The Pharma Innovation Journal 2018; 7(8): 205-211

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(8): 205-211

www.thepharmajournal.com
Received: 04-06-2018
Accepted: 06-07-2018

Harithalakshmi Jandhyam
Department of Pharmacology &Toxicology, College of Veterinary Science and Animal husbandry, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, India

Soumya Ranjan Rout
Department of Pharmacology &Toxicology, College of Veterinary Science and Animal husbandry, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, India

Subas Chandra Parija
Department of Pharmacology &Toxicology, College of Veterinary Science and Animal husbandry, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, India

Correspondence
Subas Chandra Parija
Department of Pharmacology &Toxicology, College of Veterinary Science and Animal husbandry, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, India

Vascular remodelling increases nitric oxide mediated vasorelaxation of uterine artery in pregnant goat (C*apra hircus*)

Harithalakshmi Jandhyam, Soumya Ranjan Rout and Subas Chandra Parija

Abstract
The present study investigates the role of NO in vasorelaxation of middle uterine artery obtained from non-pregnant (NP) and pregnant (P) C*apra hircus (Ch).* Uterine artery rings (1.5-2 mm) were mounted in a four chambered automatic organ bath containing 20 ml MKHS, maintained at pH 7.4. Following 1hr equilibration, Phenylephrine (PE) was added to bath in cumulative dose (1ηM-10μM) or in single dose (1μM) to induce contraction. ACh (10ηM-10μM) was added cumulatively to the bath either in absence or presence of L-NAME or Indomethacin or combination of L-NAME and Indomethacin or SNP (10ηM-10μM) to relax PE-precontracted rings. Isometric contraction was recorded using highly sensitive force transducer connected to power Lab (8/16) data acquisition system. The maximal contraction **(**E_{max}) obtained from CRC of PE elicited in MUA of P *Ch* (2.19± 0.26g) was significantly greater than that of NP *Ch* (1.61±0.01g). PE-induced sustained contraction (100%) was reduced to 67.77%, 57.88% by ACh and 47.28%, 31.30% by SNP in MUA of NP and P *Ch*, respectively. Endothelium removal in MUA rings almost abolished ACh –contraction in NP and P *Ch*. In presence of L-NAME or Indomethacin or L-NAME and Indomethacin, ACh-induced contraction was augmented to 90.54%, 74.74%, 91.43% in NP and 87.38%, 88.21%, 85.82% in P *Ch*, respectively. In conclusion, vascular remodeling of uterine artery in pregnant goat (i) increased vascular resistance is due to increased sensitivity of α_1 adrenoceptor, (ii) augmented vasorelaxation to ACh due to endothelial activation of eNOS-NO-cGMP /COX-NO-cAMP pathways. We observed for the first time that vascular remodelling in pregnancy augmented the contraction and relaxation of uterine artery of goat is due to increased sensitivity of α_1-adrenergic receptor and EDRF.

Keywords: Uterine artery, pregnancy, goat, no, SNP

1. Introduction
The endothelium plays a role in vascular tonus control through the production of vasoactive substances, i.e, endothelium-derived relaxing factor (EDRF) which was later identified as nitric oxide (NO) and was named "Molecule of the Year" in 1992 [1]. The NO is an important regulator and mediator of numerous processes in the nervous, cardiovascular and immune systems, including smooth muscle relaxation, thus resulting in vasodilatation of the artery and increasing blood flow, neurotransmission in the nervous system and has been associated with neuronal activity and various functions like avoidance learning, macrophage mediated cytotoxicity for microbes and tumor cells. Apart from normal activity, NO have been associated in pathophysiological states as diverse as septic shock, hypertension, stroke, and neurodegenerative Diseases [2]. At present, exogenous NO sources constitute a potent way to supplement NO when the body cannot generate enough for normal biological functions. In recent developments of novel NO donors, NO releasing devices as well as innovative improvements to current NO donors have been investigated [3]. However, not all reactions involving free radicals are damaging to cells. The identification of NO as an endothelial-derived relaxing factor (EDRF) was the first example of a physiological non-toxic biological activity of a free radical.
Normal pregnancy is associated with an increase in uterine blood flow and a decrease in uterine vascular resistance [4].The low resistance is attributed to a loss of smooth muscle in myometrial resistance vessels as well as augmented dilation of the larger uterine arteries. The dilation of the uterine arteries due to pregnancy induced vascular remodelling could be due to an increased role of EDRF or EDHF. Considerable evidence indicates that NO plays a role in pregnancy-induced uterine vasodilation.

Acetylcholine is more potent and efficacious in producing dilation of isolated uterine arteries from pregnant than from non-pregnant patients and abolition of this vasorelaxation by NO synthase (NOS) inhibitors confirmed the predominant role of NO in ACh mediated vasodilation in uterine artery [5]. Increase in basal NO production in pregnant uterine artery have been demonstrated in rats [6, 7], guinea pigs [8] and sheep [9, 10]. The augmented vasodilatation observed during vascular remodelling during pregnancy could be due to increased expression of endothelial nitric oxide synthase (eNOS) that lead to increased synthesis and release of NO from the endothelium [11, 12]. The importance of NO and NO signaling during pregnancy is underscored by the vascular and reproductive implications evident in mouse knockouts for endothelial NO synthase and in rats treated with the NO inhibitor *N*G-nitro-L-arginine methyl ester (L-NAME) during pregnancy [13]. L-NAME decreased reproductive performance by interfering NO production during vascular remodelling that reduces uterine arterial vasodilatation. Both small and large uterine artery outward remodeling are significantly reduced in this animal model, although the post-NO signaling mechanisms are not known [11]. NO and eNOS deficient animals have shown increased blood pressure, proteinuria, decreased fetal and neonatal weights, and a reduced number of viable newborn pups [14]. In contrast, a reduced NO-signaling has been suggested during pregnancy that could be resulting from concurrent expansion of vascular matrix to increase its resistance [15]. Mechanisms by which vascular NO signaling may be altered include changes in mechanical forces (e.g. strain/tensile deformation) secondary to increased intravascular pressure or by agonists/hormonal signaling.

There have been two opposite evidences suggesting that vascular remodelling during pregnancy increases or decreases endothelial NO. We selected the goat model considering that it conceives twice in year, is multiparous and have typical physiology of reproduction for evaluation of functional role of endothelial NO in uterine vasculature during pregnancy. In the present study as there is no information on either pattern of contraction-relaxation uterine muscles or uterine vasculature in response to endogenous mediators, hormones, agonists during non-pregnancy and pregnancy. Considering that goat middle uterine artery (MUA) undergoes remodelling from non-pregnant state to pregnancy and there is alteration of NO signalling in maintaining vascular resistance, we want to establish contribution of endothelial relaxing factors (NO/ PGI_2) by cholinergic stimulation between non pregnant and pregnant MUA rings by assessing the sensitivity to either L-NAME, a eNOS inhibitor, or Indomethacin, a PGI_2 inhibitor. This work has been well established in human and rat, but no such work has been done on goat till now with such a clarity. This will focus on the NO signaling pathways involved in pregnant goat model and thus helps in future drug discovery.

2. Materials and Methods

2.1 Ethical guidelines

This work has been approved by institutional animal ethical committee (Registration No: 433/CPCSEA/CVS vide ID. No. 1586(6)/CVS/dt.03.05.2016 for conducting randomized *ex vivo* animal tissue experiments.

2.2 Preparation of middle uterine artery and functional study

Non-pregnant and pregnant uterus with broad ligament intact along with uterine artery were obtained in an aerated ice-cold (4-6 °C) Modified Krebs-Henseleit Saline (MKHS) solution (mM): NaCl 118, KCl 4.7, CaCl2 2.5, MgSO4 1.2, NaHCO3 11.9, KH2PO4 1.2 and Dextrose 11.1, (*p*H 7.4). Secondary branch of uterine artery supplied to the uterine horn carefully cleared of fascia and connective tissue in MKHS solution under continuous aeration. The arteries were cut into segments of circular rings measuring 1.5-2 mm in length were then mounted between two fine stainless steel L-shaped hooks and kept under a resting tension of 1.5 gm in a thermostatically controlled (37.0±0.5 ºC) automatic organ bath (Pan Lab) of 20 mL capacity bubbled with carbogen (95% O_2 +5% CO_2). The change of isometric tension was measured by a highly sensitive isometric force transducer (Model: MLT0201, AD instrument, Australia) and analysed using chart 7.1.3 software.

2.3 Drugs

Acetyl Choline, Phenylephrine (Sigma, USA), L-NAME, Indomethacin (Cayman Chemical, USA) were employed in this study.

2.4 PE-induced concentration-related contractile response in MUA rings

After equilibrating the arterial ring in MKHS for 60 min, PE (1nM -10µM) was added to bath in a cumulative manner at an increment of 1.0 log unit to obtain concentrated-related contractile response. Net tension (gm) at each concentration was recorded and maximal percent response at each concentration was calculated. Graphs were plotted against-Log (M) concentration of PE (X-axis) and percent maximal response (Y-axis) in order to elicit a sigmoid concentration related response curve. Mean maximal response (E_{max}), mean threshold concentration and pEC_{50} were calculated for MUA rings of NP and P *Ch*.

2.5 ACh/SNP-induced vasorelaxation in PE-precontracted arterial rings with intact or denuded endothelium.

ACh/SNP (10nM-10µM) was added to bath cumulatively with 0.5 log unit increment at 4 min interval in order to relax PE pre-contracted rings with either intact or denuded endothelium. The percent contractile response at each concentration of ACh/SNP was calculated by taking the net plateau tension (gm) induced by PE as 100%. $E_{max}/E_{Bmax,}$ mean threshold concentration and $-logEC_{50}/pEC_{50}$ were calculated for MUA rings for non-pregnant and pregnant groups and compared.

2.6 ACh-induced vasorelaxation in PE-precontracted arterial rings either in presence of L-NAME or Indomethacin or L-NAME and Indomethacin

The arterial rings were pre incubated with either 10µM of L-NAME (10µM) or Indomethacin or L-NAME and Indomethacin for a period of 10 min prior to PE pre contraction. ACh (10nM-10µM) was added with increment of 0.5 log unit in a cumulative manner into the bath at 4min interval after attaining a plateau contraction induced by PE. The concentration-related contractile response curves (CRCs) of ACh was elicited in presence of L-NAME or Indomethacin or L-NAME and Indomethacin and shift of the CRCs were compared with non-treated control. E_{max}/ E_{Bmax}, mean threshold concentration and $-logEC_{50}/EC_{50,}$ of antagonists were calculated for MUA rings for non-pregnant and pregnant groups and compared.

2.7 Data analysis
The data was expressed as percentage of the maximum contractile response to agonist obtained in the absence of antagonist (control) and analyzed by the interactive non-linear regression through the computer program Graph Pad Prism (Graph Pad Prism Software, San Diego, CA, USA). E_{max}/E_{Bmax}, mean threshold concentration and -logEC_{50} were calculated through Graph Pad Prism. GraphPad Quick Calcs't' test was used to calculate the P value to determine the level of significance and to analyse the data. A 'p' value < 0.05 and <0.001 were considered statistically significant.

3. Results
3.1 Effect of PE-induced concentration related contractile response in MUA of NP and P Ch.
The pEC_{50} and E_{max} for PE (1nM-10μM) obtained from CRC response curve elicited in MUA were 6.4±0.05, 1.61±0.01g in NP and 5.0±0.04 and 2.19±0.26g, in P *Ch*, respectively (Fig. 1). PE (10μM)-induced a contractile response which consists of first phasic contraction followed by sustained tonic contraction. The mean peak and plateau tension to PE in MUA rings of NP (1.65±0.12g, 1.57± 0.10g, n=20) was significantly (p<0.05) increased in that of P *Ch* (2.25±0.14g, 2.13±0.12g, n=20) (Fig. 2A). Mean time to peak and time to plateau obtained from PE-induced a contractile response in MUA of NP (371±18.78 sec, 558±20.31 sec, n=20) was not altered significantly in P *Ch* (343±18.2 sec, 505±19.6 sec, n=20) (Fig. 2B).

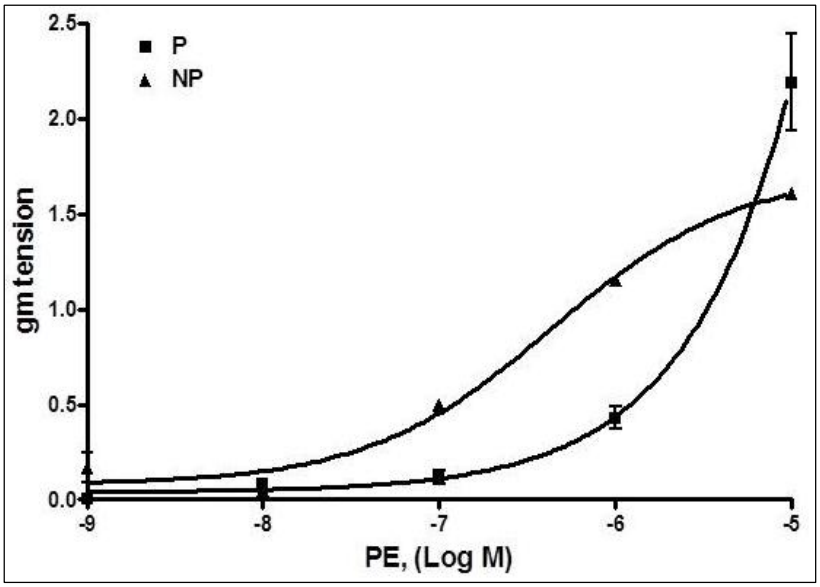


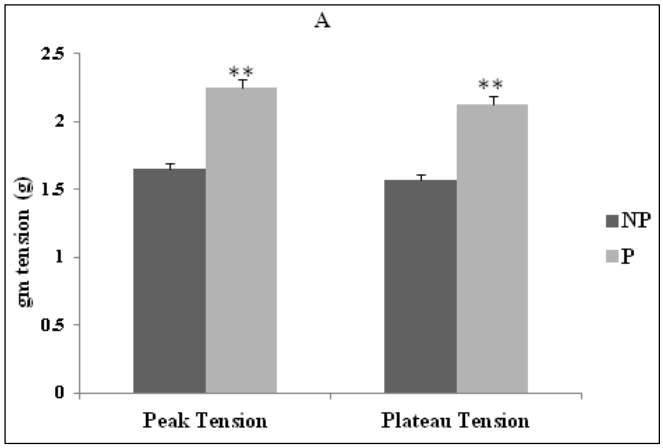
Fig 1: PE (10μM)-induced concentration response curve in ED+ MUA of non-pregnant (NP) and pregnant (P) *Ch*.

3.2 ACh/SNP-induced concentrated related response curve in PE-precontracted arterial rings
Table 1 presents the E_{max} and pEC_{50} of ACh-induced vasorelaxation in MUA of NP and P *Ch*. ACh/SNP (10nM-10μM) inhibited PE induced sustained contraction differentially in both NP and P *Ch*. ACh-induced CRC response curve in MUA rings of P *Ch* was shifted to left with significant (p<0.001) increase in -logEC_{50} value (6.83±0.09) and decrease in E_{max} (57.88±1.36%) as compared to that of MUA of NP *Ch* (-logEC_{50} 6.48 ± 0.07, E_{max} 67.77±0.55%). The removal of endothelium shifted the ACh CRC to right in MUA of NP *Ch* with significant (p<0.001) increase in E_{max} (99.37±1.45%) with complete blunting of ACh response curve as compared to endothelium intact arterial rings (Fig. 3A). Similarly, the removal of endothelium shifted the ACh-induced CRC to right in the MUA of P *Ch* with significant

Table 1: E_{max} and pEC_{50} of ACh (10nM-10μM) in endothelium intact (ED+) or in endothelium denuded (ED-) or in absence (R_{max}) or in presence (R_{Bmax}) of L-NAME (10μM) or Indomethacin (10μM) or L-NAME(L-NAME+Indo, 10μM) and Indomethacin (10μM) in PE (10μM)-precontracted MUA rings of NP and P *Ch*. The values are expressed as Mean ±SEM, N= Total number of MUA rings used in the experiments.

Treatment (ACh)	N value	E_{max}/E_{Bmax} (%)		pEC_{50}	
		NP	P	NP	P
Control (ED+)	30	67.77 ± 0.55	57.88 ± 1.36	6.48 ± 0.07	6.83 ± 0.09[c]
ED-	6	99.37 ± 1.45[a]	93.45± 0.12[ac]	Blunted[a]	5.80 ± 0.33[a]
L-NAME	6	90.54 ± 1.47[a]	87.38 ±0.86[ac]	7.28 ± 0.24[a]	6.96 ± 0.15[bc]
Indomethacin	6	74.74 ± 1.57[a]	88.21 ± 1.22[ac]	6.99 ± 0.25[b]	6.43 ± 0.42[bc]
L-Name + Indo	6	91.43 ± 0.93[a]	85.82 ± 0.92[ac]	6.46 ± 0.24	Blunted[a]

[a] (p<0.001), [b] (p<0.05) represents level of significance between the rows within each column. Data of each row (treated) is compared with the data of Control (ED+) within corresponding column. [c] (p<0.001), [d](p<0.05) represents level of significance between the sub-columns (NP and P) within each column. Data of each 'P' column in a particular row is compared with the corresponding data of 'NP' column.



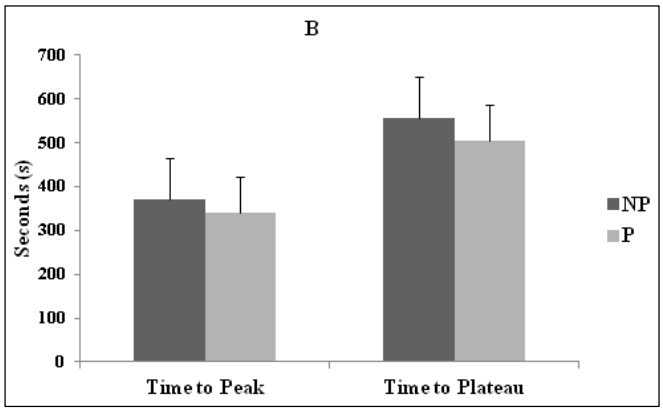
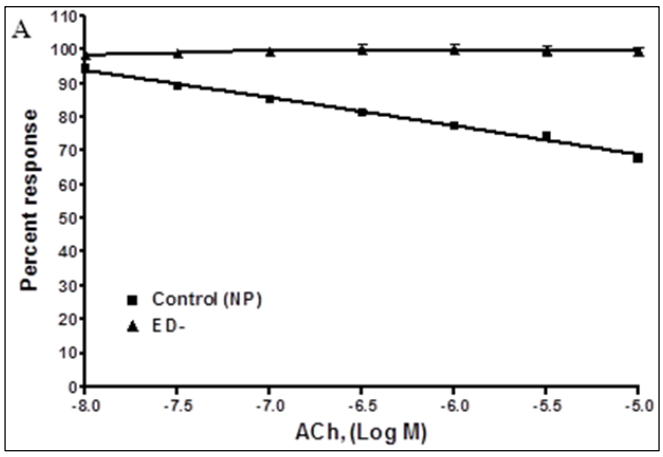


Fig 2: **A**. PE (10µM)-induced mean peak and plateau contractile response in ED+ MUA rings of NP and P *Ch* (** represents level of significance *P*<0.05 between NP and P *Ch*). **B**. PE (10µM)-induced time to peak and time to plateau in ED+ MUA rings of NP and P *Ch.*

(p<0.05) decrease in -logEC_{50} value (5.80±0.33) and increase (p<0.001) in E_{max} (93.45±0.12%) as compared to endothelium intact arterial rings (Fig. 3B).SNP (10nM-10µM) inhibited PE-induced sustained contraction differentially in both NP and P *Ch.* The SNP-induced response curve (E_{max} 47.28± 4.77%; pEC_{50} 6.43±0.03) elicited in MUA rings of NP was shifted to left with significant (P<0.05)



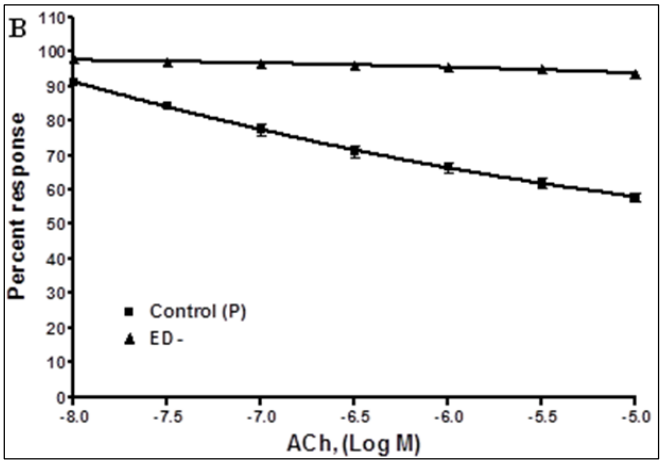


Fig 3: ACh (10nM-10µM)-induced concentration response curve elicited in ED+/- MUA ring of **A)** NP *Ch* and **B)** P *Ch.* decrease in E_{max} (31.30± 4.90%) and pEC_{50} (6.35±0.02) in that of P *Ch* (Fig. 4).

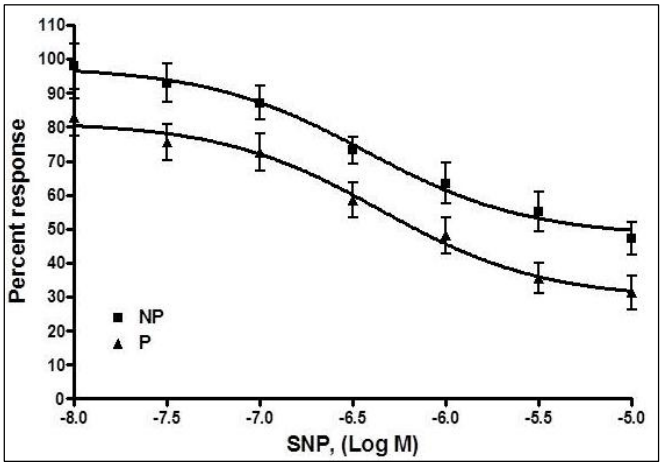


Fig 4: SNP (10nM-10µM)-induced concentration response curve elicited in ED+ MUA ring of NP and P *Ch.*

.

3.3 Effect of ACh-induced vasorelaxation in PE-precontracted arterial rings in absence or presence of L-NAME or Indomethacin or L-NAME and Indomethacin

In MUA of NP *Ch*, ACh-induced CRC response curve was shifted to right with significant (p<0.001) increase in -logEC_{50} (7. 28±0.24) and E_{Bmax} (90.54±1.47%) in presence of L-NAME (Fig.5A), significant (p<0.05) increase in both -logEC_{50} (6.99±0.02) and E_{Bmax} (74.74±1.57%) in presence of Indomethacin, non-significant decrease in -logEC_{50} (6.46±0.24) and significant (p<0.001) increase in E_{Bmax} (91.43±0.93%) in presence of both L-NAME and indomethacin (Figure 5A). In MUA of P *Ch*, ACh-induced CRC response curve was shifted to right with significant (p<0.05) increase in -logEC_{50} (6.96±0.15) and E_{Bmax} (87.38±0.86%) in presence of L-NAME, significant (p<0.05) increase in both -log EC_{50} (6.43±0.42), E_{Bmax} (88.21±1.22%) in presence of Indomethacin, significant (p<0.001) decrease in E_{Bmax} (85.82±0.92%) and completely blunted ACh-induced CRC response curve in presence of L-NAME and indomethacin (Figure 5B).

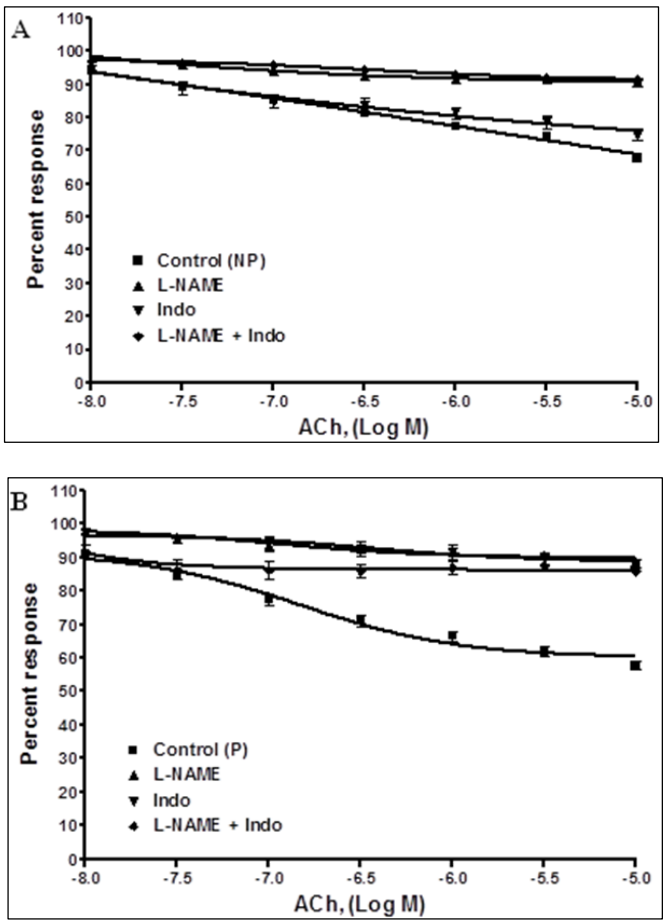


Fig 5: ACh (10nM-10µM)-induced concentration response curve elicited in or absence (control) or in presence of L-NAME (10µM) or Indomethacin (Indo, 10µM) or L-NAME and Indomethacin (L-NAME+Indo, 10µM) in MUA ring of **A)** NP *Ch* and **B)** P *Ch.*

4. Discussion

This study aimed to explore the mechanisms of vasorelaxation to ACh in middle uterine arteries of *Capra hircus*. In order to validate the normal vasoreactivity pattern of the MUA rings of NP and P *Ch*, the α_1- adrenergic receptor agonist (PE) mediated vasocontraction, Muscarinic-receptor (NO) mediated vasorelaxation response in PE precontracted tissues were examined. The major findings are (i) The E_{max} of PE was significantly augmented in P (2.19±0.26g) as compared to that of NP *Ch* (1.61±0.01g) ii) E_{max} of ACh in NP (67.77%) was decreased in P *Ch* (57.88%) and vasorelaxation to ACh was almost abolished in both ED-MUA rings of NP (99.37%) and P (93.45%) *Ch* (iii) E_{max} obtained from SNP response curve of NP (47.68%) and P (31.30%) (iv) L-NAME or both L-NAME and Indomethacin inhibited E_{max} of ACh almost identically to 90.54% and 91.43% and Indomethacin inhibited E_{max} to 74.74% in NP *Ch*. In MUA rings of P *Ch*, L-NAME, Indomethacin and both L-NAME and Indomethacin attenuated significantly E_{max} of ACh curve almost identically to 87.38%, 88.21%, 85.82% respectively.

α_1-adrenergic agonist mediated vasoconstriction obtained from isometric contraction elicited in uterine artery of rat [16], sheep [17] guinea pig [18], calf digital artery [19] and bovine mammary artery [20] demonstrated that the sensitivity of α_1-adrenergic receptor to its agonists varies with respect to species, type and location of vasculature. In earlier studies we observed that sensitivity of α_1-adrenergic receptor to PE varies greatly with respect to E_{max} and EC_{50} in ruminal artery [21], superior mesenteric artery [22] and pulmonary artery [23] in goat. E_{max} and EC_{50} of PE was reported to be greater in mesenteric artery (2.18±0.24g, 5.58±0.20) [22] than in pulmonary arteries (0.6±0.01g, 5.18±0.09) [23]. The E_{max} and pEC_{50} for PE obtained from CRC response curve elicited in MUA were (1.61±0.01g, 6.4±0.05) in NP and (2.19±0.26g, 5.0±0.04) in P *Ch* demonstrate that there is increased sensitivity α_1-adrenergic receptor to PE with advancement of pregnancy in uterine artery of goat. The uterine circulation exhibits increased responses to α_1-adrenergic stimulation compared with the systemic circulation, and this adrenergic contraction in uterine artery depends on α_1-adrenergic receptors [24]. Pregnancy significantly increased the contractile response to the α_1-agonist phenylephrine in rats [16]. This difference appears consistent with the increased sensitivity to α-adrenergic stimulation in pregnant uterine arteries compared with that of non-pregnant one and may be related to the great degree of remodeling that vascular bed undergoes during gestation [25]. In conclusion, vasoconstrictor response to PE in uterine artery of goat clearly demonstrates that there is increased sensitivity of α-adrenergic receptor to PE is augmented from NP to P *Ch* and this could be due to vascular remodeling of uterine artery in pregnancy

Acetylcholine produces an endothelium-dependent relaxation of blood vessels mediated primarily by nitric oxide (NO) which is synthesized from the L-arginine in the vascular endothelial cells [1]. ACh-induced vasorelaxation has been reported to be endothelium dependent in uterine artery of NP guinea pig [26] (Tare *et al*. 1990) human [5] and canine species [27]. Sensitivity of uterine artery to ACh vasorelaxation differ greatly among different species of animals like non pregnant rat (-log EC_{50} 7.71± 0.04 and E_{max} 96±1%) [28], and human (-log IC_{50} 7.4±0.02 and R_{max} 77.5±6.3%) [29]. similarly, the dilatory response to ACh was observed to be minimal in the uterine vascular bed of non-pregnant guinea pigs but increased markedly (~10-fold) during pregnancy [18]. The ACh (0.1nM-0.1µM) -induced maximal relaxation in uterine artery of NP (23%) was observed to be augmented significantly in pregnant human and this vasorelaxation was abolished in absence of endothelium [5]. In our present study, the E_{max} obtained from ACh concentration response curve elicited in MUA rings of NP (67.77%) was decreased in that of P *Ch* (57.88%). ACh has a greater vasorelaxation effect in PE-precontracted MUA rings (ED+) of PG than NPG. AVR was almost abolished in ED- MUA rings of NP and P *Ch* confirms that vasorelaxation to ACh is exclusively mediated *via* endothelium dependent mechanisms.

During pregnancy vasculature undergoes significant expansive remodelling to accommodate the dramatic increase in uteroplacental blood flow that is requisite for normal pregnancy outcome. Nitric oxide (NO) is a key molecule involved in vascular remodelling during pregnancy and that expression of endothelial nitric oxide synthase (eNOS) is increased during pregnancy, leading to increased synthesis and release of NO from the endothelium. In MUA of NP *Ch*, ACh-induced an identical vasorelaxation but pregnancy increased vasore laxation to ACh by about 10% clearly support that pregnancy induced remodelling differentially modulate endothelium dependent NO pathways. Similarly, removal of endothelium inhibited the E_{max} of ACh (99.37% and 93.45%) in MUA of NP and P *Ch*, suggesting that cholinergic receptor stimulated endothelium dependent vasorelaxation via NO signalling during the process of remodelling in pregnancy. It has been established that nitric oxide (NO) is a key molecule involved in vascular remodeling

during pregnancy and that expression of endothelial nitric oxide synthase (eNOS) is increased during pregnancy [11], leading to increased synthesis and release of NO from the endothelium [12].The increased vasorelaxtion by ACh in MUA of P *Ch*, are in line findings of [11, 12].

Further, to examine the altered NO signaling pathways involved due to increased release of NO from the endothelium during pregnancy, we compared the inhibition of vasorelaxation to ACh and histamine by L-NAME and Indomethacin that are considered as well-known inhibitors of NO signaling molecules namely eNOS and PGI_2. We observed that vasorelaxation to ACh in presence of L-NAME, Indomethacin and combination of both E_{max} of ACh was inhibited to 90.54%, 74.74%, 91.43% in MUA of NP and 87.38%, 88.21%, 85.82% in MUA of P *Ch* respectively, indicating that cyclooxygenase (COX) exhibited an increased sensitivity to indomethacin sensitive component with unaltered sensitivity of eNOS to L-NAME in pregnant MUA. This clearly suggests that pregnancy induced remodeling greatly enhances the cholinergic receptor activated endothelial dependent indomethacin sensitive COX and increased production of PGI_2, which in turn mediated greater vasorelaxation of vascular smooth muscle cells (VSMC) via cyclic AMP dependent mechanisms in goat MUA. The importance of NO and NO signalling during pregnancy is underscored by the vascular and reproductive implications evident in mouse knockouts for endothelial NO synthase and in rats treated with the NO inhibitor *N*G-nitro-L-arginine methyl ester (L-NAME) during pregnancy [11, 13]. The exact mechanisms of the rise in uteroplacental perfusion and accompanying decrease in vascular reactivity with remodelling of uterine artery remain unclear but plasma levels of nitrates/nitrites (NOx) and 6-keto- $PGF_{1\alpha}$, the stable metabolites of nitric oxide (NO) and PGI_2, are increased during pregnancy, suggesting that endogenous vascular NO and PGI_2 production is increased in gravid animals [6, 30]. PGI_2 production is elevated during normal pregnancy in women and reduced in preeclampsia, suggesting important clinical significance to endothelial activation in pregnancy and dysfunction in diseased states [31]. NOx have also been reported to be elevated in human pregnancy, pregnant rats and ovine due to increases in uterine cGMP secretion (via elevations in uterine endothelium-derived NO) [7, 32]. It has been reported that changes observed in eNOS, $cPLA_2$, and COX-1 expression (with vs. without endothelium) and elevated in pregnant guinea pigs [8] sheep [9] than non-pregnant.

Sodium nitroprusside is a potent vasodilator preferentially at arterial smooth muscle. Sodium nitroprusside breaks down in circulation to release NO by binding to oxyhaemoglobin to release cyanide, methaemoglobin and nitric oxide [33]. SNP-induced relaxation of uterine artery observed in non-pregnant and pregnant women [34], guinea pig, rat [35], sheep [36], mice [37] and other arteries like rat tail artery [38] and rabbit mesenteric artery [39]. SNP (1 nM-100 μM) produced a significant relaxation compared to ACh (0.1-100 μM) on 5-HT (10 μM) and NA (10 μM)-induced contraction in endothelium intact rings of goat ruminal artery [40]. Uterine artery of pregnant mice were significantly more sensitive to SNP than NP mice, indicated by a left shift in the dose-response curve and a reduced EC_{50} concentration but in non-pregnant and pregnant mesenteric arteries there was no difference in SNP-induced relaxation [36]. In the present study, we observed that the E_{max} obtained from SNP (10nM-10μM)-induced response curve elicited in PE- precontracted ED+ MUA rings of NP (47.28%) was increased that of P *Ch* (31.30%). This observation clearly shows that the uterine artery of P *Ch* is more sensitive to SNP than that of NP *Ch* and SNP has a greater vasorelaxation effect in PE-precontracted MUA rings (ED+) of P *Ch* than NP *Ch*. In conclusion, goat uterine artery is highly sensitive to exogenous NO and this NO mediated vasorelaxation is prominent in MUA of P than NP *Ch.*

5. Conclusion

i) Contractile response in uterine artery is mediated by α_1-adrenergic receptor. ii) Pregnancy increased the sensitivity of α_1-adrenergic receptor in MUA. iii) Vasorelaxation to ACh involves exclusively endothelium dependent mechanisms in MUA of both NP and P *Ch*. iv) Greatly enhances the cholinergic receptor activated endothelial dependent Indomethacin-sensitive COX via cyclic AMP dependent mechanisms v) NO liberator, SNP caused a greater vasorelaxtion in P than NP *Ch*.

6. Acknowledgment

The authors expresses their thankfulness to UGC, GOI, for student fellowship grant during the research work of one of the author Harithalakshmi Jandhyam (fellowship ID: NFO-2015-17-OBC-AND-33897) and to OUAT for providing necessary infrastructure and logistics for smooth conduct of research work.

7. References

1. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 1980; 288:373-376.
2. Davies SA, Stewart EJ, Huesmaan GR, Skaer NJ. Neuropeptide stimulation of the nitric oxide signalling pathway in Drosophila melanogaster Malpighian tubules. Am. J Physiol. 1997; 273:823-827.
3. Hou YC, Janczuk A, Wang PG. Current trends in the development of nitric oxide donors. Curr. Pharm. Des. 1999; 6:417-471.
4. Sladek SM, Magness RR, Conrad KP. Nitric oxide and pregnancy. Am J Physiol Regul Integr Comp Physiol. 1997; 272:441-463.
5. Nelson SH, Steinsland Suresh MS, Lee NM. Pregnancy augments nitric oxide-dependent dilator response to acetylcholine in the human uterine artery. Human Reproduction. 1998; 13(5):1361-1367.
6. Conrad KP, Jaffe GM, Kruszyna H, Kruszyna R, Rocehlle LG, Smith RP *et al*. Chavez JE and Mosher MD. Identification of increased nitric oxide biosynthesis during pregnancy in rats. Federation of American Societies for Experimental Biology Journal, 1993; 7:566-571.
7. Conrad KP, Kerchner LJ, Mosher MD. Plasma and 24-h NO(x) and cGMP during normal pregnancy and preeclampsia in women on a reduced NO(x) diet. American Journal of Physiology-Renal Physiology. 1999; 277:48-57.
8. Weiner C, Lui KZ, Thompson L, Herrig J, Chestnut D. Effect of pregnancy on endothelium and smooth muscle: their role in reduced adrenergic sensitivity. American Journal of Physiology and Heart Circulation Physiology. 1991; 261:1275-1283.
9. Li P, Tong C, Eisenach JC. Pregnancy and ephedrine

increase release of nitric oxide in ovine uterine arteries. Anesth Analg. 1996; 82:288-293.
10. Magness RR, Rosenfeld CR. Systemic and uterine responses to alpha-adrenergic stimulation in pregnant and nonpregnant ewes. American Journal of Obstetrics and Gynecology. 1986; 155:897-904.
11. Osol G, Barron C, Gokina N, Mandala M. Inhibition of nitric oxide synthases abrogates pregnancy-induced uterine vascular expansive remodeling. J Vasc Res. 2009; 46:478-486.
12. Matsubara K, Matsubara Y, Hyodo S, Katayama T, Ito M. Role of nitric oxide and reactive oxygen species in the pathogenesis of preeclampsia. J Obstet Gynaecol Res. 2010; 36:239-247.
13. Van der, Heijden OW, Essers YP, Fazzi G, Peeters LL, De Mey JG, van Eys GJ. Uterine artery remodeling and reproductive performance are impaired in endothelial nitric oxide synthase-deficient mice. Biol Reprod. 2005; 72:1161-1168.
14. Standley CA, Batia L, Yueh, G. Magnesium sulfate effectively reduces blood pressure in an animal model of preeclampsia. J Matern Fetal Neonatal Med. 2006; 19:171-176.
15. Hale SA, Weger L, Mandala M, Osol G. Reduced NO signaling during pregnancy attenuates outward uterine artery remodeling by altering MMP expression and collagen and elastin deposition Am J Physiol Heart Circ Physiol. 2011; 301:1266-1275.
16. Wang SY, Datta S, Segal S. Pregnancy alters adrenergic mechanisms in uterine arterioles of rats. Anesthesia and Analgesia. 2002; 94(5):1304-1309.
17. Magness RR, Sullivan JA, Li Y, Shaw CE, Phernetton TM, Bird IM. Endothelial vasodilator production by uterine and systemic arteries. VI. Ovarian and pregnancy effects on eNOS and NOx. American Journal of Physiology-Heart and Circulatory Physiology. 2001; 280:1692-1698.
18. Bell C. Dual vasoconstrictor and vasodilator innervation of the uterine arterial supply in the guinea pig. Circulation Research. 1968; 23(2):279-290.
19. Belloli C, Badino P, Arioli F, Odore R, Re G. Adrenergic regulation of vascular smooth muscle tone in calf digital artery. Journal of Veterinary Pharmacology and Therapeutics. 2004; 27:247-54.
20. Pereira FJ, Will JA. Functional characterization of post-junctional adrenergic receptor subtypes in bovine intra-mammary arteries. Journal of Veterinary Pharmacological Therapeutics. 1997; 20:434-441.
21. Kathirvel K, Behera PC, Pallai S, Mohanty J, Parija SC. Pharmacological and molecular identification of α1A/D adrenoceptor in goat ruminal artery. International Journal of Drug Development Research. 2010; 2:643-653.
22. Mohanty I, Suklabaidya S, Parija SC. Acidosis reduces the function and expression of α_{1D}-adrenoceptor in superior mesenteric artery of *Capra hircus*. Indian Journal of Pharmacology. 2016; 48(4):399-406.
23. Palai S, Parija SC. Effect of quercetin, naringenin and EGCG on α1-adrenergic receptor-mediated vascular contraction in pulmonary artery of goat (*Capra hircus*). Journal of Pharmacognosy and Phytochemistry. 2017; 6(6):170-175.
24. Isla M, Dyer DC. Characterization of alpha-adrenoceptors in the late pregnant ovine uterine artery. European Journal of Pharmacology. 1990; 178:321-331.
25. Mandala M, Osol G. Physiological remodelling of the maternal uterine circulation during pregnancy. Basic and Clinical Pharmacology and Toxicology. 2012; 110(1):12-18.
26. Tare M, Parkington HC, Coleman HA, Neild TO, Dusting GJ. Hyperpolarization and relaxation of arterial smooth muscle caused by nitric oxide derived from the endothelium. Nature. 1990; 346:69-71.
27. Matsumoto T, Kanamaru K, Sugiyama Y, Murata Y. Endothelium-derived relaxation of the pregnant and nonpregnant canine uterine artery. The Journal of Reproductive Medicine. 1992; 37:529-533.
28. Mazzuca MQ, Tare M, Parkington HC, Dragomir NM, Parry Wlodek LJ. Uteroplacental insufficiency programmes vascular dysfunction in non-pregnant rats: compensatory adaptations in pregnancy. The Journal of Physiology. 2012; 590(14):3375-3388.
29. Jovanovic S, Grbovic I, Jovannovic A. Pregnancy does not alter the response of uterine arteries to vasoactive intestinal polypeptide. Molecular Human Reproduction. 2000; 6:361-368.
30. Magness RR, Parker CR, Rosenfeld CR. Systemic and uterine responses to chronic infusion of estradiol-17 beta. Am J Physiol Endocrinol Metab. 1997; 265:690-698.
31. Reynolds LP, Redmer DA. Utero-placental vascular development and placental function. Journal of Animal Science. 1995; 73:1839-1851.
32. Rosenfeld CR, Cox BE, Roy T, Magness RR. Nitric oxide contributes to estrogen-induced vasodilation of the ovine uterine circulation. Journal of Clinical Investigation. 1996; 98:2158-2166.
33. Friederich JA, Butterworth JF. Sodium Nitroprusside: Twenty Years and Counting. Anesthesia and Analgesia. 1995; 81(1):152-162.
34. Nelson SH, Suresh MS. Comparison of nitroprusside and hydralazine in isolated uterine arteries from pregnant and nonpregnant patients. Anesthesiology. 1988; 68:541-547.
35. Ni Y, Meyer M, Osol G. Gestation increases nitric oxide-mediated vasodilation in rat uterine arteries. American Journal of Obstetrics and Gynecology. 1997; 176:856-864.
36. Cooke CM, Davidge ST. Pregnancy-induced alterations of vascular function in mouse mesenteric and uterine arteries. Biology of Reproduction. 2003; 68:1072-1077.
37. Xiao D, Pearce W, Zhang L. Pregnancy enhances endothelium-dependent relaxation of ovine uterine artery: role of NO and intracellular Ca^{2+}. American Journal of Physiology and Heart Circulation Physiology. 2001; 281:183-190.
38. Cheung D, MacKay MJ. The effects of sodium nitroprusside and 8-bromo-cyclic GMP on electrical and mechanical activities of the rat tail artery. British Journal of Pharmacology. 1985; 86(1):117-124.
39. Murphy ME, Brayden JE. Nitric oxide hyperpolarizes rabbit mesenteric arteries via ATP-sensitive potassium channels. The Journal of Physiology. 1995; 486(1):47-58.
40. Dash JR, Parija SC. Spasmolytic effect of curcumin on goat ruminal artery is endothelium independent and by activation of sGC. Research in Veterinary Science. 2013; 95(2):588-593.