

Examination of prenatal development of endocrine glands in companion animals.

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Introduction and aim. Endocrine system is one of the first organs developing in fetal life. As our knowledge is limited from prenatal endocrine functions and its maternal interactions in companion animals, aim of our study to follow prenatal development and maturation of endocrine organs and metabolism.

Materials and Methods. Samples were collected from healthy bitches that are subjects of ovariohysterectomy for treatment of unwanted pregnancy. Bitches are allocated in different groups depending upon their pregnancy age, blood samples were collected from mothers before induction of anaesthesia and fetuses after remove. Allantois and amnion fluid were collected separately for same hormones determination. Serum is separated and frozen until assayed for serum progesterone (P₄), 17β-estradiol (E₂), thyroxin (T₄), cortisol and insulin levels. Utero-placental tissue (1cm³) was collected at the time of surgery and fixed in 5–8% neutral-buffered formalin, dehydrated and embedded in paraffin wax. The 4 μm thick sections were immunostained using the EnVision System. Placental 11β-hydroxysteroid dehydrogenase (11βHSD) is examined by antigen prepared by Abcam (Cambridge, UK). T₄, cortisol and E₂ were measured by ELISA assays, (DRG International Inc., New Jersey, USA). Insulin was measured by canine Insulin assay (Merkodia Inc, Uppsala, Sweden). Progesterone was measured by Quanticheck (SzIU, Faculty of Veterinary Science, Budapest) The means and SD were calculated and subjected to ANOVA and Student's t-test. Significance of differences was set at p < 0.01. Correlations were analyzed using SPSS 13.0.

Results. P₄ concentrations in fetal serum, allantois and amnion were less than maternal levels at any time of the pregnancy. Highest concentrations in fetal serum were measured at 7th week of pregnancy (7.48±0.79 ng/ml) which differed from same parameters measured at 5th and 8th weeks at level of significance. T₄ concentrations of allantois and amnion fluids exceeded the fetal serum concentrations except the 9th week of pregnancy. T₄ levels in fetal fluids and serum were below the physiologic levels of adult animals and for preparing the parturition it was elevated at the late pregnancy. Fetal serum glucose concentrations at 8th week of pregnancy were extremely higher (10.43±3.39 mmol/l) than physiologic range in adult animals. Glucose concentrations of fetal fluids were in same range as healthy adult serum levels. The cortisol concentrations in allantois and amnion fluids in each examined period were same as physiologic range in maternal serum, fetal serum cortisol concentrations exceeded the maternal levels. P₄ and E₂ had mild correlation to insulin in fetal fluids and serum. The means of P₄, T₄, were below the maternal concentrations. Placental 11βHSD positivity of cytotrophoblast cells increased parallel with progress of pregnancy age, mainly in chorion however syncytiotrophoblasts of labyrinth zone did not shown any positivity.

Conclusions. The placenta plays a role as a barrier between the maternal and fetal circulation and serves the enzymatic and metabolic environment to keep the higher concentrations in fetal side. The increase of foetal serum T₄ level at 9th week of pregnancy helps to prepare on parturition and individual metabolism. It is conceivable that the considerable differences between the maternal and foetal serum T₄ concentrations might account for the placental impermeability of T₄. The difference among maternal, fetal serum and fetal fluid cortisol concentration and placental activity of 11βHSD demonstrated that transplacental transport is regulated by them and dependent from gestational ages. The small glucose molecule and endotheliochorial placenta indicate a close connection, our results demonstrate an independent GNG pathway in fetal life as source of increased glucose level in fetal serum. In conformity to human studies, our results indicate suspected insulin production of fetal membranes. Immunohistochemical examinations of fetal endocrine organs are in progress.