contain higher amounts of PLCζ protein than murine sperm. This is consistent with the differences in $[Ca^{2+}]_i$-releasing activity observed following injection of stallion or mouse sperm (ICSI) into mouse oocytes in our laboratory. Immunofluorescence was performed in sperm from three different stallions of proven fertility, before and after incubation in capacitating conditions. Phospholipase Cζ consistently localized to the equatorial and acrosomal regions, as well as the connecting piece between the head and midpiece of the sperm, with no significant differences observed between non-capacitated and capacitated sperm. For cloning of equine PLCζ, total equine testis RNA was isolated and cDNA amplification of specific sequences was performed by RT-PCR. Comparing PLCζ sequences of different species against partial sequence fragments of the equine gene demonstrated regional homology (88.6%) with bovine PLCζ. Therefore, primers were designed using conserved areas of the bovine and equine sequences (GenBank accession number NM_001011680; 5′-TGA AAA TAT GGA GAA CAA AT-3′ 5′-CTA TCT GAT GTA CCA AAC AT-3′). Alignment of the predicted peptide PLCζ sequence (derived from our initial clones), with that of multiple mammalian species showed that equine PLCζ displayed the highest homology (80.6%) with porcine PLCζ in regions known to be conserved among species. Conversely, the predicted peptide sequence displayed lesser homology in the region corresponding to the linker between the two catalytic subunits (X-Y) of the protein, as shown between other species. Interestingly, the X-Y linker region has been shown to be important in determining species-specific differences in PLCζ activity. Our next step is to synthesize complementary RNA and protein from our clones in order to investigate their $[Ca^{2+}]_i$-releasing activity. The results of this study will not only contribute to the advancement of assisted reproduction technologies in the horse, but also enhance our knowledge in basic reproductive physiology in mammalian species.

514. Diversity of Motility in Fish Spermatozoa Depends on Distinct Energy Metabolism. Tatsuo Harumi, Tadashi Andoh, Takahiro Matsubara, Norihiko Shimizu, Toshiyuki Hayakawa, Yokichi Hayashi, Ryuzo Yanagimachi. Asahikawa Medical College, Asahikawa, Japan; Hokkaido National Research Institute, Kushiro, Japan

Fish spermatozoa are released in water during spawning, and therefore they must swim through it utilizing their intracellular energy substrates before reaching egg. To clarify the relation between motility and energy sources of fish spermatozoa, we compared the moving and energy metabolisms in spermatozoa freshly collected from two flounders (Liopsetta obscura and Verasper moseri) and the Pacific herring (Clupea pallasii). Flounder spermatozoa swam linearly, and turned their moving direction near the opening of egg miclopyle before entering it. Herring spermatozoa, which were almost motionless in seawater, began to move near the micropyle and entered it after gliding the surface of chorion in a circular manner. When diluted in seawater, L. obscura spermatozoa swam vigorously before stopping by one minute. V. moseri spermatozoa kept swimming for more than ten minutes. Unlike flounder spermatozoa, herring spermatozoa, which began to move near the micropyle, kept moving in the micropyle region for hours even after. In seawater, herring spermatozoa were almost motionless, yet their cAMP level was elevated having the peak at five second after their dilution. Flounder spermatozoa did not show such cAMP elevation during their movement in seawater. Before swimming, flounder spermatozoa had high levels of ATP, while herring spermatozoa had only a trace amount of ATP. After started to swim, L. obscura spermatozoa depleted their ATP, whereas V. moseri spermatozoa retained 40% of ATP they had before swimming. V. moseri spermatozoa contained glycogen which was consumed during their swimming in seawater. Both L. obscura and herring spermatozoa contained only trace amounts of glycogen even before and after swimming. These results indicate that the motility and metabolic pattern of fish spermatozoa vary from one species to another.

515. The Adult Boar Testicular and Epididymal Transcriptome. Benoit Guyonnet, Jean-Louis Dacheux, Florence Jaffrezic, Anne Lacoste, Guillemette Marot, Marie-Jose Mercat, Sandrine Schwob, Jean-Luc Gatti. Institut National de la Recherche Agronomique, Nouzilly, France; Institut National de la Recherche Agronomique, Jouy en Josas, France; Institut du Porc, Le Rheu, France

Our laboratory has established the cartography of secreted proteins (secretomes) and proteins present (proteomes) in the epididymal fluid of different mammals and has demonstrated their regional variations along the epididymis. To extend the understanding of epididymal function in large mammals, we undertook a transcriptomic study of differentially expressed genes along the boar epididymis using the testis as organ of comparison. Our analysis focused on genes considered to be markers of a single organ or a transcriptomic region and over-expressed mainly in this organ or region. Tissues of four Large White adult boars (including the testis, efferent ducts, nine morphologically distinct segments of the epididymis and deferent duct) were isolated and their mRNA extracted. The gene expression of each of these samples was analyzed using a pig generic micro-array (GEO Accession Number GPL3729). After data processing, 7676 transcripts were retained, 2115 of which were differentially expressed in these 12 tissue types according to a modified F-test with a Benjamini & Hochberg (BH) correction of 1/100,000 on raw p-values. Classification analysis with these 2115 transcripts clustered the 12 tissue types in 7 transcriptomic regions, corresponding to the testis, the efferent ducts, the anterior caput (zones 0-1), the median caput (zone 2), the posterior caput (zones 3-4), the corpus (zones 4-7), and the cauda (zone 8/9 and the deferent duct). A total of 1772 transcripts were classified in 3 main clusters by two statistical methods (Partitioning Around Medoids (PAM) and Hierarchical Clustering (HCL)) that were representative of the testis, caput, corpus and a cluster formed by the efferent ducts/cauda genes. Transcripts over-expressed in the same 7 transcriptomic regions were selected by a modified t-test with BH=1/100,000. Of these, 1243 transcripts were over-expressed in a single region. By combining the results of these analyses, 459 potential markers were identified for the testis, 33 for the efferent ducts, 91 for the anterior caput, 5 for the median caput, 8 for the posterior caput, 174 for the corpus and 77 for the cauda. The expression profiles of certain of these markers were confirmed by RT-PCR. These markers will help us to find new transcripts coding for secreted proteins or contributing to specific functions of the organ studied. The results of this study will also be valuable for further analysis of the epididymal transcriptome during post-natal development and to search for differentially expressed genes in boars of contrasting fertility.

516. Antiandrogenic Signaling of Bufalin and Cinobufagin on Human Prostate Cancer Cells. Ching-Han Yu, Paulus Wang. National Yang-Ming University, Taipei, Taiwan

Androgen and androgen receptor (AR)-modulated signaling are essential for the development and function of the normal prostate as well as the prostate cancer. The men with deficiency in 5α-reductases, the enzyme that convert testosterone to its active metabolite 5α-dihydrotestosterone, are rarely found prostate cancer, which indicates the importance of androgen in prostate cancer development. Targeting androgen and AR signaling are rational strategies for the therapy of prostate cancer. Traditional medicine has been used since ancient times to remedy malignancies. Recent studies have reported that decursin, evodiamine, and digitalis extracted from herb inhibit the growth of various cancer cells. Bufalin and cinobufagin are extracts of poison glands from toads, and are traditionally used as treatments of heart failure. Our previous studies indicated that digitalis-like bufalin and cinobufagin exerted antiproliferative effects on prostate cancer cells. In the present study, the antiandrogen and AR activities of bufalin and cinobufagin in the androgen-sensitive LNCaP and 22RV1 human prostate cancer model. The effects of bufalin and cinobufagin on cell growth were examined by MTT assay. After treating with bufalin or cinobufagin for 24 hours at the concentration of 10^{-10} to 10^{-5} M, the cell growth of two prostate cancer cell lines was inhibited at 10^{-6} and 10^{-5} M. The activity of caspase 3 was observed after the treatment at the concentration of 10^{-7} to 10^{-5} M for 4 to 24 hours. Caspase 3 activity was elevated after 18-hour of treatment at the 10^{-6} and 10^{-5} M. The protein expressions of AR and prostate specific antigen (PSA), the marker of AR activity, were determined by Western blot after treating with bufalin and cinobufagin for 4, 8, and 12 hours at the concentration of 10^{-7} to 10^{-5} M. The intracellular PSA expression of LNCaP cells was inhibited after the treatment for 4, 8 amd 12 hours at the 10^{-6} and 10^{-5} M. However, there was no significant intracellular PSA expression in 22RV1 cells. Bufalin and cinobufagin didn't affect the expression of AR after 4-hour treatment in both cell lines. Nevertheless, after 8- and 12-hour treatment, AR level was downregulated at the concentration of 10^{-7} to 10^{-5} M in both cell lines. To analyze whether the effects of bufalin and cinobufagin occurred at the transcription level, the mRNA levels of PSA and AR were examined. The mRNA levels of both PSA and AR were inhibited after 8- and 12-hour treatment at the concentration of 10^{-7} to 10^{-5} M in LNCaP cells. These data suggested that bufalin and cinobufagin showed the antiandrogenic effect via downregulating both protein and mRNA levels of the PSA and AR at the administrated concentration and duration, which didn't induce cell apoptosis. Therefore, bufalin and cinobufagin might inhibit prostate cancer cell growth by inducing cell apoptosis and targeting androgenic signaling.

517. Effect Of Angiotensin II On Bovine Oocyte Nuclear Maturation Mediated by PGE_2 and $PGF_{2\alpha}$. Marcos Barreta, Joao Oliveira, Alfredo Antoniazzi, Rogerio Ferreira, Luciano Sandri, Paulo Goncalves. Universidade Federal de Santa Maria, Santa Maria, Brazil

In mammals, it is well know that resumption of meiosis occurs after the preovulatory LH surge and results in germinal vesicle breakdown (GVBD), initiating the so-called oocyte maturation. However, the pathway by which this gonadotrophin acts is not completely clear. We have recently demonstrated that AngII plays an important role on the onset of ovulation in cattle, potentially acting as an intrafollicular LH mediator. We also observed that AngII prevents the inhibitory effect of follicular cells during