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Metabolism of arachidonic acid in sheep uterus: *In vitro* studies

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Arachidonic acid (AA) metabolism in the non-pregnant sheep uterus was studied *in vitro* using conventional chromatographic and HPLC techniques. High expression of both lipoxygenase (LOX) as well as cyclooxygenase (COX) enzymes and their activities was found in the uterine tissues. On incubation of uterine enymes with AA, the LOX products formed were identified as 5-hydroperoxyeicosatetraenoic acid (5-HPETE), 12- and 15-hydroxyeicosatetraenoic acids (12- and 15-HETEs), based on their separation on TLC and HPLC. By employing differential salt precipitation techniques, the LOXs generating products 5-HPETE (5-LOX), 12-HETE and 15-HETE (12- and 15-dual LOX) were isolated. Based on their analysis on TLC, the COX products formed were identified as prostaglandins — $PGF_2\alpha$ and prostacyclin derivative 6-keto $PGF_1\alpha$. The study forms the first report on the comprehensive analysis on the metabolism of AA in sheep uterus *in vitro* via the LOX and COX pathways.

Keywords: Arachidonic acid metabosism, Lipoxygenase, Cyclooxygenase, Prostaglandins, HPETEs and HETEs

Lipoxygenases are a group of enzymes that catalyze the conversion of arachidonic acid (AA) into bioactive lipids, hydroperoxyeicosatetraenoic acids (HPETEs). The animal lipoxygenases (LOXs) are classified as 12-LOX (E.C.1.13.11.31), 15-LOX (E.C.1.13.11.33) and 5-LOX (E.C.1.13.11.34) etc., depending on the position of insertion of molecular oxygen on the AA. The HPETEs can further be reduced or oxygenated to a variety of compounds. The reduction of peroxy group to an alcohol results in the formation of corresponding hydroxy-eicosatetraenoic acids (HETEs). On the other hand, lipoxygenation of the HPETE results in formation of either a dihydroperoxyeicosatetraenoic acid (diHPETE) or trihydroxyeicosatetraenoic acid (lipoxin)[1]. Further, the HPETEs can be transformed to leukotrienes by dehydration of the peroxy group. The primary metabolites of AA generated by the 5-LOX are the leukotrienes (LTs) and lipoxins, while the 12- and 15-LOXs produce hydroxy-eicosatetraenoic acids (HETEs)[2].

The 12- and 15-LOX-derived eicosanoids are implicated in diverse inflammation-related and other physiological pathways such as lymphocyte activation and migration, thrombocyte aggregation, chemotactic stimulation of leukocytes, synaptic transmission, tumor cell metastasis and cellular apoptosis[3,4]. In the uterus, the localized changes in cell proliferation and differentiation, reorganization of extracellular matrix, recruitment of leukocytes and migration of immune cells that are associated with implantation and decidualization have been reported[5]. In humans, 12-HETE is the main LOX product in placenta, decidua and myometrium before labour, whereas 5-HETE is found to be main product in fetal membranes[6]. The LOX products are found to be elevated, prior to ovulation and inhibition of this pathway also inhibits ovulation, suggesting strongly a role for these metabolites in the ovulation[7]. Delayed luteolysis is observed after intra-uterine infusion of nordihydroguaiaretic acid (NDGA) in the ewe[8]. Earlier, we reported characterization of a dual LOX (12- and 15-LOX) with 14, 15-leukotrieneA_4 synthase activity in sheep uterus[9].

Cyclooxygenases (E.C.1.14.99.1) catalyze the conversion of AA to prostaglandins (PGs). Two different catalytic activities have been reported for

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Abbreviations: LOX, lipoxygenase; COX, cyclooxygenase; AA, arachidonic acid; HPETE, hydroperoxyeicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid; TMPD, N,N,N′, N′-tetramethyl-*p*-phenylene diamine; NDGA, nordihydroguaiaretic acid; PMSF, phenyl methyl sulphonyl fluoride; DDC, diethyl dithio carbamate; TLC, thin layer chromatography; HPLC, high-performance liquid chromatography; UV-VIS, ultra violet-visible.

this enzyme: a) one which catalyzes the formation of prostaglandin G_2 (PGG_2) from AA and b) a peroxidase activity which reduces PGG_2 to prostaglandin H_2 (PGH_2)[10]. PGH_2 is the precursor of all other prostaglandins of types PGD_2, PGE_2, $PGF_2\alpha$, prostacyclin (PGI_2) and thromboxanes (TXA_2 and TXB_2). PGE_2 through the EP_2 receptor on ovarian cells is responsible for ovulation, fertilization processes, while $PGF_2\alpha$ is responsible for uterine smooth muscle contraction and parturition[11].

PGs generated in most cell types act as autocrine and paracrine mediators of several cell functions including vascular permeability, pain generation, febrile response and uterine contractility[11,12]. Since they are involved in several uterine functions, their regulation is important for successful pregnancy and parturition[13,14], and prevention of preterm labor[15]. PGs are also found to be essential in ovum maturation and inhibition of their synthesis results in ovulation failure[7]. In sheep ovary, PGE_2 is the major PG formed in the ovarian follicles, whereas $PGF_2\alpha$ is the major product in the corpus luteum[16]. $PGF_2\alpha$ is an important regulator of the estrous cycle, as it controls the regression of the corpus luteum[17]. Although number of studies have been conducted on both LOX and COX systems separately[7,9,11], no detailed studies on the metabolism of AA by these enzyme systems simultaneously and the subsequent product analysis, have been reported in animal systems.

In order to have comprehensive analysis on the metabolism of AA via the LOX and COX pathways in sheep uterus, the products of AA formed by these two enzyme systems have been analyzed by TLC and HPLC. Also, the 5-LOX and 12- and 15-dual LOX, the enzymes involved in the metabolism of AA in sheep uterus have been isolated by employing differential salt precipitation techniques.

Materials and Methods

Nordihydroguaiaretic acid (NDGA), hematin, phenyl methyl sulphonyl fluoride (PMSF), diethyl dithio carbamate (DDC), Triton X-100 and N,N,N′,N′-tetramethyl-*p*-phenylene diamine (TMPD) were purchased from Sigma Chemicals Co., St. Louis, USA. DE-52 was from Whatman, PG standards and anti COX-1 antibody were from Cayman Chemicals, Ann Arbor, USA. Rabbit polyclonal anti 15-LOX antibody was from Santa Cruz Biotechnology, Inc., USA and arachidonic acid (AA) was from Nu Chek Prep, USA. Prestained protein markers were from MBI Fermentas, Inc., USA. All other chemicals were of analytical grade from Qualigens, India.

Tissue collection

Sheep uteri at different stages were collected from a local slaughterhouse, transported on ice to the laboratory and immediately used. The tissues were processed free of fat tissue, ovaries and cervix.

Extraction of uterine lipoxygenase (LOX)

The non-pregnant sheep uterine tissue was minced and homogenized to 20% homogenate in 150 m*M* potassium phosphate, *p*H 7.4 buffer containing 1 m*M* ascorbic acid, 1 m*M* EDTA, 2 m*M* $CaCl_2$, 2 m*M* $MgCl_2$, 1 m*M* KCl, 10 m*M* sodium metabilsufite, 1 m*M* PMSF and 250 m*M* sucrose. The homogenate was filtered through two layers of cheese cloth and centrifuged at 12,000 rpm for 20 min. The resulting supernatant was centrifuged at 33,000 rpm for 1 h and the supernatant (cytosol) was loaded on to DE-52 in batch-wise fashion and flowthrough was collected and checked for activity and protein. The matrix was washed with three volumes of homogenization buffer and then eluted with one volume of the same buffer containing 0.5 *M* NaCl and then checked for activity and protein. Since flowthrough contained most of the activity, washing and elution was discontinued in the later experiments. Proteins in the flowthrough were precipitated by subjecting to ammonium sulfate $(NH_4)_2SO_4$ fractionation as 0-50% and 50-100% and the fractionated proteins were subjected to activity assay. Protein concentrations were measured by Lowry's method[18].

Immunoblot of LOX

Proteins (100 µgs) in the total cytosol were separated on a 10% SDS-PAGE[19] and transferred on to nitrocellulose membrane. Western blot analysis was done according to the previously described method[20]. The blots were incubated with polyclonal 15-LOX specific antibodies and developed with goat anti-rabbit IgG-horseradish peroxidase.

Assay of LOX

Enzyme activity was measured spectrophotometrically by basically following the previously described method[21]. The reaction mixture contained 30-100 µg of uterine LOX in 150 m*M* citrate phosphate, *p*H 5.5 buffer at room temp. The reaction was initiated by addition of 250 µ*M* AA and followed for 1 min at 235 nm for the formation of conjugated diene. Enzyme activity was defined as µmoles of hydroperoxides formed per min per ml enzyme.

Inhibitory study on LOX

The reaction mixture contained the same components as above except by incubating the enzyme-buffer mixture with 1 μ*M* NDGA for 10 min, before addition of AA.

Extraction, separation and identification of LOX products of AA

The active enzyme was incubated with 250 μ*M* AA in 150 m*M* citrate phosphate buffer, *p*H 5.5 for 2 min in 100 ml reactions. The reaction was terminated by acidifying the reaction mixture to *p*H 3.0 with 6 *N* HCl. The products (HPETEs/HETEs) were extracted twice with equal volumes of hexane: ether mixture. The organic phase was evaporated under rotary evaporator to total dryness. The residue was dissolved in either ethanol or straight-phase HPLC mobile system consisting of hexane: propane-2-ol: acetic acid in 1000:15:1.

The compounds were separated both on TLC with mobile phase of diethyl ether: hexane: acetic acid (60:40:1) and on straight-phase HPLC (Shimadzu model equipped with SPD 6AV detector and CR4A chromatopac) using CLC-SIL (25 × 0.4 cm) column under isocratic conditions employing mobile phase (hexane: propane-2-ol: acetic acid in 1000: 15:1) at a flow rate of 1 ml/min. The column eluant was monitored at 235 nm and peaks were analyzed on a Hitachi scanning spectrophotometer. Peaks showing the conjugated diene spectra were collected and identified based on co-chromatography with standards. The standards were generated in the laboratory using potato 5-LOX and green gram 15-LOX, following the protocol developed by Reddanna *et al*[22].

Extraction of uterine cyclooxygenase (COX)

The uterine tissue was homogenized in 100 m*M* Tris-HCl, *p*H 8.0 buffer containing 5 m*M* EDTA and 5 m*M* DDC. The homogenate was centrifuged at 12,000 rpm for 30 min at 4°C. The supernatant was re-centrifuged at 33,000 rpm for 1 h in an ultracentrifuge. The microsomal pellet thus obtained was solubilized in 0.5% Triton X-100 for 30 min at 4°C at slow stirring and the sample was again centrifuged at 42,000 rpm for 1 h and the resulting supernatant was used as the enzyme source.

Immunoblot of COX

The microsomal extract containing COX enzyme (100 μg) was subjected to SDS-PAGE and immunoblotting was done by using COX-1 specific antibodies as described previously for LOX.

Assay of COX

COX activity was measured spectrophotometrically by using a chromogenic assay based on the oxidation of TMPD during the reduction of PGG_2 to PGH_2. The assay mixture contained Tris-HCl buffer (100 m*M*, *p*H 8.0), hematin (1 μ*M*), EDTA (5 m*M*) and COX enzyme source (100 μg). The reaction was initiated by the addition of 250 μ*M* AA and 170 μ*M* TMPD in the total volume of 1.0 ml. Change in absorbance was monitored over a period of 25 s and the activity was calculated as the change in absorbance per min and specific activity as the change in absorbance per mg protein.

Extraction, separation and identification of COX products of AA

The uterine COX was incubated with 250 μ*M* AA in 100 m*M* Tris-HCl, *p*H 8.0 buffer containing 5 m*M* EDTA, 1 μ*M* hematin for 2 min at room temperature with constant stirring. The reaction was terminated by acidifying the mixture to *p*H 3.0 with 6 *N* HCl. PGs were extracted twice with an equal volume of ethyl acetate: petroleum ether mixture (1:1 v/v). The organic phase was evaporated under dry N_2 gas to total dryness. The residue was dissolved in minimum volume of diethyl ether. The extracted PGs were separated on silica gel TLC plates using the solvent phase of water: saturated ethyl acetate: acetic acid: isooctane (51:80:25:80). The separated PGs were developed in iodine chamber and compared with the standards.

Results

Isolation and Western blot analysis of LOX

High activity of the LOX was found in 50-100% $(NH_4)_2SO_4$ precipitate of the DE-52 flowthrough fraction. The identity of the enzyme was also confirmed by Western blot using LOX-specific antibodies and the molecular mass of the protein (66 kDa) was in accordance with the known animal 15-LOXs[9] (Fig. 1A).

Analysis of AA products

Two bands (Fig. 1B, lane 2) were observed when the enzyme was incubated with AA and products were analyzed on TLC plate. One of them was tentatively identified as 15-HETE, as it co-migrated with the standard 15-HETE (lane 3). The other band showed migration between standard 15-HETE and 5-HETEs (Fig. 1B, lane 2). These observations

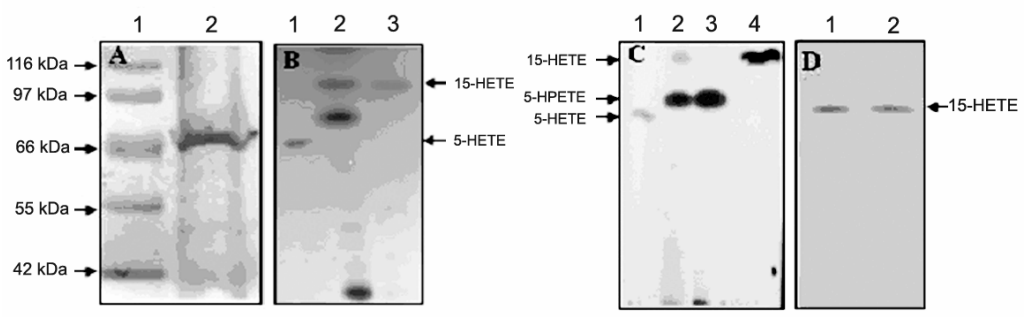

Fig. 1—(**A**): Immunoblot analysis of sheep uterine LOX [Lane 1, prestained protein markers; and lane 2, LOX separated on SDS-PAGE and cross-reacted with commercial antibodies against 15-LOX]; (**B**): TLC separation of AA metabolites produced by LOX (50-100% $(NH_4)_2SO_4$ fraction) [Sheep uterine LOX was incubated with AA in 150 m*M* citrate phosphate buffer, *p*H 5.5 and the products generated were extracted into hexane: ether (1:1) and were separated on silica gel TLC using hexane: ether: acetic acid (40:60:1). Lane 1, standard 5-HETE; lane 2, 50-100% fractionated LOX generated products; and lane 3, standard 15-HETE]; (**C**): TLC separation of AA metabolites produced by LOX (50-70% $(NH_4)_2SO_4$ fraction) [The methodology used was same as in Fig. 1B, except that 50-70% $(NH_4)_2SO_4$ fraction was used. Lane 1, Standard 5-HETE; lane 2, standard 5-HPETE; lane 3, 50-70% fractionated LOX products; and lane 4, standard 15-HETE]; (**D**): TLC separation of AA metabolites produced by uterine LOX (70-100% $(NH_4)_2SO_4$ fraction) [The methodology used was same as in Fig. 1B, except that 70-100% $(NH_4)_2SO_4$ fraction was used. Lane 1, 15-HETE formed by enzyme action; and lane 2, standard 15-HETE]

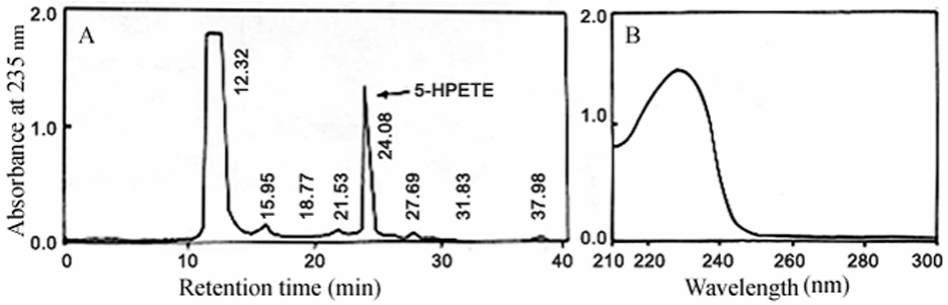

Fig. 2—(**A**): Straight-phase HPLC separation of the products generated as in Fig.1 C [The products were separated on a Schimadzu CLC-SIL (25 × 0.4 cm) column with hexane: propanol: acetic acid (1000:15:1) at a flow rate of 1 ml/min. The effluent was monitored at 235 nm]; and (**B**): Ultraviolet spectrum of peak with retention time of 24.08 of (A).

indicated that these bands were formed by the action of two different enzymes. To separate these enzymes, the DE-52 treated cytosol was fractionated in three steps — 0-50%, 50-70% and 70-100% $(NH_4)_2SO_4$ precipitations. The 0-50% fractionated protein did not show any enzyme activity. However, 50-70% and 70-100% fractionated enzymes exhibited significant LOX activity and were incubated separately with AA to obtain the products.

The 50-70% fractionated enzyme gave a single product on TLC which co-migrated with the standard 5-HPETE, but not with the standard 5-HETE (Fig. 1C). The products of this enzyme were further subjected to HPLC and peaks were analyzed for typical conjugated diene spectra using UV-VIS scanning spectrophotometer. The peak with retention time of 24.08 min (Fig. 2A) gave the typical conjugated diene spectrum (Fig. 2B), which co-migrated with the standard 5-HPETE (data not shown). The 70-100% fractionated enzyme gave a single product, which co-migrated with standard 15-HETE on TLC (Fig. 1D). On further analysis by HPLC, the products were separated into two peaks with retention times of 5.7 and 8.11 min (Fig. 3). The products generated by 50-70% and 70-100% $(NH_4)_2SO_4$ fractions were of enzymatic origin only, as no products were formed on heat-inactivation (data not shown). Also no products were formed when 1 μ*M* NDGA, a known inhibitor of LOXs was incubated in the assay mixture (Fig. 4). These observations suggested the involvement of LOXs in the metabolism of AA in sheep uterus.

Isolation and Western blot analysis of COX

COX was extracted from the microsomal fraction of sheep uterus and its identity was confirmed by

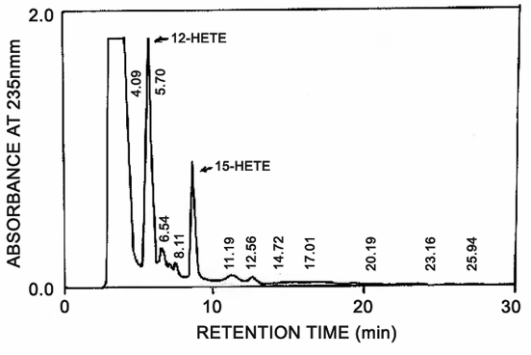

Fig. 3—Straight-phase HPLC separation of the products generated as in Fig. 1D [The products were separated on a Schimadzu CLC-SIL (25 × 0.4 cm) column with hexane: propanol: acetic acid (1000:15:1) at a flow rate of 1 ml/min. The effluent was monitored at 235 nm]

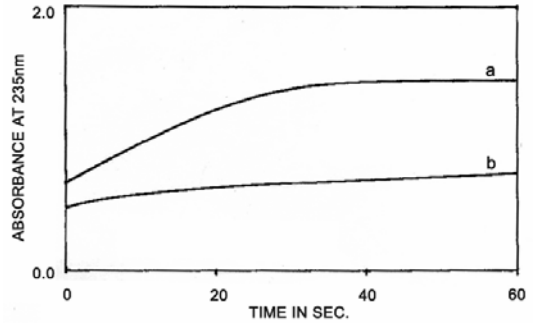

Fig. 4—Inhibitory activity of NDGA on LOX [Active LOX enzyme from 50-70% or 70-100% $(NH_4)_2SO_4$ fractions were incubated with AA in 150 m*M* citrate-phosphate buffer, *p*H 5.5 with or without NDGA (1 μ*M*) and reaction was monitored for 1 min in UV/VIS scanning spectrophotometer at 235 nm. (a): Without NDGA; and (b): with NDGA]

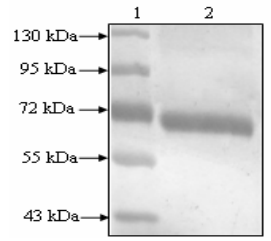

Fig. 5—Immunoblot analysis of sheep uterine microsomal COX [Lane 1, prestained protein marker; and lane 2, sheep uterine COX separated on SDS-PAGE and cross-reacted with commercial antibodies against COX-1]

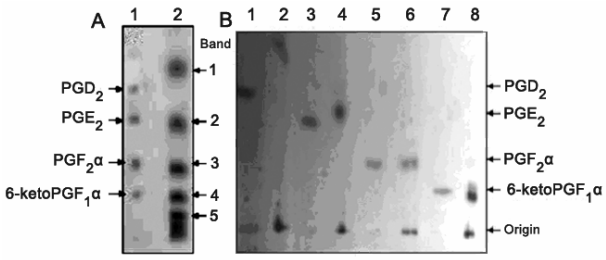

Fig. 6—(**A**): TLC separation of uterine microsomal COX generated prostaglandins [Sheep uterine COX was incubated with AA in Tris-HCl, *p*H 8.0 buffer containing EDTA and hematin. The products prostaglandins (PGs) were extracted into equal volumes of ethyl acetate: petroleum-ether mixture (1:1 v/v). The products were separated on silica gel TLC plates by using the solvent phase of water: saturated ethyl acetate: acetic acid: isooctane (51:80:25:80). Lane 1, standard PGs; and lane 2, microsomal-generated PGs; (**B**): Comparison of sheep uterine PGs with standards [The individual bands (1 to 4) which correspond to the respective standards of Fig. 6A were scrapped from the TLC plate, extracted into methanol and re-chromatographed on TLC plate in the same solvent system along with the individual standards. Lane 1, standard PGD_2; lane 3, standard PGE_2; lane 5, standard $PGF_2\alpha$; lane 7, standard 6-keto$PGF_1\alpha$; and lanes 2, 4, 6 & 8, sheep uterine PGs (bands 1-4 of Fig. A)]

activity assay and Western blot using COX-specific antibodies. The molecular mass of the enzyme was 70 kDa, which was in accordance with other known COX enzymes[10] (Fig. 5).

Analysis of AA products

The PGs formed by the microsomal COX were separated on TLC along with the standards (Fig. 6A). Four prominent bands (bands 1-4) along with some lower non-resolved bands were observed on the TLC plate. The individual bands, which correspond to the respective standards, were scrapped from the TLC plate, extracted into methanol and re-chromatographed on TLC plate in the same solvent system along with the individual standards. On re-chromatography, band 1 (lane 2) migrated much faster than the standard PGD_2 (lane 1). On the other hand, band 2 (lane 4) migrated very close to that of the standard PGE_2 (lane 3). Bands 3 (lane 6) and 4 (lane 8) co-migrated exactly with the standard $PGF_2\alpha$ (lane 5) and 6-keto $PGF_1\alpha$ (lane 7) respectively (Fig. 6B). From these observations, it could be concluded that sheep uterine COX system generated prostaglandin $PGF_2\alpha$ and prostacyclin derivative 6-keto $PGF_1\alpha$. The band which was little above the standard PGE_2 could be a modified form of PGE_2. No band was observed corresponding to PGD_2.

Discussion

Eicosanoids, specifically PGE_2 and $F_2\alpha$ are known to play a key role in uterine functions. The role of

LOX products, however, is not well defined. Also, there are no comprehensive studies on the type of LOX and COX metabolites formed in the uterus. In the present study, analysis of the AA products and their separation was done by a combination of both TLC and HPLC techniques which resulted in a clear identification of AA products. These studies demonstrated formation of 5-HPETE via the 5-LOX pathway, which was confined to the 50-70% $(NH_4)_2SO_4$ fraction and formation of 12-HETE and 15-HETE via the 12- and 15-dual LOX pathway, which was confined to the 70-100% $(NH_4)_2SO_4$ fraction of the uterus. As 5-LOX catalyzes the first committed step in the synthesis of leukotrienes[23], their formation in the uterine tissue was expected. However, there are no studies on the formation of 5, 6-leukotrienes, although the existence of 14, 15-leukotrienes was reported in the sheep uterus[9].

The expression of 5-LOX and 12-LOX genes is altered at different gestational ages prior to and at onset of labour[24]. The eicosanoids are shown to regulate gene expression through direct interaction with α and γ peroxisome proliferators activated receptor (PPAR)[25], the members of nuclear hormone receptor superfamily[26]. The significance of the LOX enzymes in reproduction was confirmed at the gene level in the mice with null mutation in L-12/15-LOX, which had significantly reduced uterine levels of AA metabolites and exhibited a partial impairment of implantation. Complete blockade of uterine 12/15-LOX activity by a specific inhibitor led to greater than 80% reduction in number of implantation sites, compared to untreated controls[27].

We observed that the metabolites formed by 5- and 12/15-LOX were different in their oxidation states, as 5-LOX produced hydroperoxy metabolite (5-HPETE), whereas 12 and 15-dual LOX produced the hydroxy derivatives (12 and 15-HETEs). At this point, we only could suggest a possible physiological role for these products, as 5-HPETE would be diverted for the formation of 5, 6-leukotrienes, whereas 12 and 15-HETEs could take part as potent chemotactic factors in several aspects of uterine physiology. In the light of above, it would be interesting to analyze the formation of 5, 6-leukotrienes formed in the sheep uterus.

The present study demonstrated high-level expression of COX-1 protein in sheep uterus. Similar expression of COX-1, but not COX-2 was reported in the myometrium of non-pregnant pigs[12]. The major

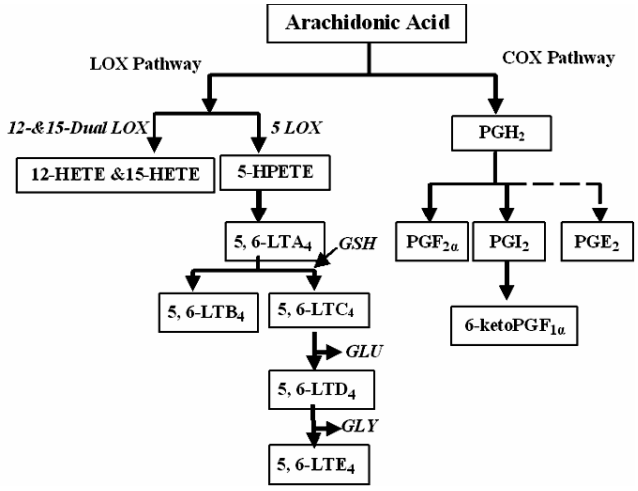

Fig. 7—Proposed model for the metabolism of arachidonic acid in sheep uterus

sheep COX products were identified as $PGF_2\alpha$ and 6-keto $PGF_1\alpha$ found in equal amounts by using the commercial PG standards on TLC. PGs are generally considered to be stimulators of the physiologies of labor and PG receptor inhibitors prevent preterm labor[15]. $PGF_2\alpha$ and its receptor FP ($PGF_2\alpha$ receptor) are shown to activate and prepare the decidua for its role in parturition[28]. It also increases the sensitivity of the contractile proteins to Ca^{2+} in myometrium[29]. The diverse effects of PGs are normally mediated through their G protein-coupled, cell-surface receptors that are linked to different signaling pathways[30].

Based on the above observations, we propose a model for the metabolism of AA in sheep uterus (Fig. 7). AA in the sheep uterus is metabolized by two enzyme systems – LOX and COX. The LOX pathway includes 5-LOX and 12,15-dual LOX enzymes. 5-LOX produces 5-HPETE, which would lead to the formation of 5, 6-leukotrienes. On the other hand, 12-, 15-dual LOX produces 12- and 15- HETEs. The COX pathway leads to the formation of $PGF_2\alpha$, 6-keto-$PGF_1\alpha$. The present study thus forms the first report on the comprehensive analysis on the metabolism of AA in sheep uterus.

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References

1 Rokach J & Fitzsimmons B (1988) *Int J Biochem* 20, 735-758
2 Brash A R (1999) *J Biol chem* 274, 23679-23682
3 McDonnell M, Davis W Jr, Li H & Funk C D (2001) *Prostaglandins Other Lipid Mediat* 63, 93-107
4 Dailey L A & Imming P (1999) *Curr Med Chem* 6, 389-398
5 Carson D D, Bagchi I, Dey S K, Enders A C, Fazleabas A J, Lessey B A & Yoshinaga K (2000) *Dev Biol* 223, 217-237
6 Schafer W R, Zahradnik H P, Arbogast E, Wetzka B, Werner K & Breckwoldt M (1996) *Placenta* 17, 231-238
7 Downey B R, Mootoo J E & Doyle S E (1998) *Anim Reprod Sci* 49, 269-279
8 Cooke R G & Ahmad N (1998) *Anim Reprod Sci* 52, 113-121
9 Sailesh S, Kiran Kumar Y V, Prasad M & Reddanna P (1994) *Arch Biochem Biophys* 315, 362-368
10 Smith W L (1992) *Am J Physiol* 263, F181-F191
11 Funk C D (2001) *Science* 294, 1871-1875
12 Cao J, Kitazawa T, Takehana K, Taneike T (2006) *Prostaglandins Other Lipid Mediat* 81, 93-105
13 Poyser N L (1995) *Prostaglandins Leukotrienes Essent Fatty Acids* 53, 147-195
14 Jenkin G (1992) *J Reprod Fertil* 45, 97-117
15 Olson D M, Ammann C (2007) *Front Biosci* 12, 1329-1343
16 Padmavathi O, Pandu Ranga Reddy G, Ramesh Reddy G & Reddanna P (1990) *Biochem Int* 20, 455-461
17 McCracken J A, Custer E E & Lamsa J C (1999) *Physiol Rev* 9, 263-323
18 Lowry O H, Rosenbrough N J, Farr A L & Randall R L (1951) *J Biol Chem* 193, 265-275
19 Laemmli U K (1970) *Nature* 227, 680-685
20 Towbin H, Staehelin T & Gordon J (1979) *Proc Natl Acad Sci* (USA) 76, 4350-4354
21 Ben Aziz S, Grossman I A & Budowoki B (1970) *Anal Biochem* 34, 88-100
22 Reddanna P, Whelan J, Maddipati K R & Channa Reddy C (1990) *Meth Enzymol* 187, 268-276
23 Samuelsson B, Dahlen S E, Lindgren J A, Rouzer C A & Serhan C N (1987) *Science* 237, 1171-1176
24 Smith G C, Wu W X, Nathanielsz P W (2001) *Mol Hum Reprod* 7, 587-594
25 Kliewer S, Sundseth S S, Jones S A, Brown P J, Wisely G B, Koble C S, Devchand P, Wahil W, Wilson T M, Lenhard J M & Lehmann J M (1997) *Proc Natl Acad Sci* (USA) 94, 4318-4323
26 Desvergne B & Wahli W (1999) *Endocr Rev* 20, 649-688
27 Quanxi Li, Yong-Pil C, Kannan A, Shanker S, Bagchi I C & Bagchi M K (2004) *J Biol Chem* 279, 11570-11581
28 Makino S, Zaragoza D B, Mitchell B F, Robertson S & Olson D M (2007) *Semin Reprod Med* 25, 60-68
29 Woodcock N A, Taylor C W & Thornton S (2006) *Am J Obstet Gynecol* 195, 1404-1406
30 Negishi M, Sugimoto Y & Ichikawa A (1995) *Biochem Biophys Acta* 1259, 109-120