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# Hormonally Modulated S-Nitrosation of Profilin-1 and Myosin Regulatory Light Polypeptide 9

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By

Andrew N. Tschernia

Dr. Iain L. O. Buxton, Pharm D., Thesis Advisor

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Dr. Iain L. O. Buxton, Pharm D., Regents Professor and Chairman, **Department of Pharmacology, UNSOM**

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Tamara Valentine, Ph.D., Director, **Honors Program**

**Abstract:**

The roles of estrogen and progesterone are highly regulated during the different phases of pregnancy. The roles of these hormones and the cellular signal transduction of labor contractions are well understood; however, the initiation of spontaneous preterm labor contractions is still unknown in research today. The levels of estrogen and progesterone are known to increase, and myosin regulatory light polypeptide 9 and profilin-1 is known to be upregulated and S-nitrosated in preterm laboring myometrium. We hypothesize that levels of estrogen and progesterone may have an effect on posttranslational modification of myosin regulatory light polypeptide 9 and profilin-1. Estrogen and Progesterone are highly regulated during the different phases of pregnancy. Using western blot and biotin switch technique, we isolated S-nitrosated proteins, myosin regulatory light polypeptide 9 and profilin-1, from hormonal and non hormonal growth arrested cells lines of Pregnant Human Uterine Smooth Muscle Cell human Telomerase Reverse Transcriptase (PHUSMC hTRT) for analysis. The results indicated that myosin light polypeptide 9 had 1.45 and 1.68 fold increases (hormone and non-hormone) in S-nitrosation. Profilin-1 had 2.94 and 5.07 fold increases (hormone and non-hormone) S-nitrosation.

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**Introduction:**

The existence of preterm labor in today's society is no doubt saddening and should be unimagined in modern medicine. It is both stressful on the fetus and the parent. Financially it is a heavy burden for all parties involved for the development of the fetus, the U.S. alone spends at least $26.2 billion a year or $51, 600 per preterm infant.[1] In recent studies, the United States percentage of premature birth has reduced. The premature birth rate has fallen to 11.99% in the United States, [2] but further action is needed to reduce the percentage even more. In 2010, an estimated 14.9 million babies were born preterm, with 5% in several European countries, 18% in some African countries, and 60% in southern Asia and sub-Saharan Africa.[1] Preterm is still the single biggest cause of neonatal deaths, claiming 1.1 million infants every year.[1]

Preterm labor is classified as labor occurring before 37 completed weeks of pregnancy, which could lead to premature birth.[3] Babies born prematurely generally face a series of health problems. Also included are a variety of other causes of death closely associated with prematurity, such as Respiratory distress, bacterial sepsis, necrotizing

enterocolitis of newborn, and others.[3] Many of the newborn deaths are caused by underdevelopment of major organ systems. The exact mechanism that leads to early onset labor is still not well known. During pregnancy estrogen and progesterone increase with advancing gestational age (5, 35, 36).[22] So it is well known that the levels of hormones estrogen and progesterone contribute to the normal pregnancy and may lead to early onset labor.

From current research, it is know that myosin regulatory light polypeptide 9 and profilin-1 play a critical role in smooth muscle contraction.[4,5] Myosin light polypeptide is a regulatory subunit that has an important role in regulating smooth muscle contraction through its phosphorylation. Profilin-1 is an actin monomer-binding protein in the profilin family. These proteins are some of the first to be phosphorylated and dephosphorylated in a cascade of signaling reactions. It has been suggested that profilin-1 facilitates the polymerization of actin.[6] Profilin-1 binds to soluble monomeric G-actin creating a profilin-actin complex, which facilitates the exchange of ADP for ATP.[7] To provide the smooth muscle contraction after polymerization of the actin filament, the release of calcium ions creates an action potential and causes a cascade of molecular signaling through various messenger molecules (e.g. IP3, DAG) and phosphorylation of transmembrane ion channels.[8] Eventually the signaling leads to the activation of myosin light chain kinase (MLCK), which in turn causes the phosphorylation of myosin light polypeptide 9, thus initiating cross-bridge cycling and allowing the smooth muscle to contract.[10] Phosphorylation has been linked with levels of estrogen in vascular smooth muscle stimulating extracellular signal-regulated kinases (ERK).[23] This evidence of

smooth muscle phosphorylation could suggest that hormone levels effect posttranslational modification of proteins in uterine smooth muscle cells.

Similarly, the influence of nitric oxide (NO) on cellular signal transduction is well known.[11] S-nitrosated proteins play key roles in NO-dependent signaling that is vital to almost all aspects of cell proliferation, homeostasis, and to programmed cell death. The base reaction for s-nitrosated proteins is when a cysteine thiol reacts with NO in the presence of an electron acceptor to form an S-NO bond.[12] This is a posttranslational modification, which affects the function of a wide array of cellular proteins.[12] Much research has suggested that the products of S-nitrosation, S-nitrosothiols (SNOs), play key roles in human health and disease.[13] The disruption or dysregulation of SNO signaling leads to impairment of cellular function.[13] NO production has been found in endothelial cells from an exogenous S-nitrosothiol donor or from endogenous production of NO by endothelial NO synthase.[20] NO can diffuse freely through cell membrane or can use an amino acid transporter system L (L-AT), which uptakes NO through CysNO.[21] This recent insight regarding the bioactivities, molecular signaling pathways, and metabolism of endogenous s-nitrosothiols has suggested new therapies for diseases ranging from cystic fibrosis to pulmonary hypertension.[12] In relation to nonpregnant PHUSM, NO relaxes the smooth muscle in a dose-dependent manner.[14] In nonpregnant myometrium, NO increases cGMP (guanosine 3',5'-cyclic monophosphate) causing relaxation. In pregnant myometrium, individuals in labor appear less sensitive to the relaxant effects of NO.[15] During pregnancy state, there have been different techniques to prove the production of NO by Nitric Oxide Synthase (NOS) in uterine smooth muscle. One technique used radiolabeled L-arginine-to-L-citrulline in homogenates of pregnant

rat, guinea pig uteri, and pregnant human myometrium.[16] NO metabolites were also measured during gestation in rat and rabbit uterine explants which also confirmed NOS activity.[16] Though uterine-generated NOS is still debated and being studied. There is evidence that profilin-1 (and beta-actin) are excessively S-nitrosated after an increased synthesis of reactive species by NOS.[17] It has been shown that myosin can be extensively S-nitrosated.[18] It was hypothesized that critical proteins (e.g. myosin light polypeptide 9, profilin-1) are S-nitrosated disparately in pregnant and nonpregnant USM with the development of regulation of contraction/relaxation of signaling.[14] The purpose of this study will be to investigate the role of hormone regulation (e.g. estrogen and progesterone) and the expression of S-nitrosation of myosin light polypeptide 9 and profilin-1 in telemorized uterine smooth muscle cells.

**METHOD & MATERIALS:**

**PHUSMC hTRT Cell Culture:** - Human Telomerase Reverse Transcriptase Pregnant Human Uterine Smooth Muscle Cells (PHUSMC hTRT) were grown in Dulbecco's Modified Eagle Medium (D-MEM) (1X), liquid (high glucose) (gibco® cat. #11995), 10% Fetal Bovine Serum (FBS), progesterone (200ng/ml), estrogen (15ng/ml), Penicillin (60 µg/ml) on six 150mm growth dishes. Cell lines were grown to at least 90% confluency then three of the 150mm growth dishes were growth arrested in Dulbecco's Modified Eagle Medium/High Modified (DMEM/High Modified, liquid (high glucose) ((-) Phenol Red) (HyClone® cat. #SH30284.01), progesterone (200ng/ml), estrogen (15ng/ml), Pencillin (60 µg/ml) and 1% Insulin-Transferrin-Selenium (ITS) for 8 days. The three other 150mm dishes were growth arrested for 8 days with the same media, however no hormones treatment was added.

**Protein Isolation of PHUSMC Cell Lines:** - HENS buffer (250 mM HEPES pH 7.7, 1mM Ethylenediaminetetraacetic acid (EDTA), 0.1mM Neocuproine, 1% SDS) with 0.4% CHAPs (10% (W/V) in 1 ml HEN buffer). 2% EDTA/protease inhibitor was added to 1 ml of HEN buffer with 0.4% CHAPs solution. The 8-day growth arrested media was removed and each dish was washed in ice-cold sterile phosphate buffered saline (PBS) twice. One hormone and no hormone growth arrested line were incubated 20 minutes in 100 mM S-nitrosoglutathione (GSNO), and a second set of hormone and hormone-free growth arrested lines were incubated 20 minutes in 100 mM S-nitrosocysteine (CysNO) before isolation. 10 mL CysNO were made by adding 100mM cysteine, 105 mM Sodium Nitrite, 100 mM HCl. It was then quenched 10 minutes in the dark with 100 mM NaOH. The sample was then immediately run for absorbance reading of 543 nm. 500µl HENS/CHAPs was added to each dish ((3) hormone and (3) no hormone growth arrested cells) in the dark. The dishes were scraped and the cell lysate was collected in 1.5 ml amber epindorff tubes. The cell lysates were sonicated and then centrifuged for 20 minutes at 10,000xg. Supernatants were transferred to new tubes.

**EZQ® Protein Quantitation:** - EZQ® protein quantification was provided by Nevada Proteomics Center and performed based on manufactures instructions (Molecular Probes $^{TM}$). 1:10 dilutions of each sample were prepared for quantification in HEN buffer. Standardized samples were provided by the Nevada Proteomics Center at concentrations of: 0.0 µg/µl (no protein control), 0.02 µg/µl, 0.10 µg/µl, 0.50 µg/µl, 1.00 µg/µl, 1.5 µg/µl, 2.50, µg/µl, and 5.00 µg/µl. A full sheet of assay paper was placed over a microplate, and aligned the paper with the inner tabs of the top, bottom, and left sides of the plate. The stainless steel backing plate was inserted on top to secure the assay paper.

1 µl of standardized and isolated protein samples were applied in triplicate through the backing plate onto the assay paper. After spotting samples, the assay paper was allowed to dry completely. The assay sheet was placed into a plastic staining tray and ~40 mL of 100% methanol was poured into the tray. For 5 minutes, the assay sheet soaked in the methanol with gentle agitation. This will fix the proteins and remove any contaminating substances from the samples. After washing and the assay paper was dried again. ~40 mL of the EZQ® protein quantitation reagent was poured into the staining tray and gently agitated overnight. The assay paper was then washed in ~40 mL of 1X destaining reagent and rinsed three times after with $dH_2O$. The proteins spots were visualized and analyzed using a laser-based scanner (532-560 nm).

**Biotin Switch Assay:** The biotin switch assay was performed on the 6 protein samples, a positive control and a negative control. The positive control was made up of 300 µM GSNO added to 1330 µg albumin, and incubated for 10 minutes at room temperature. The negative control was contained 2 mL $H_2O$ and 1330 µg albumin and treated in UV light for 1 hour. 1330 µg of each sample and the controls were precipitated in (4) volumes of 100% acetone. The 8 samples were cooled -30°C for protein precipitation overnight in 15 mL amber conical tubes. The samples were centrifuged at 10, 000xg for 1-hour to pellet the protein. 3 gentle washes with 70% acetone were performed that the pellets air-dried for 15 minutes. The pellets were re-suspended in HEN buffer, 25% SDS, and 3M NEM (N-ethlymaleimide dissolved in N, N-Dimethylformanide, anhydrous (Sigma 227056, Batch 0869KK)). NEM is a non-reducing agent that binds free thiols. The samples were incubated at 50°C in the dark for 20 minutes with frequent vortexing. (4) Volumes of -30°C 100% acetone were added to

each sample. Protein samples were precipitated at -30°C overnight. Samples were again centrifuged at 3,000xg for 1 hour. (3) Gentle washes with 70% acetone were performed and pellets air dried for 15 minutes. The pellets were re-suspended in HENS buffer (HEN with 1% SDS) and transferred into 2 mL amber tubes. 463.15µM Biotin-HPDP (30 µl of 2.5 mg/ml) was followed by 20mM ascorbate. The samples were incubated on a rotor for 1 hour at room temperature. (4) Volumes of -30°C 100% acetone were added to each sample and precipitated at -30° overnight to remove excess biotin-HPDP in solution. (3) Gentle washes of 70% acetone, air-dried for 15 minutes, and centrifuged at negative pressure for 10 minutes. Each sample was re-suspended in HENS buffer and neutralization buffer (protein stability). 50 µl of magnetic beads were added to each tube, incubated on rotor for 15 minutes at room temperature, vortexed (2X), and magnetically separated for 10 minutes. The samples were gently washed with (10) volumes of neutralization buffer with NaCl (each wash solution was kept for non-SNO protein future use). The samples were removed from magnetic separator and incubated for 5 minutes in Elution buffer (20 mM HEPES-NaOH ph 7.7, 100 mM NaCl, 1mM EDTA, 100 mM β-Me) to recover bound proteins. The elution buffer contained additional fresh 14.3 M β-mercaptoethanol to reduce disulfide bonds (β-Me). The samples were returned to the magnetic separator for 10 minutes to collect the solution that contained SNO proteins.

**Western Blot of SNO Protein:** - 4X SDS sample buffer added to the 1330 µg of each protein sample. 2.96 µL of a total protein sample (PHUSMC hTRT with hormone10.12 µg/µl, 10/12/12) was mixed with 4X SDS sample buffer, dd$H_2O$ and 14.3 M β-Me. The nine total samples were boiled for 3 minutes, immediately cooled on ice

and quickly spun down. The samples were loaded onto a 4-20% acrylamide gel, and into the electrophoresis container. The gel ran for 45 minutes at 200 volts. The gel was immediately transferred onto nitrocellulose membrane and placed in 1X phosphate-buffered saline (PBS) for SYPRO® ruby staining. After SYPRO® ruby staining and scanning, the blot was continued with western blot protocol. The membrane was not destained prior to the blocking stage. The membrane was blocked with 1:1 dilution of Licor Blocking Buffer and 1X PBS for 1 hour. After the hour, a dilution of 1:1000 1° profilin mouse monoclonal IgM antibody (Santa Cruz Biotechnology, SC-137236) was applied and shook overnight at 4°C. The 1° antibody (Santa Cruz Biotechnology, SC-137236) was removed and the blot was quickly washed with 1X phosphate buffered saline with 0.1% Tween (PBST) to remove any excess antibody. Then washed the membrane for 4X 5 minutes each in a sufficient volume of 1X PBST at 4°C while shaking. A 1:25,000 dilution of 2° antimouse antibody (Santa Cruz Biotechnology) was applied. The secondary antibody was removed and the blot was quickly washed with 1X PBST to remove any excess antibody. Then washed the membrane for 4X 5 minutes each in a sufficient volume of 1X PBST at room temperature while shaking in the dark. After a dilution of 1:1000 1° myosin light polypeptide 9 rabbit polyclonal IgM antibody (Santa Cruz Biotechnology, SC-15370) was applied and shook overnight at 4°C. The 1° myosin light polypeptide 9 polyclonal antibody (Santa Cruz Biotechnology, SC-15370) was removed and the blot was quickly washed with 1X PBST to remove any excess antibodies. Then washed the membrane for 4X 5 minutes each in a sufficient volume of 1X PBST at 4°C while shaking. A 1:25,000 dilution of 2° antirabbit antibody (Santa Cruz Biotechnology) was applied. The secondary antibody was removed and the blot was

quickly washed with 1X PBST to remove any excess antibody. Then washed the membrane for 4X 5 minutes each in a sufficient volume of 1X PBST at room temperature while shaking in the dark. The membrane was then destained with 150 mM Tris, pH 8.8/20% methanol for 20 minutes.

**SYPRO® Ruby Staining:** - SYPRO® ruby staining was performed in protocol (Molecular Probes, S-11791) in Nevada Proteomics Center. After electroblotting proteins to nitrocellulose membrane, the membrane was immersed in gel fixing agent (7% acetic acid, 10% methanol) and incubated at room temperature for 15 minutes while agitated. The fixing agent was drained and the membrane was incubated 4X with $ddH_2O$ changes, each 5 minutes. The membrane was completely immersed in SYPRO Ruby blot stain reagent for 15 minutes. The membrane was washed 4-6 times for 1 minute in $ddH_2O$ to remove excess dye. The membrane was scanned and analyzed. The membrane was washed face down on a solution of 150 mM Tris, pH 8.8/20% methanol for 20 minutes. The blot was rinsed 4X for 1 minute in $ddH_2O$.

**Results:**

**SYPRO® Ruby Staining of SNO Protein:** The stain indicated that SNO labeled proteins were present in some lanes. The blot is not fully conclusive to all SNO proteins visible in each lane. However, there is indication that there were SNO proteins isolated. The positive control did not show any protein in lane 2. Procedural error is the most likely explanation for lack of a positive control. The positive control was GSNO treated albumin. The GSNO treated experimental lanes displayed SNO proteins (Lane 4 & 7). The CysNO treated experimental lanes did not have any visual indication of SNO

proteins (Lane 5 & 8). The two lanes (Lane 6 & 9) with no SNO treatment displayed SNO proteins. In lane 10, isolated total protein correlated that there was SNO protein in to the experimental lanes.

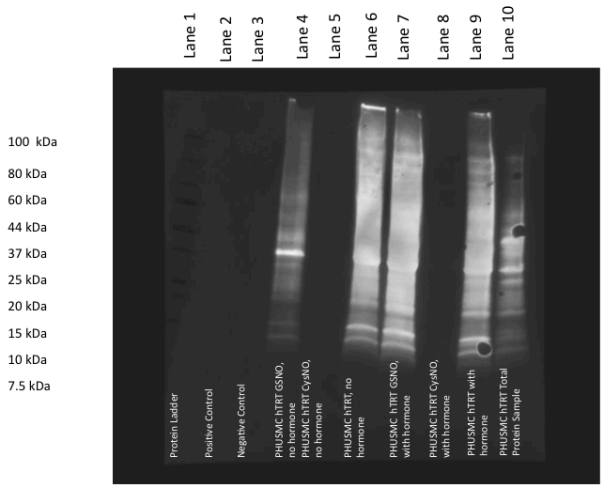


Figure 1) Total Protein Stain of Growth Arrested Nitric Oxide Treated Telemorized PHUSM Cells

Total SNO protein was isolated, collected, separated on a 4-20% Acrylamide gel, and stained with SYPRO® Ruby gel stain following the rapid stain protocol. The positive control (Lane 2) was a GSNO treated Albumin sample that did not work (procedural error). The negative control (Lane 3) was a GSNO treated Albumin sample in 1 hour UV incubation. Lane 10 was a total protein sample from PHUSMC (10/12/12) that was used for to confer SNO proteins were collected.

**Western Blot of SNO Protein:** To determine whether or not myosin light polypeptide 9 and profilin-1 were s-nitrosated the blot was treated with antibodies specific for both myosin light polypeptide 9 and profilin-1. Both antibodies attached and displayed at the correct molecular weights of myosin light polypeptide 9 (20 kDa) and profilin-1 (15 kDa) in figures 2 and 3, respectively. These indicated that both proteins were S-nitrosated and at different amounts with hormone and non-hormone treated cells during growth arrest

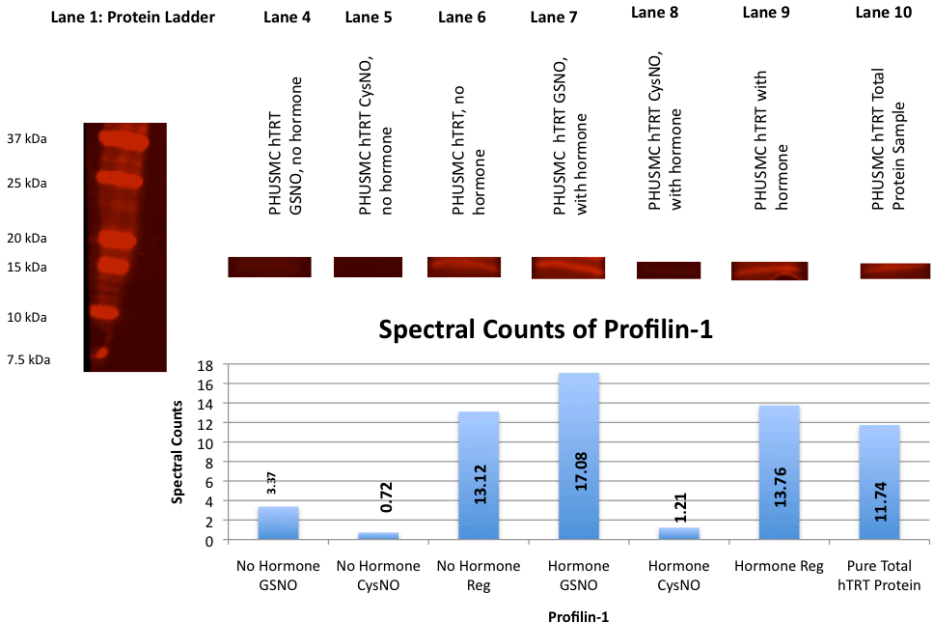


Figure 2a: Western Blot of SNO Profilin-1

PHUSMC hTRT were grown up and growth arrested for 8 days in 100uM treated GSNO and CysNO. The blot indicated that Profilin-1 was S-nitrosated differencing in concentration.

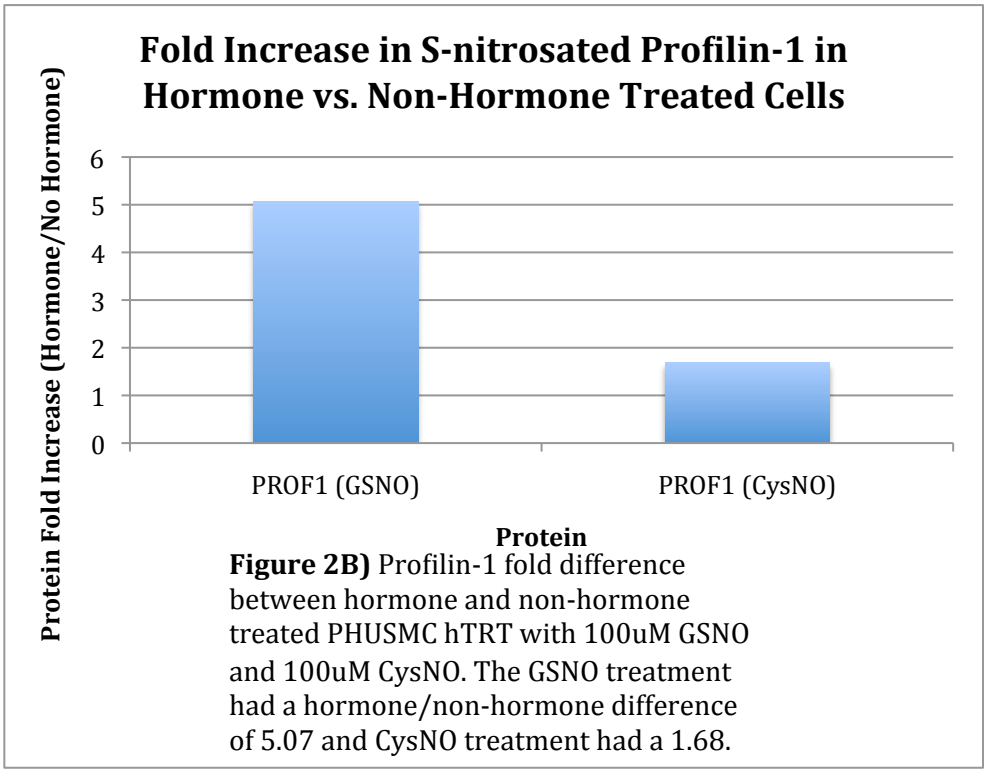


**Figure 2B)** Profilin-1 fold difference between hormone and non-hormone treated PHUSMC hTRT with 100uM GSNO and 100uM CysNO. The GSNO treatment had a hormone/non-hormone difference of 5.07 and CysNO treatment had a 1.68.

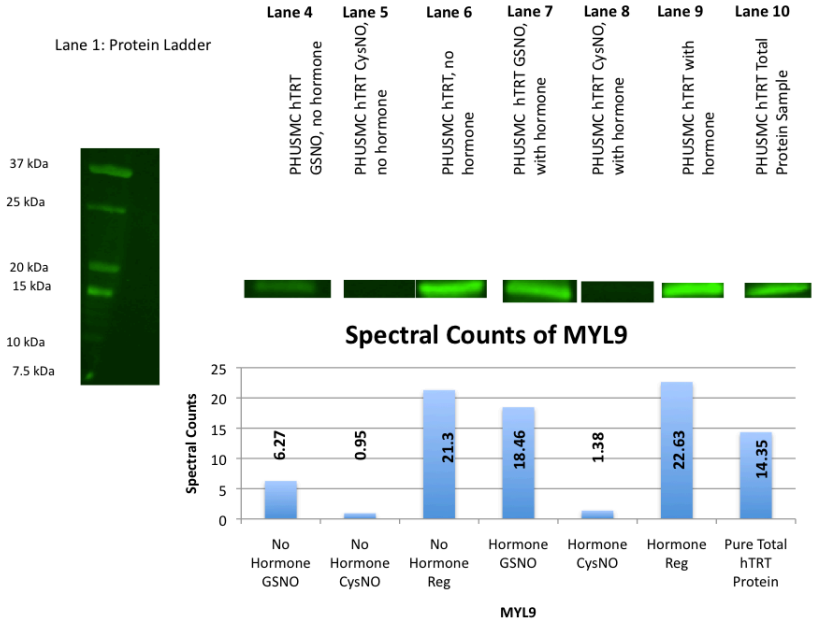


Figure 3a: Western Blot of SNO Protein (Myosin Light Polypeptide 9)

PHUSMC hTRT were grown up and growth arrested for 8 days in 100uM treated GSNO and CysNO. The blot indicated that Myosin Light Polypeptide 9 was S-nitrosated differencing in concentration.

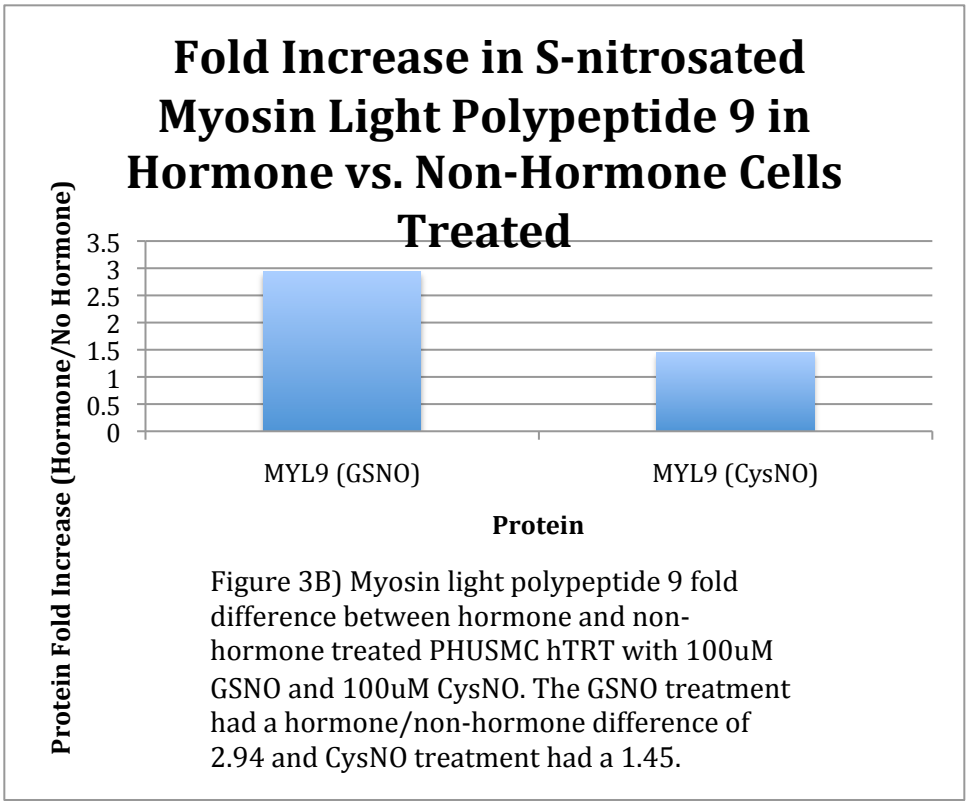


Figure 3B) Myosin light polypeptide 9 fold difference between hormone and non-hormone treated PHUSMC hTRT with 100uM GSNO and 100uM CysNO. The GSNO treatment had a hormone/non-hormone difference of 2.94 and CysNO treatment had a 1.45.

**Discussion:**

This research could suggest that based on the fold differences in S-nitrosated proteins, the amount of hormone being produced could have an affect on S-nitrosation of myosin light polypeptide 9 and profilin-1. However, procedural error occurred in between protein isolation and preparation of the samples for western blotting. During the washes throughout the experiment, protein could have been washed away from the precipitated pellet. For example, the positive control was not seen at after pull down. This would indicate the protein was lost at some point during washing cycles or at some other time. Naturally there are levels of estrogen and progesterone in the non-hormone growth arrested cells, but the addition of hormones may mirror USM during pregnancy. During pregnancy estrogen and progesterone increase with advancing gestational age (5, 35, 36).[22] Based on spectral counts of the band of the western blot, the myosin light polypeptide 9 and profilin-1 had a 2.94 and 5.07 fold, respectively; increase in GSNO treated cells with hormones. Myosin light polypeptide 9 and profilin-1 had a 1.45 and 1.68 fold, respectively, increase in CysNO treated cells with hormones. The difference in fold increase between GSNO and CysNO may be attributed to each transport mechanism. GSNO is directly transported in the cell, while CysNO uses the L-AT cotransport mechanism.[20] It might take longer periods of time for the cotransport of CysNO to enter the cell. Both were only incubated 20 minutes in each NO solution. CysNO read at 543 nm, which correlates with previous studies.[21]

Profilin-1 and myosin light polypeptide 9 are know to have a critical role in the smooth muscle contraction through posttranslational modification. They are involved in signal transduction muscle cells that help direct the contraction of a cell [4,5]. In many

cellular proteins posttranslational modification can occur through S- nitrosation, which has been studied as a way of protein modification. Profilin-1 has been excessively S-nitrosated in high oxygen which increased synthesis of reactive species by NOS.[17] It has been shown that skeletal myosin can be extensively S-nitrosated.[18] Profilin-1 and myosin light polypeptide 9 could have vital roles in S-nitrosated pregnant and non-pregnant uterine smooth muscle. In relation to nonpregnant PHUSM, NO relaxes the smooth muscle in a dose-dependent manner. [14] NO can enter USMC in two ways. NO can directly cross the membrane and interact with GSH to form GSNO. It can also be transported via L-AT system were it is bound to a cysteine (CysNO) from GSNO in the blood. [19]

This research has led to the idea that hormones could possibly have a great effect on S-nitrosation of the various proteins involved in uterine smooth muscle cell contraction. Further research will be done to look at this more in-depth at how the possibility of stretch affects S-nitrosation of these proteins along with hormones. The RNA will also be analyzed by qPCR to look for any indication of regulation pre-translation of protein. This could lead to further understanding of preterm labor and drive future research interests.

**References:**


1 Martin, J.A., Hamilton, B.E., Ventura S.J., Osterman M.J.K., et al. (2010). Births: Final Data for 2010. National Vital Statistics Reports, 61(1). Atlanta: U.S. Centers for Disease Control and Prevention.

2 Blencowe, H., Cousens S., Oestergaard M.Z., Chou D., et al. (2012) *The Lancet* - 9 June 2012 (Vol. 379, Issue 9832, Pages 2162-2172) DOI: 10.1016/S0140-6736(12)60820-4

3 Mathews, T.J., MacDorman, M.F. (2007). Infant Mortality Statistics From the 2007 Period Linked Birth/Infant Death Data Set, 59(6). Atlanta: U.S. Centers for Disease Control and Prevention.

4 Kamm K.E., Stull J.T., (1985) The Function of Myosin and Myosin Light Chain Kinase Phosphorylation in Smooth Muscle. Ann. Rev. Pharmacol. Toxicol. 1985. 25:593-620

5 Yarmola E.G., Bubb M.R., (April 2006). Profilin: Emerging Concepts and Lingering Misconceptions. TRENDS in Biochemical Sciences, 31(4).

6 Gutsche-Perelroizen I., Lepault J., Ott A., Carlier M.F., (1998). Filament Assembly from Profilin-Actin. The Journal of Biological Chemistry 274(10). Issue of March 5th. Pp 6234-6243. 1999. The American Society for Biochemistry and Molecular Biology, Inc.

7 Kang F., Purich D.L., Southwick F.S., (1999). Profilin Promotes Barbed-end Actin Filament Assembly without Lowering the Critical Concentration. The Journal of Biological Chemistry, 274(52) Issue of December 24, pp. 36963-36972. The American Society for Biochemistry and Molecular Biology, Inc.

8 Bursztyn L., Eytan O., Jaffa A.J., Elad D., (2007). Modeling Myometrial Smooth Muscle Contraction. Ann. N.Y. Academy of Sciences. 1101: 110-138 (2007).

9 Morgan K.G., Gangopadhyay S.S., (2001). Cross-bridge Regulation by Thin Filament-associated Proteins. Journal of Applied Physiology 91, pp. 953-962, 2001. The American Physiological Society

10 Webb R.C., (2003). Smooth Muscle Contraction And Relaxation. Advances in Physiology Education, 24(4), pp.201-206 December 2003.

11 Foster M.W., Hess D.T., Stamler J.S., (2009). Protein S-Nitrosylation in Heath and Disease: A Current Perspective. Trends in Molecular Medicine 15(9), pp. 391-404, 2009.

12 Gaston B.M., Carver J., Doctor A., Palmer L.A., (2003). S-nitrosylation signaling in cell biology. Molecular Interventions 3(5). Pp. 253-263. Aug 2003.

13 Foster M.W., Hess D.T., Stamler J.S., (2003). S-Nitrosylation in Heath and Disease. Trends in Molecular Medicine 9(4), pp. 160-168, 2003.

14 Ulrich C., Quillici D.R., Schegg K., Woolsey R., Nordmeier A., Buxton I.L., (2011). Department of Pharmacology, University of Nevada School of Medicine and Nevada Proteomics Center, University of Nevada, Reno, Nevada. Uterine Smooth Muscle S-Nitrosylproteome in Pregnancy. Molecular Pharmacology 81, pp. 143-153, 2012. The American Society for Pharmacology and Experimental Therapeutics
15 Buxton I.L., Kaiser R.A., Malmquist N.A, Tichenor S., (2001). Department of Pharmacology, University of Nevada School of Medicine, Reno, NV 89557, USA. NO-induced Relaxation of labouring and non-labouring Human Myometrium is not mediated by cyclic GMP. British Journal of Pharmacology 134(1), pp. 206-214. September 2001.
16 Sladek S.M., Magness R.R., Conrad K.P., (1997). Nitric oxide and pregnancy. American Journal of Physiology 272 (Regulatory, Integrative and Comparative Physiology. 41) pp. 441-463. 1997. The American Physiological Society.
17 Thom S.R., Bhopale V.M., Mancini D.J., Milovanoca T.N., (2008). Actin S-Nitrosylation Inhibits Neutrophil ß2 Integrin Function. The Journal of Biological Chemistry 283(16), pp. 10822-10834. 2008 Apr 18th.
18 Nogueira L., Figueiredo-Freitas C., Casimiro-Lopes G., et al. (2009). Myosin is reversibly inhibited by S-nitrosylation. Biochemial Journal 424, pp. 221-231. (2009). Biochemical Society.
19 Yang Y., Loscalzo J. (2005). Whitaker Cardiovascular Institute and Evans Department of Medicine, Boston University of Medicine, Boston, MA 02118. S-nitrosoprotein formation and localization in endothelial cells. Proceedings of the National Academy of Sciences of the United States of America, pp. 117-122. (2005).
20 Zhang Y., Hogg N., (2004). Department of Biophysics and Free Radical Research Center, Medical College of Wisconsin, Milwaukee, WI 53226. The mechanism of transmembrane S-nitrosothiol transport. Proceedings of the National Academy of Sciences of the United States of America, pp. 7891-7896. (2004).
21 Ryuman N., Watanabe N., Arai T., (2011). Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science, 2461 Yamazaki, Noda, Chiba 278-8510, Japan. S-Nitrosation of Cellular Proteins by NO Donors in Rats Embryonic Fibroblast 3Y1 Cells: Factors Affecting S-Nitrosation. Hindawi Publishing Corporation, Oxidative Medicine and Cellular Longevity, Volume 2011, Article ID 450317, pp. 8. (2011).
22 Peck J. D., Hulka B. S., Poole C., et al., (2002). Department of Epidemiology, University of North Carolina School of Public Health, Chapel Hill, N. C. 27599; Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, N. C. 27709; Department of Veterinary Anatomy and

Public Health, Division of Public Health, Texas A&M University, Texas 77802. Steroid Hormone Levels during Pregnancy and Incidence of Maternal Breast Cancer. Cancer Epidemiology, Biomarkers Prevention, pp. 361-368. (2002).

23 Ding Q., Gros R., Limbird L.E., Chorazyczewski, J., Feldman R. D., (2009). Departments of Medicine and of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, and Vascular Biology Research Group, Robarts Research Institute, University of Western Ontario, London, Canada; and Department of Cardiovascular Biology, Meharry Medical College, Nashville, Tennessee. Estradiol-mediated ERK phosphorylation and apoptosis in vascular smooth muscle cells requires GPR 30. American Journal of Physiology. Cell Physiology., pp. 1178-87. (2009)