

## LECTURES

# SCREENING AND VACCINATION: A SYNERGY FOR CERVICAL CARCINOMA

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Cervical cancer is one of the rare cancers where there is easy access to the organ and the screening test is simple and relatively inexpensive. In addition, slow progression of the disease means that there is a long period of time, 10 years, from infection to cancer. Cervical smear has proved very successful. However, this test is also limited in terms of sensitivity and low reproducibility. Reading smear tests remains an art and they are not always interpreted in the same way. Because of the low sensitivity there is a requirement to repeat the test regularly and even if this is the case in developed countries the coverage is never 100%. In developing countries, the coverage is very low because of the lack of resources and the lack of pathologists and cytologists.

HPV is a necessary but insufficient cause of cervical cancer. It is insufficient because most HPV infections will regress spontaneously without treatment. The prevalence in the normal population is approximately 10%, with notable age variation but low geographical variation. However, in patients with precancerous lesions or cervical cancer the prevalence is extremely high, with the high risk HPV 16/18 types present in 70% of cancer cases.

Vaccination is considered to be primary prevention whereas cervical smear and other such tests are forms of secondary prevention. Vaccination aims to prevent infection before it can become persistent and provoke lesions. The HPV vaccine has been developed thanks to virus-like particles (VLPs) which are non-infectious but which provoke the production of neutralizing antibodies. These antibodies prevent the penetration of the virus in the epithelium and provoke a very strong immune reaction whilst at the same time remaining non-infectious for the vaccinated individual. The results of the clinical studies relating to the vaccine against the HPV types 16 and 18 are very promising: there is a remarkable immune re-

sponse of nearly 100%, much higher than with natural HPV infection. They show no major side effects linked to vaccination. The efficacy of the vaccine against the HPV types 16 and 18 is also remarkable with nearly 100% protection against persistent infection and precancerous lesions. However, the vaccine gives little or no protection against infections and lesions associated with HPV types which are not included in the vaccine. This implies that a vaccinated woman will be protected against 70% of cervical cancers. The duration of the protection seems to be very good (the study data covered five years), so there may be no need to do a repeat injection. The vaccine does not protect women who are already infected by HPV. This implies vaccinating much earlier than the average age of the first sexual experience. The peak of HPV infection is around age 20-25 and drops sharply after age 30. The peak incidence of cervical cancer is between age 40 and 50. If women are vaccinated between age 10 and 15, the time necessary for the impact of vaccination on the incidence of cervical cancer in the vaccinated population will be at least 20 years. The time necessary to see the total impact of vaccination, with the entire generation of women having been vaccinated, will be closer to 30-50 years. It is therefore evident that there can be no question of ending screening when vaccination is introduced because there will be a time shift in the impact of a vaccination program of at least 20 years and in any case the current vaccines only provide protection against 70% of cervical cancers. There is a possibility that the vaccination will have a favorable effect on screening because doctors and the general public will be made more aware of cervical cancer through vaccination and there will be renewed interest in the disease. In addition, as the media have started taking interest in the disease, politicians are also taking more interest in the subject.

## ESTIMATION OF TIME AND CAUSE OF PERINATAL DEATH IN STILLBORN INFANTS

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Skillfully performed perinatal autopsy is extremely important because it provides informations relevant to the family, the physician and the community. Standardized protocols that should be followed to gain reliable informations contain photographs, radiograms, karyotyping (in selected cases), gross and histologic examination of the placenta, gross examination and autopsy of the fetus (newborn). According to the literature, stillbirth (defined as delivery of a fetus who has died in utero after 20 weeks' gestation) accounts for about 50% of all perinatal deaths. Similar data were obtained also for Croatia in the past few years (stillbirth defined as gestational age >22 weeks and birthweight of 500g and more). Gross examination should be performed in all

cases of perinatal death, as well as the autopsy, because it reveals the cause of death in the majority of cases. In cases of stillbirth not enough attention is paid to estimation of time of fetal death. Gross examination of the stillborn and histological examination of samples of fetal organs taken during the autopsy (with pathological examination of the placenta) enables us to assess fairly accurately the duration of time period between intrauterine death and birth. This can also have important medico-legal implications. The best gross and histologic predictors of the time between fetal death and delivery are listed, as well the histologic features of placenta that can be used as good predictors when timing intrauterine death.

## COMPARATIVE PATHOLOGY OF THE PLACENTA IN DOMESTIC ANIMALS

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Placentas are classified by their gross and histologic appearance. Regarding the macroscopic features the placenta can be discoid, cotyledonary, diffuse, and zonary. The histologic classification takes in consideration the intimacy of fetal-maternal contact classifying placentas in epitheliochorial, syndesmochorial, endotheliochorial, and hemochorial types, denominating the layers between fetal trophoblast and maternal endometrial surface. Some placentas (e.g. ruminants) may have some combination of these types of "invasion" and strict classification has often demanded ultrastructural studies for verification. In primates, hemo-monochorial, hemo-dichorial, and hemo-trichorial placental implantation have been identified, since ultrastructural examination revealed that, frequently, more than one layer of trophoblastic cells makes up the villous surface. In addition, the aspect of the development of the villi structure made necessary a different classification: folded, lamellar, trabecular, labyrinthine and villous. Insectivores have a discoid or diffuse, labyrinthine, hemochorial placenta. Chiropters are discoid, labyrinthine, endotheliochorial and occasionally hemochorial. In primates it varies: bidiscoid, labyrinthine endotheliochorial in *Tupaia*, diffuse, folded, epitheliochorial in *Galago*, bidiscoid, villous, hemochorial in Rhesus, discoid, villous, hemochorial in apes and humans. Lagomorphs are discoid, labyrinthine and hemochorial. In rodents the placenta is also variable: discoid, labyrinthine and hemochorial in guinea pigs, discoid labyrinthine and hemotrichorial in rats and mice and discoid, labyrinthine and hemodichorial in the beaver. Canids and felids have a zonary, labyrinthine endotheliochorial placenta. In artiodactyls (bovids, ovids caprids, cervids, suids) the placenta is diffuse, folded, epitheliochorial in suids, cotyledonary, villous, epithelio- and syndesmochorial in ovids, cotyledonary, villous and ep-

itheliochorial in bovids, caprids and cervids. Cervids have very few placentomes. Peryssodactyls (e.g. equids) are diffuse, villous, epitheliochorial and so are tylopods (e.g. camels, llamas, alpacas, vacunas). Sirenids (manatees) are zonary, labyrinthine and hemochorial, whereas cetaceans are diffuse, villous and epitheliochorial. Placentomes are the functional placental unit in artiodactyls comprised by the uterine caruncle and the chorionic cotyledon. The diffuse placenta of other species has microplacentomes (comprised by macrocotyledons and microcaruncles).

The lesions of the placenta can be frequently caused by infectious agents and are much less frequently congenital, toxic and very rarely neoplastic. Infectious agents can be viral, bacterial, protozoal and mycotic. Infectious agents can be species specific (many viruses) or may infect a few species (a few viruses, some bacteria and protozoa) and other agents target many species (many bacteria and fungi). Some protozoa (e.g. *Toxoplasma gondii*, *Neospora* spp. and *Sarcocystis* spp.) are able to cross the chorioendometrial barrier and colonize the fetus causing minimal placental and fetal damage guaranteeing in this way their vertical transmission. Although they may cause sporadic and epizootic abortions, this is not the rule. The investigation of placental and fetal pathology in carnivores is difficult and lack data because the dam promptly devours the placenta and often the fetus after the abortion.

Viruses known to be able to cross the placenta and to infect fetuses of domestic animals are classical swine fever pestivirus (suids), bovine virus diarrhea pestivirus (bovids and suids), border disease pestivirus (ovids), equine arteritis virus (equids and new world camelids), Japanese B encephalitis flavivirus (suids), Wesselbron disease flavivirus, Rift Valley fever phlebovirus (ovids

and other ruminants), feline panleukopenia parvovirus (felids), porcine parvovirus (suids), bovine parvovirus (bovids), minute parvovirus of canine (canids), bluetongue orbivirus (bovids and ovids), epizootic hemorrhagic disease orbivirus (bovids), Chuzan orbivirus (bovids), Akabane bunyavirus (bovids, ovids, caprids), Cache valley bunyavirus (ovids), Aino bunyavirus (bovids), Nairobi sheep disease bunyavirus (ovids), equine herpesvirus 1 and 4 (equids and new world camelids), canine herpesvirus 1 (canids), bovine herpesvirus 1 (bovids), bovine herpesvirus 5 (bovids), caprine herpesvirus 1 (caprids), suid herpesvirus 1 (suids), porcine cytomegalovirus (suids), rinderpest paramyxovirus (cattle), encephalomyocarditis picornavirus (suids). Abortigenic viruses in horses are equine herpesvirus 1 (EHV-1) and 4 and equine arteritis virus (EAV). In the pregnant viremic mares EHV-1 transported by leukocytes is able to colonize endothelia and epithelia thus invading progressively into endometrium, chorion and eventually the fetus. EHV-1 uses the chorion mainly as a bridge to reach the fetus and placental lesion are generally mild edema and vascular necrosis. In the fetus EHV-1 causes multifocal necrosis in lung, liver, adrenal gland and lymphoid organs. EHV-1 is also able to cause endometrial vasculitis and necrosis, which is also a feature of EAV. EAV seldom colonizes chorion and fetus; abortion is caused by its endometrial cytopathic effect. If it reaches the chorion EAV can be detected within the trophoblastic epithelium and chorionic mesenchymal cells. EHV-1 and EAV can also rarely cause abortion in new world camelids and these animals can be target of several equine and bovine pathogens. Abortigenic bovine viruses are bovine herpesvirus 1 (BHV-1) and bovine pestivirus (BVDV). BHV-1 produces, like many herpesviruses, the fetal lesions described for EHV-1 but in addition it constantly causes a florid chorionic vascular necrosis where the virus can be detected within endothelial cells and blood vessel wall. BVDV is a pantropic virus and is able to colonize all the chorionic and fetal cells. Chorionic lesions are mild and may include chorionitis and vascular necrosis. In small ruminants the ovine pestivirus is characterized by similar distribution and lesions. Suid herpesvirus 1, the etiologic agent of Aujeszky's disease (pseudorabies) in pigs, which is very neurotropic and can infect other species, is able to cause various degrees of necrotizing placentitis. Lesions are characterized by coagulative necrosis of the chorionic fossae with intranuclear acidophilic inclusions in trophoblast cells and occasionally in mesenchy-

mal cells. A mild inflammatory cell reaction was observed in the mesenchyma. Fetuses are affected by the typical herpesviral coagulative necrosis of liver, spleen, adrenal glands, and visceral lymph nodes with inclusions similar to those in the chorionic placenta. Porcine cytomegalovirus is able to cross the placenta and colonize the embryos, where it localizes in leptomeningeal cells, hepatic sinusoidal cells, peritoneal macrophages, periosteal cells and occasional alveolar cells; however the placenta is not a primary site of viral replication, like for other viral agents in many species. The same is true for porcine circovirus type 2 (PCV-2) and porcine arterivirus (PRRSV). PCV-2 infected sows may suffer abortion and premature farrowing. PCV-2 can be detected in lymph node, spleen, thymus, lung, tonsil and liver from both stillborn and liveborn piglets. The aborted fetuses of PRRSV infected sows may present with necrotizing umbilical arteritis with periarterial hemorrhage. The virus can be detected within the endothelial cells and macrophages of fetal membranes but especially in the fetal lung, lymphoid tissue and kidneys. African swine fever virus (ASFV) can be detected within the chorion of infected sows, and is associated with inconsistent lesions such as mild focal placentitis, fetal mild hepatic degeneration and necrosis, and mild interstitial pneumonia. These changes are not considered to be sufficiently specific to have diagnostic significance. In marked contrast to these changes in the fetal tissues, maternal tissues contain abundant virus. Specific diagnosis of abortion resulting from ASFV infection should, therefore, be based on examination of maternal tissues, rather than fetal tissues. The pregnancy failure seems to result from the effects of the virus infection on the dam more so than from direct viral damage to the placenta or fetus. Classical swine fever pestivirus crosses the placenta in viremic sows and colonizes the concepti where is able to produce various deleterious effect such as embryonic or fetal death, or persistent infections in case of early infections. In case of early to midgestation infections can cause malformations such as pulmonary hypoplasia, pulmonary artery malformation, micrognathia, arthrogryposis, and central nervous system malformations including cerebellar hypoplasia, microcephaly and defective myelination in brain and spinal cord. Infections during the last trimester may produce no abnormalities or may result in mummification and or stillbirths. BVDV is also able to reach the porcine fetuses by crossing the placenta. Porcine parvovirus infection causes reproductive failure in pigs and manifests as embryonic

death, mummification, stillbirth and reduced litter size. Lesions are most commonly observed in older fetuses and include nephritis, hepatitis, encephalitis and placental chorionitis with accumulation of mononuclear cells. In experimental infection canine herpesvirus 1 causes necrosis of the chorionic labyrinth in addition to the multifocal organ necrosis frequently observed in natural infection. Feline herpesvirus 1 is probably able to produce similar changes in cats. Pregnant cats acutely infected with feline immunodeficiency virus may transmit the virus to their offspring via both prenatal and postnatal routes. *In utero* transmission led to several pathogenic consequences including arrested fetal development, abortion, stillbirth, subnormal birth weights, and birth of viable, virus-infected, and asymptomatic but T cell-deficient kittens.

Numerous bacteria are able to cause endometritis and placentitis. The agents may be able to cross the placenta causing fetal septicemia or may exert their deleterious effect on endometrium and placental chorion causing lesions such as neutrophilic, lymphocytic and plasmacytic, necrotizing, necrosuppurative chorionitis, allanto-chorionitis, thrombosis, vasculitis, edema, trophoblast hyperplasia, squamous metaplasia, and mineralization. In certain cases even in absence of fetal septicemia the chorion is so compromised that the endometrial nourishment is unable to reach the fetus and abortion occurs. Well described causes of bacterial placentitis in ruminants are *Arcanobacterium pyogenes*, *Brucella* spp., *Campylobacter* spp., *Chlamydophila* spp., *Listeria monocytogenes*, *Salmonella* spp., *Coxiella burnetii*, *Escherichia coli*, *Leptospira interrogans*, *Bacillus* spp., *Bacteroides* spp., *Deltaproteobacterium* spp., *Ureaplasma* spp, and *Mycoplasma* spp. *Brucella* and *campylobacter* induce the formation of a placental exudate grossly similar so "soft caramel candy", *coxiella*'s chorionic exudate is chalky; the *chlamydophila* infected placenta may have a leather-like appearance. Many of these agents cause very similar gross and histologic lesions and the final diagnosis will be supported by ancillary procedures performed of chorionic and fetal tissue. Many of these agents are dangerous zoonotic agents. Causes of bacterial placentitis in equids are *Streptococcus equi zooepidemicus* and *equi*, *Escherichia coli*, *Leptospira interrogans gryppotyphosa* and *kennewicki*, *Crossiella equi*. Regarding some suggestive gross lesions, *Streptococcus* causes and ascending cervical star chorionitis characterized by thickening of the fetal membrane and similar to mycotic placentitis, and the nocardiform *crossiella* causes a large area of placentitis on the placental

body with a characteristic sticky brown exudate. It is proposed that the Mare Reproductive Loss Syndrome observed in Kentucky USA, which includes early fetal loss, late fetal loss, uveitis, pericarditis, and encephalitis, is associated with tissue penetration by septic barbed setal fragments (septic penetrating setae) from Eastern tent caterpillars (*Malacosoma americanum*). Once ingested, these barbed setal fragments migrate through moving tissues, followed by rapid hematogenous spread of bacteria, bacterial emboli, and/or septic fragments of setae (septic penetrating setal emboli). Aborted fetuses present with funisitis. Bacteria that may cause endometritis, placentitis, fetal septicemia and abortion in pigs are *Leptospira interrogans*, *Brucella suis*, *Erysipelothrix rhusiopathiae*, *Campylobacter* spp. *Chlamydophila*, *Eperythrozoon suis*, *Escherichia coli*, *Streptococcus suis*, *Staphylococcus*, *Arcobacter* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*, *Pasteurella multocida*. *Brucella canis*, *Campylobacter* spp., *Pasteurella multocida*, beta-hemolytic *Streptococcus* spp. group G, and *Escherichia coli* can be responsible of endometritis and pregnancy failure and perhaps placentitis in the bitch.

Protozoa that are vertically transmitted and able to colonize placenta and fetus are *Toxoplasma gondii*, *Neospora caninum* and *Sarcocystis* spp. Equids are not susceptible to these agents but the majority of the other species are, especially ruminants. Chorionic cotyledonary lesions of toxoplasma in small ruminants are highly suggestive and consist of multiple white foci which are the results of necrosis and adhesion of the villi. Histologically it is possible to detect chorionitis and recognize the agents with ancillary procedures. *Leishmania* spp. colonizes the canine placenta causing necrotizing chorionitis with numerous *Leishmania* spp amastigotes withing the trophoblasts. Experimental infection of *Neospora* spp. in a queen revealed severe necrotizing placentitis, metritis, hepatitis, and nephritis and chorion necrosis and mineralization in queens experimentally infected with *Toxoplasma gondii*.

Mycetes can cause placentitis and abortion especially in cattle and equids. In cattle the placental gross lesions are highly suggestive with cupping of the cotyledons, in horses the cervical star may be rather thickened; histologically it is possible to detect the fungal hyphae or pseudohyphae within the necrosuppurative inflammation using conventional or special stains. In addition to the relatively common *Aspergillus*, other causes of mycotic abortion include *Mucor* spp., *Histoplasma*

*capsulatum*, and *Candida spp.*, whereas *Allescheria boydii* and *Coccidioides immitis* are rare.

Fescue toxicosis is a cause of perinatal death of foals. It is associated with the endophyte *Neotyphodium spp.*. In these cases fetal membranes are congested and very edematous; histologically the chorionic mesenchyma is expanded and basophilic.

In case of torsion of the umbilical cord of the equine fetus, which is predisposed by excessive length, the placenta may be edematous with fibrin thrombi and mineralization of the microcotyledonal capillaries. The same changes may be observed in case of equine fetus cardiac anomalies and secondary systemic passive congestion. Adventitial placentation in cattle is the development of intercotyledonary placentation as a mechanism of compensation for inadequate development of placentomes. Hydramnios and hydrallantois are excessive accumulation of fluid in the amniotic and allantoic sacs. They occur most often in cattle and are rare in other species. Hydramnios (hydrops of the amnion) is usually associated with inherited or acquired malformations of the fetus. Hydrallantois in cattle is most often associated

with uterine disease with inadequate numbers of caruncles and the development of adventitial placentation. The disease occurs rarely in mares. In sheep it can be associated with Rift Valley fever and Wesselbron's disease viral infections. Adenomatous hyperplasia-dysplasia of the equine allantois is a mass due to an adenomatoid proliferation of the allantois under the influence of stimuli such as placentitis and unknown stimuli. Amniotic plaques, placental mineralization and avascular chorion are changes of little significance due to unknown and should not be confuse with placentitis.

Rarely the equine placenta is affected by neoplasms such as multiple fibromas, teratoma, teratocarcinoma, lymphoma, metastatic hepatoblastoma. Trophoblastic moles are extremely rare in animals.

The placenta is an important organ for the diagnosis of abortion and endometrial diseases. The evaluation of the placenta should include a detailed gross and histologic examination of all the fetal membranes and if necessary the use of ancillary procedures such as histochemistry, immunohistochemistry, conventional and molecular microbiology, mycology and virology and toxicology.

## FOREFRONTS OF VETERINARY MEDICAL EDUCATION: TEACHING PATHOLOGY IN PROBLEM BASED LEARNING (PBL)-CURRICULUM

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One of the missions of the College of Veterinary Medicine at the Western University of Health Sciences is to serve the society and animals by preparing the students for the practice of veterinary medicine, veterinary public health, and /or veterinary research through the commitment to student-centered, life-long learning. The unique learning environment of Problem Based Learning (PBL) in veterinary education presents an education challenge for a Pathology Content Expert. Along with the concept of “one medicine” there is a strong emphasis on the interdisciplinary approach and comparative medicine/pathology. Pathology is well represented in pre-clinical and clinical phases of the curriculum. In the phase I, all of the clinical cases used in the “Veterinary Basic Sciences” part of the curriculum contain clinical pathology and/or gross/histopathology data. This phase is supported by the visual pathology learning materials, pathology expert’s Power Point (PPT) presentations and contributions to discussions at the weekly Grand Rounds, weekly Path Quizzes, interac-

tive PPT programs and wet labs (“MDCologies”). Clinical Skills Course also provides a niche for a simulated practice of pathology skills through a set of exercises called “Pathology psychomotor skills”. Pathology in the Clinical years of the curriculum is structured through the strategic partnerships and alliances that involve animal shelter rotations, diagnostic services, medical research organizations, field necropsies, on-campus necropsies, and Zoo rotations. Educational efforts are constantly monitored by the classroom assessment techniques that also provide basis for the improvement of the curriculum to meet student needs and enhance learning.

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## POLYMYALGIA RHEUMATICA

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The term polymyalgia rheumatica (PMR) is a well-defined syndrome occurring in individuals older than 50 and most over 65 years and consisting of pain and morning stiffness in the proximal muscles. The very high erythrocyte sedimentation rate (ESR) is a key to diagnosis, often being found to reach up to 100 mm/h (Westergren method). Bilateral pain and stiffness are noted in the neck and back as well as in the shoulder girdle and may radiate into the gluteal muscles and thighs.<sup>1</sup>

We examined electron-microscopically the skeletal musculature of 21 patients with clinically confirmed PMR and classified the ultrastructural changes on the basis of 15 criteria.<sup>2</sup> These criteria include changes in nuclei, myofilaments, mitochondria, and in the T-system, furthermore glycogen depositions as well as lipid, lipofuscin, and myelin figures. The functionally most important ultrastructural change concerns the mitochondria (in the form of crystal depositions, deformations, new formation, accumulation).

The changes found in muscles are focal, unspecific, and regressive in nature. The systemic assessment of all criteria results in a conspicuous accumulation of certain features: a constellation of characteristics, by which the ultrastructural picture of PMR obtains a specific profile.

Giant cell arteritis (GCA) affects medium and large muscular arteries with a well-developed internal and ex-

ternal elastic lamina. The affliction is segmental and multilocal, and it preferentially affects the temporal artery and other branches of the carotid artery. The special morphological sign of GCA is the formation of multinuclear giant cells.

The connection between PMR and GCA seems to be close. There are varied clinical reports about the coincidence of both phenomena. But it has to be considered that they have progress curves, which do not have to coincide with time. We ourselves have the impression that the burnt-out arterial process can escape observation if the minimal traces it leaves behind are overlooked and misinterpreted, respectively.

The question whether there is any association between PMR and GCA or not in literature still remains open. But in our bioptic material (1991–2006) from 261 clinically as well as pathologically confirmed PMR patients we can demonstrate a collateral consisting GCA in 46 cases (17,62%).

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