Summary
Many disorders of human health are linked to an unbalanced overproduction of hormone like materials called eicosanoids. Eicosanoid is the term given to a group of oxygenated derivatives of eicosapolyenoic fatty acids, especially arachidonic acid. Arachidonic acid, the most abundant polyunsaturated fatty acid in cell membrane phospholipids, once released, will be oxygenated by two important pathways: the cyclooxygenase pathway producing prostaglandins, thromboxanes and prostacyclin, and the lipoxygenase pathway leading to the production of hydroperoxides, leukotrienes and lipoxins. These are extremely potent biologically active compounds with bewildering variety of actions. There is not a single system or process that is not affected, one way or the other, by the eicosanoids, including reproduction. There is extensive literature and many reviews on the role of prostanoids in reproduction. However there is very little information on lipoxygenase pathway in reproduction. In the present study an attempt is made to analyze the lipoxygenase pathway in sheep uterus.

Purification of sheep uterus lipoxygenase

An abundant lipoxygenase activity, as high as 10.7 units/g wet tissue was observed in the cytosolic fraction of sheep uterus. This is one of the most abundant sources for lipoxygenases ever reported in animal tissues. The protein was purified by conventional chromatographic as well as HPLC techniques. Since the lipoxygenase activity was in the flowthrough of both cation and anion exchange columns, the enzyme was passed through both the columns connected in series. On HPLC using PA-DEAE ion exchange column also, the protein eluted in the flowthrough fractions.

The enzyme was purified to apparent homogeneity with 14.6 fold purification and an overall yield of 42.66%. The highly purified enzyme showed specific activity as high as 14.0 units/mg protein.
Structural characterization

The purified protein on SDS polyacrylamide gel electrophoresis showed a single band with molecular weight of 66 kDa. The same molecular weight was observed when the protein was run on HPLC using gel filtration column (Diol-300). However, on non-denaturing gels, the highly purified lipoxygenase resolved into two bands: 132 kDa and 66 kDa. Both the bands cross reacted with polyclonal antibodies raised against the highly purified lipoxygenase on Western blotting, indicating that the high molecular weight band could be the dimeric form of the lipoxygenase. In order to find out the catalytically active form of the enzyme, the proteins resolved on non-denaturing gel was incubated with arachidonic acid (substrate) solution and the hydroperoxides formed were later reacted with O-dianisididine color reagent. These studies revealed that only the dimeric form to be catalytically active.

The uterus lipoxygenase was found to be a neutral protein with a Pi value of 7.0 as indicated by non-binding to both anionic as well as cationic exchangers and electrofocussing. The enzyme expresessed maximum activity at pH 5.5. The substrate dependent kinetics with arachidonic acid as the substrate revealed a high Km i.e 180 μM. The affinity towards the γ-linolenic acid (GLA) was much higher when compared to that of arachidonic acid as evidenced by low Km for GLA (98 uM). Further the enzyme was found to be unique in that no cofactors like Ca^{2+}, Mg^{2+}, ATP are required for expression of maximum activity.

Functional characterization

In order to identify the type, the purified uterus lipoxygenase was incubated with arachidonic acid as the substrate and the products were extracted into organic layer and then separated on straight phase HPLC. The products separated were identified based on their UV/Vis spectral characterization and GC/MS analysis. From these studies it was observed that both 12-HETE and 15-HETE were formed in almost equal concentration when highly purified enzyme was incubated with arachidonic acid as the substrate,
indicating dual lipoxygenase nature of the enzyme. The relative concentration of 12- and 15- HETEs, however, varied with the pH of the incubation medium. At pH below the optimum, 15-HETE was formed in higher concentration. At alkaline pH, the reaction favoured the formation of 12-HETE when compared to 15-HETE. Similar to arachidonic acid, the enzyme showed dual lipoxygenase activity with GLA producing 13-HOTrE and 10-HOTrE in equal concentration at the optimum pH. The enzyme thus, is unique in exhibiting dual regiospecificity. The dual regiospecificity of lipoxygenase can be explained based on the dimeric nature of the enzyme. Since only the dimer is catalytically active, two substrate molecules should be binding to the two catalytic centres, one facilitating the formation of 12-HETE and the other catalytic centre producing 15-HETE

As a result of dual regiospecificity, the enzyme should exhibit LTA4 synthase activity similar to other known dual lipoxygenases reported. Leukocytes which exhibit 5- and 8- lipoxygenase activities, exhibit 5,6-LTA4 synthase activity also (Shimizu et al., 1985). In order to test the formation of leukotrienes, uterus lipoxygenase was incubated with 15-HPETE and the products formed were separated on reverse phase HPLC and identified basing on the UV/VIS spectra and GC/MS analysis. Two major products (peak I and II) were formed, with typical conjugated triene spectra and absorption maximum of 268 nm, with 15-HPETE as the substrate. Both the products (peak I and II) were identified as 8,15-diHETEs based on GC/MS analysis. The elution pattern was similar to the hydrolyzed products of 14,15-LTA4. Further peak I and peak II co-eluted with 14,15-LTA4 hydrolyzed 8(S), 15(R)- and 8(S), 15(S)-diHETEs, confirming the formation of 14,15-LTA4 when 15-HPETE was incubated with uterine lipoxygenase. These studies have thus demonstrated that the uterine dual lipoxygenase exhibits 14,15-LTA4 synthase activity. This is the first report on the formation of 14,15-series of leukotrienes in mammalian reproductive tissues.
Physiological role of lipoxygenases in uterus

In order to understand the physiological role of lipoxygenases in uterus, PUFAs were analyzed in different phases of estrous cycle on GC. Arachidonic acid was found to be the predominant unsaturated fatty acid present both in the cytosol and microsomal membrane fractions.

Lipoxygenase activity was the highest in luteal phase as compared to the other phases of estrous cycle. Also the endogenous products extracted from uterus, at different phases of estrous cycle, were higher in the luteal phase. In luteal phase, uterus is under the influence of estrogen and progesterone with active mitogenic activity. From this it appears that lipoxygenases may be involved in the mediation of steroid hormone actions.

In order to identify the lipoxygenase products involved in uterine functions, the endogenous products were extracted, separated on HPLC and identified. Among the hydroxy metabolites only 15-HETE was identified with a conspicuous absence of 12-HETE, as against expectations. The results suggest the possible diversion/utilization of 12-HETE specifically under *in vivo* conditions. 12- and 15-HETEs may be acting as chemotactic agents for leukocytes leading to their infiltration into the uterus. In addition to hydroxy metabolites, dihydroxymetabolites and leukotrienes were observed among the endogenous metabolites of arachidonic acid in the uterus. The dihydroxymetabolites were identified as 8,15-diHETEs. Since 8,15-diHETEs are known to play an important role in the mediation of immunological cross reactions, the diHETEs formed in the uterus in the present study must be involved in leukocyte infiltration and other hypersensitivity reactions.

Among the leukotrienes, a product with 280 nm absorption maximum was isolated. It was identified as 14,15-LTC₄ based on the co-chromatography with standard leukotrienes. The formation of 14,15-LTC₄ was further confirmed by demonstration of LTC₄ synthase activity in uterine microsomes. Since peptido-leukotrienes are known for...
smooth muscle contractile properties, \textbf{14,15-LTC}_4 formed in the uterus must be playing vital role in uterine contractions which are helpful either in normal parturition or in the transport of spermatozoa towards the site of fertilization and fertilized eggs to the site of implantation.

From these studies it can be concluded that a lipoxygenase, with dual regio specificity producing 12- and 15-HETEs with arachidonic acid as the substrate is present abundantly in the sheep uterus. As a result of dual \textit{regiospecificity} the enzyme also showed 14,15-LTA\textsubscript{4} synthase activity Since arachidonic acid is the major PUFA in the uterus, the arachidonic acid metabolites formed via the lipoxygenase pathway must be playing vital role in \textit{uterine} functions such as growth and \textit{contractions}