- 3) Patients unable to consent for themselves
- 4) Multiple gestation

Screening and recruitment

- Subjects undergoing cesarean section will be identified through the C-section schedule in EPIC and the schedule book in Labor and delivery.
- Epic will be reviewed to identify subjects who opted out of research.
- For subjects who did not opt out of research, obstetric clinic progress noted, and vitals including BMI will be reviewed for the subjects undergoing C-section to determine eligibility.
- Eligible participants (please see inclusion and exclusion criteria above) will be approached and recruited for the study by either Dr. Mustafa or Dr. Gill during the subject clinic visits in Riverside Professional Building clinics.
- Subjects will not be approached in clinic only after notifying their clinic provider who will ask for their permission to meet with the study team consenting them.
- During that clinic visit subjects will be given a copy of the consent form and HIPPA form.
- The consent will be obtained subsequently upon presentation to labor and delivery for cesarean delivery.

PROTOCOL TITLE: Effect of Adiponectin and TNF α on uterine contractility in GDM and obese pregnant patients

PROTOCOL DATE: 02/26/2018

- No local recruitment methods/materials(e.g. advertisement) will be used
- No payment will be given to participants

Methods

- Upon participants presentation for their scheduled CS delivery at the birth center, informed consent will be obtained .
- At the time of scheduled cesarean delivery, a strip of myometrium approximately 5 mm in diameter length will be collected from the upper border of the low transverse hysterotomy along its length.
- The biopsy will be obtained by the surgical team performing the procedure.
- The removal of this length of myometrial tissue will not significantly change the surgical procedure for the cesarean section. It is anticipated that the acquisition of this tissue will add approximately 0.5-1 minute to the surgical procedure, with a clinically insignificant increase in blood loss. Therefore, less than minimal increase in patient risk or outcome due to the acquisition of this tissue is anticipated, and the technique has been described in numerous publications previously for similar testing.
- Myometrial tissue will be immediately immersed in in physiologic Krebs buffer provided by the Visible Heart Lab, labelled with a unique code assigned for each participant. Bionet registration is anticipated in a pass-through fashion. The sample will then be transported to the Visible Heart Lab immediately for testing.
- Myometrial tissue biopsies will then be dissected free of serosa and connective tissue under light microscopy into multiple bundles approximately 2 cm in length and 2 mm in diameter. The longitudinal strips will be suspended in oxygenated physiologic Krebs solution isometrically at 37 degrees Celsius. Dilute oxytocin will be added to the tissue baths to induce contractions. The muscle bundles will be allowed to equilibrate for approximately 1 hour. Recordings of the force of contraction will be obtained over a one hour period, as previously described in similar studies [34, 35]. Data will be continuously recorded with a custom-built LabView interface.

PROTOCOL TITLE: Effect of Adiponectin and TNF α on uterine contractility in GDM and obese pregnant patients

PROTOCOL DATE: 02/26/2018

- For gestational diabetic and obese groups, after one hour of baseline data is recorded, bundles will be assigned to adiponectin infusion, TNF-alpha infusion or saline infusion. Force of contraction will again be continuously recorded for one hour.
- Myometrial biopsies from the control group will be treated in duplicate with Progesterone(P4) or 17 α -hydroxyprogesterone (HPC). Those samples will be subsequently exposed to Adiponectin. UtSMC with +/- Adiponectin will serve as positive and negative controls. IL-6 will be measured in the culture media by ELISA to assess the inflammatory response.

PROTOCOL TITLE: Effect of Adiponectin and TNF α on uterine contractility in GDM and obese pregnant patients

PROTOCOL DATE: 02/26/2018

- Following completion of data recordings, samples will be disposed by using standard biohazard disposal technique. Data regarding baseline characteristics of women enrolled in the trial will be collected by chart review by the principle investigator. These data will be maintained on a password protected departmental server utilizing REDCap. Data collected will include: name, medical record number, age, BMI at start of pregnancy, BMI at delivery, A1c level in pregnancy for GDM patients, weight gain (kg) in pregnancy, ethnicity, gravidity, parity, gestational age at delivery, and infant birth weight. Upon time for data analysis, each participant will be assigned a unique code so data will be de-identified.

5.4 Study Duration: Enrollment and statistical analysis is expected to finish by 07/2019

5.5 Individually Identifiable Health Information:
Name and MR numbers will be collected initially in REDCap; study data later will be coded (unique code will be assigned for every participant) for the statistical analysis.

5.6 Use of radiation: N/A

5.7 Use of Center for Magnetic Resonance Research: N/A

6.0 Data and Specimen Banking

6.1 Storage and Access: Data will be stored in REDCap and will be banked for future use. Myometrial specimens will be disposed by using standard biohazard disposal technique. All study team members will have access to the data. Bionet will be utilized on a “Pass-through” basis.

6.2 Data: The data that will be banked for future use include the de-identified data: BMI at start of pregnancy, BMI at delivery, A1c level for GDM patients, weight gain (kg) in pregnancy, ethnicity, gravidity, parity, gestational age at delivery, and infant birth weight.

6.3 Release/Sharing: Stored data will be de-identified for analysis using the unique code assigned for every participant.

7.0 **Sharing of Results with Participants:** results will not be shared with participants.

8.0 Vulnerable Populations

8.1 Vulnerable Populations:

- Fetuses/Neonates/Children
- Pregnant women
- Prisoners
- Adults lacking capacity to consent and/or adults with diminished capacity to consent, including, but not limited to, those with acute medical conditions, psychiatric disorders, neurologic disorders, developmental disorders, and behavioral disorders
- Approached for participation in research during a stressful situation such as emergency room setting, childbirth (labor), etc.
- Disadvantaged in the distribution of social goods and services such as income, housing, or healthcare
- Serious health condition for which there are no satisfactory standard treatments
- Fear of negative consequences for not participating in the research (e.g. institutionalization, deportation, disclosure of stigmatizing behavior)
- Any other circumstance/dynamic that could increase vulnerability to coercion or exploitation that might influence consent to research or decision to continue in research
- Undervalued or disenfranchised social group
- Members of the military
- Non-English speakers
- Those unable to read (illiterate)
- Employees of the researcher
- Students of the researcher
- None of the above

9.0 Local Number of Participants:

20 controls, 20 gestational diabetics, and 20 obese women will be recruited.

10.0 Withdrawal of Participants

Participants will be withdrawn from the study if any clinically significant event or condition is uncovered during the surgery (e.g., excessive bleeding, acute sepsis) that might render the subject medically unstable or complicate the subject's postsurgical course. Data will not be collected or used if participants were withdrawn from the study. Likewise, participants are free to withdraw their consent from the study at any time.

11.0 Risks to Participants

Myometrial biopsies will be obtained after delivery of the fetus. So no risks are anticipated to the fetus.

The removal of this length of myometrial tissue will not significantly change the surgical procedure for the cesarean section. It is anticipated that the acquisition of this tissue will add approximately 0.5-1 minute to the surgical procedure, with a clinically insignificant increase in blood loss. Therefore, a minimal increase in patient risk or outcome due to the acquisition of this tissue is anticipated, and the technique has been described in numerous publications previously for similar testing [36-38]

12.0 Potential Benefits to Participants

There is no direct benefit to individual participants.

13.0 Statistical Considerations

Anticipated numbers for each group were estimated from those compared in prior studies. No a priori data for this particular subset of patients are available, and as such a power calculation was not feasible. Sample size was determined using sample sizes from previous similar studies, and in accordance to resources available as well as number of patients available for recruitment. Using custom-built MatLab software mean and median values for maximum

PROTOCOL TITLE: Effect of Adiponectin and TNF α on uterine contractility in GDM and obese pregnant patients

PROTOCOL DATE: 02/26/2018

contractile force will be evaluated, as well as area under the curve. These values will be compared using univariate analysis of either a Mann Whitney U test for medians or t-test for means. A p value <0.05 will be considered significant.

14.0 Confidentiality:

All data will be stored in a secure data shelter available through REDCap. The data will be accessible only to study staff that has completed HIPAA compliance training through the University of Minnesota. REDCap database is password protected. All paper forms will be destroyed after finishing the study.

15.0 Provisions to Protect the Privacy Interests of Participants

- The consent process will be done in a secluded private setting.
- HIPAA authorization will be obtained from each participant to access personal health information (PHI) to obtain only the required related information to the study.
- Refusing to participate in the study will not affect their care in any way.
- A unique code will be assigned to every participant to de-identify PHI.
- If interpreter is needed during the process, subject's permission will be obtained first to discuss study details using the interpreter.
- Data will be accessed and stored using a password protected REDCap database.

16.0 Compensation for Research-Related Injury

No research related injury is anticipated in this research study, but in the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow up care as needed. Care for such injuries will be billed in the ordinary manner. No personal compensation will be provided.

17.0 Consent Process

17.1 Consent process:

PROTOCOL TITLE: Effect of Adiponectin and TNF α on uterine contractility in GDM and obese pregnant patients

PROTOCOL DATE: 02/26/2018

- Eligible participants (please see inclusion and exclusion criteria above) will be approached and recruited for the study by the PI during their clinic visits after they had being scheduled for their CS delivery by their provider, and they will be given a copy of the consent form and HIPPA form during that clinic visit.
- The consent will be obtained subsequently upon presentation to labor and delivery in Riverside Hospital for their scheduled cesarean delivery.

17.2 Waiver or alteration of consent process: N/A

17.3 Non-English Speaking Participants:

- After obtaining subject's permission to discuss study details using an interpreter. Oral instructions will be explained via the interpreter; written instructions will be given in her language.
- Written instructions will be translated to the desired language using certified interpreter.
- The language of the investigator obtaining consent is English.
- Patients will be screened for eligibility through reviewing their medical chart by the PI who has legitimate access to these patient's records as she routinely take part in cesarean deliveries in the birth place.

17.4 Participants Who Are Not Yet Adults (infants, children, teenagers under 18 years of age): Will be excluded from the study

17.5 Cognitively Impaired Adults, or adults with fluctuating or diminished capacity to consent: Will be excluded from the study

17.6 Adults Unable to Consent: Will be excluded from the study

18.0 Setting

18.1 Research Sites:

- Research team will identify and recruit potential participants during their clinic visits at Fairview Riverside Women's Clinic, Women's Health Specialists Clinic, and Maternal Fetal Medical Center in the Riverside Professional Building.
- Biopsies will be obtained at the M Health Birth Place and muscle contraction studies will take place in the Visible

PROTOCOL TITLE: Effect of Adiponectin and TNF α on uterine contractility in GDM and obese pregnant patients

PROTOCOL DATE: 02/26/2018

Heart Lab in the Mayo Building on the East Bank of the
University of Minnesota.

18.2 International Research: N/A

19.0 Multi-Site Research: N/A

20.0 References

1. Soheilykhah, S., Mojibian, M., Rahimi-Saghand, S., Rashidi, M. and Hadinedoushan, H. (2011) Maternal serum leptin concentration in gestational diabetes. *Taiwan J Obstet Gynecol* 50: 149–153
2. Buchanan, T. and Xiang, A. (2005) Gestational diabetes mellitus. *J Clin Invest* 115: 485–491.
3. Hehir MP, Morrison JJ. The adipokine apelin and human uterine contractility. *Am J Obstet Gynecol.* 2012;206:359.e1-5.
doi:10.1016/j.ajog.2012.01.032. Medline:22360921

PROTOCOL TITLE: Effect of Adiponectin and TNF α on uterine contractility in GDM and obese pregnant patients

PROTOCOL DATE: 02/26/2018

4. Rooney BL, Schauberger CW. Excess pregnancy weight gain and long-term obesity: one decade later. *Obstet Gynecol.* 2002;100:245-52.

Medline:12151145

5. Ehrenberg HM, Durnwald CP, Catalano P, Mercer BM (2004) The influence of obesity and diabetes on the risk of cesarean delivery. *Am J Obstet Gynecol* 191:969–974

6. Jensen DM, Damm P, Moelsted-Pedersen L et al (2004) Outcomes in type 1 diabetic pregnancies: a nationwide, population-based study. *Diabetes Care* 27:2819–2823

7. Dunne F, Brydon P, Smith K, Gee H (2003) Pregnancy in women with Type 2 diabetes: 12 years outcome data 1990–2002. *Diabet Med* 20:734–738

8. Department of Health (2010) NHS Maternity Statistics, England. 2002–2003 Statistical Bulletin

9. Evers IM, de Valk HW, Visser GH (2004) Risk of complications of pregnancy in women with type 1 diabetes: nationwide prospective study in the Netherlands. *BMJ* 328:915

10. Langer O, Yogev Y, Xenakis EM, Brustman L (2005) Overweight and obese in gestational diabetes: the impact on pregnancy outcome. *Am J Obstet Gynecol* 192:1768–1776

11. Ehrenberg HM, Durnwald CP, Catalano P, Mercer BM (2004) The influence of obesity and diabetes on the risk of cesarean delivery. *Am J Obstet Gynecol* 191:969–974

12. Bo S, Menato G, Signorile A et al (2003) Obesity or diabetes: what is worse for the mother and for the baby? *Diabetes Metab* 29:175–178

13. Kjos SL, Berkowitz K, Xiang A (2004) Independent predictors of cesarean delivery in women with diabetes. *J Matern Fetal Neonatal Med* 15:61–67

PROTOCOL TITLE: Effect of Adiponectin and TNF α on uterine contractility in GDM and obese pregnant patients

PROTOCOL DATE: 02/26/2018

14. Evers IM, de Valk HW, Visser GH (2004) Risk of complications of pregnancy in women with type 1 diabetes: nationwide prospective study in the Netherlands. *BMJ* 328:915
15. Dunne F, Brydon P, Smith K, Gee H (2003) Pregnancy in women with Type 2 diabetes: 12 years outcome data 1990–2002. *Diabet Med* 20:734–738
16. Fleischman AR, Oinuma M, Clark SL. Rethinking the definition of “term pregnancy”. *Obstet Gynecol.* 2010;116:136-9. doi:10.1097/AOG.0b013e3181e24f28. Medline:20567179
17. Zhang J, Bricker L, Wray S, Quenby S. Poor uterine contractility in obese women. *BJOG.* 2007;114:343-8. doi:10.1111/j.1471-0528.2006.01233.x. Medline:17261121
18. R. Gilead, S. Yaniv Salem, R. Sergienko, E. Sheiner, Maternal “isolated” obesity and obstetric complications, *J.Matern. Fetal NeonatalMed.* 25 (12) (2012) 2579–2582.
19. I. Bautista-Castano, P. Henriquez-Sanchez, N. Aleman-Perez, J.J. Garcia-Salvador, A.Gonzalez-Quesada, J.A. Garcia-Hernandez, et al., Maternal obesity in early pregnancy and risk of adverse outcomes, *PLoS One* 8 (11) (2013) e80410.
20. J.X.Wang,M.J. Davies, R.J. Norman, Obesity increases the risk of spontaneous abortion during infertility treatment, *Obes. Res.* 10 (6) (2002) 551–554.
21. T.E. O'Brien, J.G. Ray, W.S. Chan, Maternal body mass index and the risk of preeclampsia: a systematic overview, *Epidemiology* 14 (3) (2003) 368–374.
22. M. Wolf, E. Kettyle, L. Sandler, J.L. Ecker, J. Roberts, R. Thadhani, Obesity and preeclampsia: the potential role of inflammation, *Obstet. Gynecol.* 98 (5 Pt 1) (2001) 757–762.

PROTOCOL TITLE: Effect of Adiponectin and TNF α on uterine contractility in GDM and obese pregnant patients

PROTOCOL DATE: 02/26/2018

23. A.S. Poobalan, L.S. Aucott, T. Gurung, W.C. Smith, S. Bhattacharya, Obesity as an independent risk factor for elective and emergency caesarean delivery in nulliparous women—systematic review and meta-analysis of cohort studies, *Obes. Rev.* 10 (1) (2009) 28–35.

24. J. Zhang, L. Bricker, S. Wray, S. Quenby, Poor uterine contractility in obese women, *BJOG* 114 (3) (2007) 343–348.

25. Meller, M., Qiu, C., Vadachkoria, S., Abetew, D., Luthy, D. and Williams, M. (2006) Changes in placental adipocytokine gene expression associated with gestational diabetes mellitus. *Physiol Res* 55:501–512.

26. J.N. Fain, A.K. Madan, M.L. Hiler, P. Cheema, S.W. Bahouth, Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans, *Endocrinology* 145 (5) (2004) 2273–2282.

27. J. Axelsson, O. Heimburger, B. Lindholm, P. Stenvinkel, Adipose tissue and its relation to inflammation: the role of adipokines, *J. Ren. Nutr.* 15 (1) (2005) 131–136.

28. Kinałski, M., Telejko, B., Kuz' ski, A. and Kinałska, I. (2005) Tumor necrosis factor alpha system and plasma adiponectin concentration in women with gestational diabetes. *Horm Metab Res* 37: 450–454.

29. Desoye, G. and Hauguel-de Mouzon, S. (2007) The human placenta in gestational diabetes mellitus. The insulin and cytokine network. *Diabetes Care* 30(Suppl. 2): S120–S126.

30. N. Smolinska, A. Maleszka, K. Dobrzyń, M. Kieżun, K. Szeszko, T. Kaminski, Expression of adiponectin and adiponectin receptors 1 and 2 in the porcine uterus, conceptus, and trophoblast during early pregnancy, *Theriogenology* 82 (7) (2014) 951–965.

31. S. Wray, Insights into the uterus, *Exp. Physiol.* 92 (4) (2007) 621–631.

PROTOCOL TITLE: Effect of Adiponectin and TNF α on uterine contractility in GDM and obese pregnant patients

PROTOCOL DATE: 02/26/2018

32. R. Floyd, S.Wray, Calcium transporters and signalling in smooth muscles, *Cell Calcium* 42 (4–5) (2007) 467–476
33. Iaizzo PA, Wedel DJ, Gallagher WJ. In vitro contracture testing for determination of susceptibility to malignant hyperthermia: a methodologic update. *Mayo Clin Proc* 66:998-1004.
34. Hehir MP, Morrison JJ. The adipokine apelin and human uterine contractility. *Am J Obstet Gynecol* 2012;206:359.e1-5.
35. Mumtaz S, AlSaif S, Wray S, Noble K. Inhibitory effect of visfatin and leptin on human and rat myometrial contractility. *Life Sciences* 125 (2015) 57-62.
36. Zhang J, 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 Jul;14(5):456-66.
37. Arrowsmith S, Keov P, Muttenthaler M, Gruber CW. Contractility Measurements of Human Uterine Smooth Muscle to Aid Drug Development. *J Vis Exp.* 2018 Jan 26;(131). doi: 10.3791/56639.
38. Talati C, Carvalho JCA, Luca A, Balki M. The Effect of Intermittent Oxytocin Pretreatment on Oxytocin-Induced Contractility of Human Myometrium In Vitro. *Anesth Analg.* 2018 Feb 2. doi: 10.1213/ANE.0000000000002834.