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Investigation of the regulation of prostaglandin synthesis in the bovine oviduct

The oviduct plays a decisive role in reproduction as it provides a beneficial environment for oocyte maturation, capacitation, gamete transport, fertilization, and the early embryonic development. The latter are key processes in the origin of pregnancy which are regulated by hormones, e.g. sexual steroids and prostaglandins. For instance, prostaglandins are considered to be central mediators concerning ovulation, fertilization, as well as establishment and maintenance of pregnancy. Several prostaglandins were already detected in the bovine oviduct. To get a better insight into the regulation of prostaglandin synthesis within the bovine oviduct, estrous cycle-dependent changes and local distributions of the enzymes were examined on mRNA and partly on the protein level. Bovine oviducts were collected at the local slaughterhouse and classified into one of the following four phases: pre- (days 19-21) and postovulatory phase (days 1-5), early luteal phase (days 6-12), and late luteal phase (days 13-18). Moreover, the oviducts of each cow were separated into the ipsilateral (to ovulation site/corpus luteum) and the contralateral oviduct and further divided into ampulla and isthmus.

The mRNA expression of $sPLA_2$, $cPLA_2\alpha$, $cPLA_2\beta$, COX-1, COX-2, cPGES, mPGES-1, mPGES-2, PGFS, HSD, H-PGDS, L-PGDS and PGIS was investigated in flushed oviductal cells by quantitative RT-PCR. Specific mRNA transcripts of the listed enzymes were detected in the bovine oviduct. COX-1, mPGES-1, HSD, PGFS and H-PGDS were significantly higher expressed during the post-ovulatory phase at the time of early reproductive events. The highest expression of $cPLA_2\beta$ and PGIS was detected in the pre-ovulatory phase while L-PGDS showed the highest expression in the late luteal phase. In contrast, $sPLA_2$, $cPLA_2\alpha$, COX-2, cPGES and mPGES-2 were expressed estrous cycle-independently. Concerning the different regions in the oviduct, $sPLA_2$, L-PGDS and PGIS were higher expressed in the ampullary parts in contrast to mPGES-1 and HSD which showed a higher expression in the isthmic parts. No differences were observed between ipsi- and contralateral oviducts.

The protein amount of cyclooxygenases was quantified via western blot analysis in relation to the estrous cycle-phase and the region of the oviduct. COX-1 showed the highest protein expression in the early luteal phase compared with the other phases. COX-2 showed a constant expression without an evident regulation during estrous cycle. The examination of cyclooxygenase activity revealed, that COX-1 contributed the majority of the cyclooxygenase activity within the bovine oviduct.

Immunohistological stainings for COX-1, COX-2 and mPGES-1 revealed their exact localization within the oviduct. COX-1 and mPGES-1 were located in epithelial and smooth muscle cells in contrast to COX-2 which was exclusively localized in epithelial cells. Inside epithelial cells, mPGES-1 showed a nuclear staining while COX-1 was found in the

cytoplasm and nucleus. COX-2 was exclusively located in the cytoplasm. In the smooth muscle cells COX-1 and mPGES-1 showed a cytoplasmic staining.

Additionally, a potential regulation of the mRNA expression of these enzymes was investigated in cultured primary oviductal cells after treatment with 17β-estradiol, progesterone, arachidonic acid or PGE_2 in a time-dependent experiment over a period of 6 hours. Cell culture experiments revealed that $cPLA_2α$, COX-2 and mPGES-1 mRNA expression was stimulated by all treatments while PGIS mRNA was higher expressed after PGE_2 application. The other enzymes did not show any changes in their mRNA expression after treatment with 17β-estradiol, progesterone, arachidonic acid or PGE_2.

All the results of this study support the hypothesis that a fine-tuned and estrous cycle-dependent regulated system for prostaglandin synthethis exists in the bovine oviduct. That suggests that prostaglandins may directly influence survival, maturation and transport of the oocyte as well as fertilization and embryonic development. Therefore, it is likely that prostaglandins play an essential role in the genital tract of the cow concerning the origin of a new organism.