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Immunodeficiencies and immune-mediated disorders in foals

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1. Immune system in general.

I would like to discuss the development of the immune system, common immune deficiencies and immune-mediated disorders of the neonatal foal.

The immune system is a defence system that protects against pathogenic microorganisms whose aim is to invade the body. It is a complex system containing links and responses which together construct an efficient pathogenic defence. Its intricacy allows it to recognise the difference between foreign and self cells, to retain memory towards previous pathogenic agents and to suppress itself when not required. A good immune system has to be maintained. It is dependent on many effects such as; age of the animal, environment, nutrition and exposure to infectious agents. Young, old, stressed or sick animals are generally more susceptible to infectious agents as they are predisposed to a weak and/or damaged immune system. The two main lines of defence, contributing to mammalian immunity, are the innate and acquired immune systems. These two systems work through a series of steps and are linked by the dendritic cells (DC) which are also called antigen presenting cells (APC). I will discuss each part separately.

1.1 Innate Immunity

The innate immune system is the first line of immune defence. It is not specific, meaning it doesn't recognise a certain type of foreign antigen or produce a specific defence towards it but instead forms a physical and chemical barrier against all types of penetrating pathogenic microorganisms. Its barrier defence is comprised of epithelial layers covering body surfaces and body entrances where pathogens generally attack. These layers are also covered with antimicrobial secretions such as lysozymes, phospholipase, antimicrobial peptides and surfactants which further protect the body. The main cells that target non-specific pathogens, which enter the body through trauma and wounds, are the neutrophils, macrophages, dendritic cells, mast cells and natural killer (NK) cells. These cells are activated and attracted to signals released by cell-derived mediators, such as cytokines and chemokines which damaged tissue liberate as part of an inflammatory response. This defence unlike the adaptive immunity has no memory and is relatively weak especially if the pathogenic microorganism is resolute. (Day & Schultz, 2011a).

1.2 Adaptive immunity

The adaptive immune system is a far more complex arrangement. Unlike the innate system it produces a specific defence against invading pathogens. Its process is much slower, when compared to the innate immunity, but it is more vigorous and longer lasting. Its main function lies in its production of antibodies, in the case of B-lymphocytes, and its synthesis of subset-specific mediators, in the case of T-lymphocytes, which both orchestrate enhanced specificity in an immune response. The B- and T-lymphocytes develop in the bone marrow and the thymus and can be activated in secondary lymphoid tissues such as the spleen, lymph nodes and mucosal lymphoid tissues. They are activated via antigen-recognition (with or without the help of antigen-presenting cells in the case of T and B cells, respectively) in an inflammatory environment which provides the appropriate cytokine- and chemokine-milieu to enhance their activation. The adaptive immune response takes over from the innate system at approximately 4-7 days post-infection. Its chief trigger comes from dendritic cells, which first transports the pathogenic antigen to the regional lymphoid tissue and then activates the antigen specific T-lymphocyte by presenting small peptides in complex with their own MHC molecules. At the same time the B-lymphocytes are activated by soluble antigens. These activated lymphocytes are then mobilized from the lymphoid tissue and begin to circulate in the blood to reach the site of infection (Day & Schultz, 2011a)

1.2.1 T-lymphocytes

T-lymphocytes originate from the bone marrow. They leave here and travel in the blood to the thymus where they mature. Before they can leave the thymus they must have the ability to express the TCR-complex (T cell receptor) which consists of two chains responsible for mediating the contact with peptide-MHC complexes (designated α and β or γ and δ chains) and a signalling structure which is called CD3. The TCR on the T-lymphocyte has to interact with the peptide antigen presented to it together with the MHC molecules expressed by antigen-presenting cells. The cells that interact with the MHC-I molecule will become $CD8^+$ and those that interact with the MHC-II molecule become $CD4^+$ cells. $CD4^+$ cells are also called T-helper cells and $CD8^+$ cells are called cytotoxic T-cells. After leaving the thymus the T-lymphocytes reside in the lymph nodes of the body

until the antigen presenting dendritic cells carry a pathogenic antigen to the node for presentation in complex with their MHC molecules. This process along with cytokine release from the APC allows for activation of the T lymphocytes. The T cells replicate and become effector T-cells and memory T-cells, and their progeny will express the same antigen receptor specific to the antigen of the invader. The two main T-helper cell subsets' (Th1 and Th2) function by helping with humoral and cell mediated immunity. Th1 cells produce mainly interleukin-2 (IL-2) and interferon-gamma (IFN γ). They facilitate more with a cell mediated immune response rather than a humoral one. The Th2 cells produce mostly IL-4, IL-5, and IL-13 and have a big impact in assisting the humoral immunity by aiding B-cell differentiation and production of specific immunoglobulin subclasses, such as; IgG, IgA and IgE (Day & Schultz, 2011a). Subsequently these helper cells have been noted for their antagonistic nature "IFN γ produced by the Th1 cells is inhibitory of the function of the Th2 cell and the IL-4 and IL-13 produced by the Th2 cells suppresses the action of Th1 cells." (Day & Schultz, 2011b).

1.2.2 B-lymphocytes

B-lymphocytes also stem from the haematopoietic stem cells. They mature in the bone marrow and spleen. They, the B-cells, have IgM monomers on their surfaces and are so-called B-cell receptors (BCR). The initial signal for activation occurs when the BCR recognises the antigenic epitope which reaches the B-cell zone of lymph nodes via the lymph. The next step is through interaction with antigen specific Th-cells. Such an interaction occurs when the B-cells present the recognised antigens associated with their MHC-II molecules to T-cells with the same specificity, but this also requires the ligation of the B cell surface molecule CD40 with the CD40-ligand (CD40L) expressed on the surface of Th-cells, as well as some other cytokines released by neighbouring cells. After these steps the B-cells are activated and become B-lymphoblasts, which proliferate intensively in the central zone of the lymphoid follicles, in the so-called the germinal center. Meanwhile immunoglobulin class switch occurs, which changes the original IgM- and IgD-type surface receptors to IgA, IgG or IgE. Proliferating B cells can either differentiate into plasma cells or become memory B cells. The plasma cells "will synthesize and secrete immunoglobulins of the same antigen specificity as the parent cell and of the same immunoglobulin class determined by class switching" (Day & Schultz, 2011c).

1.3 Immune-deficiencies in general

The immune system has evolved to become so complicated and advanced that any blemish in its development or performance can leave an individual defenceless against pathogenic agents. This inability to produce an adequate immune defence is called an immunodeficiency. There are many factors which contribute to immunodeficiency. These factors have been classified into primary or secondary origin. Primary immune-deficiencies have a genetic cause. They are acquired through inherited defective genes or individual genetic mutations. Which immune cell is affected depends on the location of the mutation (Tizard, 2013a). Defects can be localised on humoral immune cells, cell mediated immune cells, phagocytes (neutrophils, monocytes) or complement factors (McKinnon et al., 2011a). Recognising these inherited immune-deficiencies in neonatal foals is not easy. Neonates acquire maternal immunoglobulins, through ingested colostrum, which provides them with a passive immunity and for that reason clinical signs may go unnoticed for a couple of months. It may be observed, however, that these foals don't grow or thrive as well as normal foals and after passive maternal antibodies subside then clinical presentations such as repeated infection and medication unresponsiveness may highlight the fact that an underlying immunodeficiency is responsible.

Secondary immune-deficiencies are a consequence of many different factors such as neoplastic tumours, stress, malnutrition, immunosuppressive medication, endocrine disturbances and, certainly the most important cause of secondary immunodeficiency in neonatal foals is the failure of passive transfer (FPT) (McKinnon et al., 2011a; Crisman & Scarratt, 2008). These immunodeficiencies are not as serious as primary immunodeficiencies and can be treated with good prognosis, they may even self limit.

2. The prenatal immune system.

The prenatal foetus develops an immune system in utero without antigenic stimulation. Its immune progression throughout gestation, although naive, is essential for its subsequent survival post-partum. The primary lymphoid organs; the bone marrow and thymus are the first immune organs to develop. Haematopoietic cells in these organs generate lymphocytes, both B and T cells. The B cells mature here but the T lymphocytes travel to the thymus and mature there. Secondary immune organs such as the spleen, lymph nodes, tonsil, Peyers patches and associated lymphoid tissues (mucosal, bronchial and gut) develop later on in gestation (Tizard, 2013b).

The time frame for immune development depends on the length of gestation. In equine, the gestation period is approximately 336 days. Lymphocytes in the foals thymus, mesenteric and intestinal propria can be seen at approximately 60-80 days gestation, in the blood at approximately 120 days and the spleen at 175 days. Immunoglobulin such as IgM and IgG are present much later, approximately 240 days, in gestation (Tizard, 2013b).

The innate immune system of the perinate develops but it is deficient in some areas due to the fact that there is no pathogenic antigen stimulus in the uterus. The innate systems main defence is through phagocytosis, and cytokine release. Amplification and processing of pathogens to phagocytes occurs through complementation and opsonisation. Phagocytosis and bactericidal effects such as oxidative burst are the main means by which the neutrophils and macrophages (phagocytes) function (McKinnon et al., 2011b). According to Tizard (2013b) their functional activity is similar to adults. Cytokines which are produced from the phagocytes and epithelial cells as a defence and signalling mechanism are functional in utero. The components of the innate system that do not function fully are the complement and opsonisation components. These two mechanisms work by presenting pathogens to phagocytes and without them phagocyte capacity decreases, therefore the first line of defence the innate system decreases (McKinnon et al., 2011b).

As already stated, the immune organs start to develop at certain stages during gestation. These organs start to produce immune cells as part of the adaptive immune system. Studies have found that prenatal foals have a predisposition toward Th2 immune responses. Th2 cells promote humoral responses. The overridden cells, the Th1 cells support cellular immune responses. Therefore, as part of this prejudice, the foetus is born

with immunity sided toward humoral. This mechanism has been considered incidental because the Th1 cells have been known to cause damage to the placenta. Thus, the placenta acting as a protective structure, by ascertaining this immune imbalance, produces cytokine IL-4 and IL-10, progesterone and PGE₂ which together function to inhibit Th1 immune response (Tizard, 2013b; Morein et al., 2002).

The co- stimulation of, not only the innate and adaptive immune systems but also the humoral and cellular immune systems is provided by antigen presenting cells (APC) also known as dendritic cells and macrophages. This association between systems is reduced because the foetus in utero is unchallenged and its immunity is untrained. The dendritic cells (DC) therefore have a decreased ability to express important ligands such as CD86, CD40 and lymphocytic ligand CDL40. These ligands are normally needed to increase the lymphocyte clonal production after stimulation by T cells from APC signalling (McKinnon et al., 2011b).

Probably the most important and interesting immune development in the prenatal foal is its immunoglobulin production. Immunoglobulins are structurally varied. This variability is necessary to accommodate for any possible pathogenic agent the foetus may encounter post-partum. Variation occurs during B cell development, before any antigenic encounter, in utero. It occurs on a genetic level forming various recombinations and on an immunoglobulin cell level causing junctional diversity, on the heavy and light chains. This important mechanism allows for a couple of billion different immunoglobulins to develop and thus adds great immune diversity to the developing foals' immune system (McKinnon et al., 2011b).

In order to identify whether or not the foetal immune system is functional in utero vaccines have been injected and pre-serum immunoglobulin levels tested. A vaccination at about 200 days gestation and an examination of pre-suckle IgM and IgG serum concentration postpartum determines this function. Genetically inherited immune-deficient foals cannot mount immunoglobulin production and therefore IgM and IgG levels in their pre-suckle serum are absent. This proves that the prenatal immune system while immature is functional and can react, intra utero, to antigenic triggers (Tizard, 2013b; McKinnon et al., 2011b).

3. The placenta; general functions, formation.

The placenta is a multifunctional temporary organ which provides a connection between maternal and foetal systems. Its functions are to deliver nutrients and gases to the developing foetus, to allow for the removal of metabolic waste products from the foetus to the mother, to act as a temporary endocrine organ by secreting growth factors and steroids necessary for maintaining pregnancy and to provide a barrier which protects the foetus from the maternal immune system and also from invading pathogenic microorganisms (McKinnon et al., 2011c).

The placenta is formed from foetal membranes which start to develop soon after fertilization. These membranes develop from embryonic germ cells and engulf the developing embryo. These membranes form cavities. The amniotic cavity is fluid filled and cradles the foetus throughout gestation. It has shock absorber properties. The allantoic cavity is a protuberance from the embryonic hindgut. It collects the foetal waste material. These cavity membranes fuse with the outermost membrane, the chorion, to form the foetal placenta. The chorionic membrane, composed of chorionic epithelium (trophoblast cells), fetal connective tissue and fetal endothelium (vasculature), is an important part of the foetal placenta. In fact it is the trophoblast cells of this membrane that invade the maternal tissues to form the foeto-maternal placental connection (McKinnon et al., 2011c; Borghesi et al., 2014).

3.1. Placental types

There are several different placenta types which are species specific. They are categorized based on which endometrial layer (maternal endothelium (vasculature), uterine connective tissue and uterine epithelium) the foetal trophoblast cells invade, erode and permanently appose.

The types of placenta and related species are: (1) epitheliochorial found in equine and swine, (2) endotheliochorial found in cats and dogs, (3) synepitheliochorial found in ruminants and (4) haemochorial found in humans and primates (McKinnon et al., 2011c; Borghesi et al., 2014; Day & Schultz, 2011d).

There are species differences in relation to the shape of placenta and amount of foetal-maternal apposition also. It is termed diffuse in equine and swine, zonary in dog and cat, caruncular in ruminants and discoid in humans and primates (McKinnon et al., 2011c).

3.2 The equine placenta

The equine placenta is diffuse epitheliochorial. Diffuse means that the foetal placenta apposes the entire maternal endometrium except for a few parts; the cervix forming the cervical star and the bifurcation of the uterine horns (McKinnon et al., 2011c). Epitheliochorial means that the chorionic and maternal epitheliums are apposed and there is no erosion from the maternal side (McKinnon et al., 2011c; Borghesi et al., 2014).

To briefly compare this placenta type to the others I have listed we can see that the endotheliochorial placenta, found in dogs and cats, has a more invasive character. During this placental attachment two maternal layers are lost; the uterine epithelium and connective tissue. Therefore placental attachment is formed by the chorionic epithelium and the maternal endothelium. The synepitheliochorial placenta, found in ruminants, is less invasive. There is only one maternal layer missing; the uterine epithelium. In this case the chorionic epithelium apposes uterine connective tissue. The haemochorial placenta, found in humans has the most invasiveness character. In this placenta all maternal layers are lost and the chorionic epithelium apposes the maternal circulation directly (Borghesi et al., 2014; Day & Schultz, 2011d). From this placenta type comparison it is sure that the haemochorial placenta has the most intimate connection with the maternal side. Equine and swine have the least intimate connection as all layers are intact and there is no direct contact with maternal blood. Conversely this does not mean that there is less efficient transfer of nutrients. In fact, it has been established that the equine placenta has the greatest nutrient exchange compared to other placenta types (McKinnon et al., 2011c).

3.3 Placental immunoglobulin transfer

The transfer of maternal immunoglobulins in utero is however affected by the type of placenta. Immunoglobulins are antibodies. They are macromolecules of a glycoprotein nature and they are found in the globulin portion of blood serum. There are 5 different immunoglobulins IgG, IgM, IgA, IgE, and IgD. They help trigger immune responses in the body and are responsible for neutralizing and destroying foreign microorganisms. IgG and

IgM are found in the highest and second highest concentrations in the body, respectively. IgG is the smallest in size and it can cross capillaries easily. It is therefore the only one that can pass through the placenta and into the foetus (Borghesi et al., 2014; Tizard, 2013c).

Out of the four placenta types mentioned only the haemochorial placenta can allow full transfer of IgG. These newborns have serum level similar to adult serum levels (Day & Schultz, 2011d). The endotheliochorial placenta, in cats and dogs, allows partial transfer (about 5-10%) of maternal IgG to the foetus (Day & Schultz, 2011d; Tizard, 2013d). Equine, swine and ruminants have no maternal IgG placental transfer because the chorionic epithelium has no direct apposition with maternal circulation and IgG is too big to pass through the layers. These species rely on the passive transfer of immunoglobulins. This passive transfer can be acquired through ingestion of the dam's colostrum after birth (Borghesi et al., 2014; Tizard, 2013d).

How do we know that there is no maternal antibody transfer in utero? Well, a study conducted on Combined Immune-deficient (CID) foals gives evidence that placental transfer of maternal antibodies is negligible (McGuire, 1981). CID foals, which I will detail in a later chapter, are born with a genetically defective immune system. They cannot synthesize B and T lymphocytes and therefore have no circulating immune cells in their blood serum (Crisman & Scarratt, 2008; McGuire, 1981). The immune defective foals pre-suckle serum was compared to normal foals pre-suckle serum. On examination of their serum it was found that they had no serum immunoglobulin whereas the normal foals' had (McKinnon et al., 2011b). Normal foals have a developed immune system in utero and can synthesize immunoglobulins as discussed in the previous chapter. The fact that inherent immune-deficient foals had no serum immunoglobulins proves that maternal transfer of immunoglobulins in utero is non-existent.

How does the foetus protect itself in utero if there is no maternal transfer of immunoglobulin? As previously discussed it has been recognised that the prenatal foal does develop an immune system. It has also been acknowledged that the foetus has the ability to synthesize specific immunoglobulins after in utero injection of antigens (McGuire, 1981). This proves that it is not defenceless in utero. It is however extremely immune sensitive after birth. The uterus is sterile but the environment is contaminated. The neonatal foal needs the mares' colostrum to provide itself with additional protection against challenging environmental pathogens (Borghesi et al., 2014; Tizard, 2013c).

4. Post-partum immune development and colostral transfer.

4.1 Post-partum foal; Immune situation

According to the prenatal period the fetus has an immune system that favours Th2 response. This has been stated by Tizard (2013b) and Morein et al. (2002) as a protective mechanism to prevent placental damage. However according to recent literature (Perkins & Wagner, 2015) it has been established that the neonate has in fact a Th1 bias (Perkins & Wagner, 2015). This study explains that, by measuring the actual intercellular IL-4 and INF γ in T cell subsets and not just the cytokine measurement in peripheral blood mononuclear cells, INF γ and cytotoxic T cells can be detected just days after birth. They also state that the Th2 cells producing IL-4 is essentially absent for the first few months of life. This conveys to us that the neonate can mount cellular responses and is actually more deficient in humoral responses (Perkins & Wagner, 2015). According to Breathnach (2006), which states foals are INF γ deficient at birth, under suitable conditions T cells can produce INF γ but it is the gene expression and protein synthesis of INF γ from lymphocytes that is not at adults levels so understandably there is increased susceptibility to intracellular bacteria such as *Rhodococcus equi* and certain viruses and consequently INF γ is more or less deficient in the postnatal foal (Breathnach et al., 2006).

This so called deficiency is not affected by colostrum intake either as production can begin as early as 36 hours and increase steadily until 1 year of age (Breathnach et al., 2006). Another factor involving the production and activation of Th1 responses is the amount of signalling by APCs. The more exposure the APC gets from environmental antigens the more active the cellular responses (Sun, 2011).

Perkins and Wagner (2015) state that many literature studies have discrepancies based on their cellular immune response facts because they include studies that have been performed on humans and mice instead of foals. In spite of this, actuality remains in that even though the cellular immune response is active in the post-partum neonate it is still immature up until one year of age and intracellular pathogens and viruses still pose a danger for neonates (Perkins & Wagner, 2015; Breathnach et al., 2006).

4.2 Protection via colostrum intake and absorption

From the previous chapters we know that the neonate is born with a functionally immature immune system. There are no maternal antibodies passed through the placenta and thus the fetus is born relatively immune deficient. There are small levels of IgG1 and IgM in its pre-suckle serum but this is not enough for protection in a pathogen full environment (Perkins & Wagner, 2015). In order to obtain immune protection the neonate must ingest the mares' colostrum. Colostrum is produced by the mare in the last 2 weeks of gestation. It is composed of nutrients and immune components such as immunoglobulins, cytokines and lymphocytes. Colostral immune components mirror the dams own antigenic exposure to her environment and intake is vital for obtaining protection against microorganisms and for stimulating development of the foals own immune system (Tizard, 2013d; Perkins & Wagner, 2015).

The largest immune component of colostrum is immunoglobulins. Of the five immunoglobulins IgG and IgA predominate. There are also quantities of IgE and IgM but to a lesser extent. There are 7 subclasses of IgG in the horse, IgG1-7. Studies have concentrated on these subclasses to get a better understanding of the protection levels acquired from colostrum (Tizard, 2013d). Colostrum immunoglobulin levels emulate the mares serum immunoglobulin levels (Perkins & Wagner, 2015). Previous nomenclature classified IgG1-7 into just IgG 1-4 also called IgGa, IgGc, IgG (T) and IgGb respectively. These immunoglobulin subclasses have different properties and functions and their concentration in serum differs (Tizard, 2013d).

Lymphocytes and cytokines such as IL-6 and tumour necrosis factor (TNF α) are also present in colostrum. They are important because they encourage B cell and antibody initiation and production in the foal as well as providing a cellular immune defence (Tizard, 2013d; Perkins & Wagner, 2015; Porto, 2014).

From **Table 1.** we can see that IgG dominates in the colostrum of mares and other non-ruminant species but IgA dominates in the mares' milk. In ruminants, IgG dominates in both the colostrum and milk.

Table 1: Immunoglobulin levels in colostrum and milk in domestic animals. (Data are derived from Ian R. Tizard (2013d): Veterinary Immunology 9th edition, 2013)

Species	Fluid	IgA (mg/dl)	IgM (mg/dl)	IgG (mg/dl)
Horse	Colostrum	500-1500	100-350	1500-5000
	Milk	50-100	5-10	20-50
Cow	Colostrum	100-700	300-1300	2400-8000
	Milk	10-50	10-20	50-750
Sow	Colostrum	950-1050	250-320	3000-7000
Dog	Colostrum	500-2200	14-57	120-3250
	Milk	110-620	10-54	1-3
Cat	Colostrum	150-340	47-58	4400-3250
	Milk	240-620	0	100-440

Absorption of colostrum and milk in neonatal foals is through the intestine. The intestine is made up of epithelial cell tight junctions, immune tissue and various immune cells (Vendrig & Fink- Gremmels, 2012). The post-partum neonate, however, has an intestinal epithelial structure that is unrestricting. The reason for this is to allow huge quantities of colostrum cells to reach the neonatal blood stream and provide fast immunity. Also present in the intestinal wall are neonatal Fc receptors (FcRn) (Tizard, 2013d; Cervenak & Kacskovics, 2009). The receptors bind immunoglobulin present in the intestine and also prevent their destruction (Cervenak & Kacskovics, 2009).

The permeability of the intestine and duration of the receptors is limited and in order for the neonate to achieve colostrums best effects it needs to be ingested within the first 6-12 hours after birth (Tizard, 2013d). In equine and swine, immunoglobulins are selective. IgG's and IgM's are selected and forward to the blood stream and IgA's are left in the gut (Tizard, 2013d). IgA becomes part of the intestinal luminal defence (Vendrig & Fink- Gremmels, 2012). In ruminants however there is no selectivity and all immunoglobulins are absorbed (Tizard, 2013d). This effect may lead to question the actual effect of FcRn's in the intestine since all immunoglobulins are absorbed and within a short period of time (Cervenak & Kacskovics, 2009).

4.3 Neonatal endogenous immunoglobulin production

Although maternal antibody protection is vital, it's also well known that maternal immunity prevents or slows the neonates own immune development and response to pathogens (Perkins & Wagner, 2015; Holznagel et al., 2003). Before approximately 3 days the neonatal intestinal barrier closes to antibody macromolecules and thus in turn the mares' colostrum turns to milk. There are fewer immunoglobulins and no cytokines in milk but intake is still important for nutrition and immune protection (Tizard, 2013d).

The endogenous production of certain IgG subtypes is relatively the same when comparing different literature studies (McKinnon et al., 2011b; Perkins & Wagner, 2015; Holznagel et al., 2003). IgG1 is present before birth and reaches levels higher than adults within 2-3 months (Perkins & Wagner, 2015). IgG3, IgG5 and IgA increases just after birth and reaches relatively stable levels at approximately 5-8 weeks. IgG4 and IgG7 which are equivalent to IgGb are the most abundant IgG in adult serum (McKinnon et al., 2011b; Perkins & Wagner, 2015; Holznagel et al., 2003). In the neonate however there is slow onset of IgGb and production has been observed around 16-20 weeks. It continues to rise until 1 year of age but levels are below adult levels prior to that age (Perkins & Wagner, 2015; Holznagel et al., 2003). From this we can see that endogenous production is relatively insignificant before 4 weeks of age and endogenous production starts at about 5 weeks.

Before 4 weeks however the foal is protected by maternal antibodies, through colostrum intake, and the half lives of certain antibodies can last 18, 21 and 32 days for IgG1, IgG3/IgG5 and IgG4/IgG7 respectively (McKinnon et al., 2011b; Perkins & Wagner, 2015). After this time protection in the milk diminishes and because endogenous immune production is not fully developed the foal can become susceptible to certain diseases at this time. This delayed immunoglobulin commencement is possibly due to maternal antibodies asserting a non specific immunosuppressive effect on the neonatal foals' immune system. Other factors causing the same effect maybe the long half lives of antibodies or just the inherently slow onset of antibody production (Perkins & Wagner, 2015; Holznagel et al., 2003).

5. Secondary Immune-deficiencies in Foals.

5.1 Failure of passive transfer (FPT)

Failure of passive transfer is the failure of maternal antibody protection through colostrum. It is the most common immunodeficiency affecting ~25% of neonatal foals (Baird et al., 1987) and thus the main foundation for septicaemia in neonatal foals. The reasons behind the need for passive immunity have already been outlined in the previous chapters. Adequate passive transfer is defined as an IgG serum concentration greater than 800mg/dl. Failure occurs when levels are below 400mg/dl. Levels between 400 and 800mg/dl are classified as partial failure (Crisman & Scarratt, 2008; Tizard, 2013d; Baird et al., 1987; Giguere & Polkes, 2005; Hurcombe et al., 2012; McKinnon et al., 2011d).

Pathogenesis:

The failure of passive transfer is a consequence of inadequate colostrum intake, absorption or poor antibody concentration in the colostrum after birth. Other important causes of FPT are the poor management (stress factors, inadequate disinfection of the navel) and hygiene levels of the surrounding environment (pathogenic virulence load) (Crisman & Scarratt, 2008; Tizard, 2013d; Baird et al., 1987; Giguere & Polkes, 2005). Certain issues disabling the foals suckle after birth and thus causing inadequate intake are prematurity (weakness), musculoskeletal disorders (fractured bones, tendon laxity), mares' rejection, illness, damaged or dirty teats, etc...

Inadequate intestinal absorption is frequently due to late colostrum intake. The neonate requires greater than 800mg/dl colostrum within the first 6-12 hours for adequate protection. Absorption decreases gradually after 12 hours and very little is absorbed from 18-24 hours after birth. The poor quality and concentration of IgG in colostrum can be caused by premature lactation, twins' births, illness, placentitis or uterine infection, stressful environment, caesarean section etc... (Crisman & Scarratt, 2008; Tizard, 2013d; Baird et al., 1987; Giguere & Polkes, 2005)

Diagnosis:

By measuring antibody levels in the foals' serum, post-partum, FPT can be diagnosed. The ideal time to measure serum levels is 18-24 hours after birth. There are

several methods/tests used. The most quantitative and accurate diagnostic test is the Single Radio-immunodiffusion test (SRID); It measures the precipitation reaction between the antibody, in foals serum, and antigen, on agar gel plates. However, this test is very impractical because it takes 18-24 hours before a result can be reached. Quick diagnosis and treatment is vital for FPT foals (Crisman & Scarratt, 2008; Tizard, 2013d; Baird et al., 1987; Giguere & Polkes, 2005).

Other cheaper and quicker tests are often preferred, these are: (1) Zinc sulphate turbidity test, (2) Glutaraldehyde test, (3) Latex agglutination, (4) ELISA, (5) TPP (total plasma protein) and serum globulin (Crisman & Scarratt, 2008; Tizard, 2013d; Baird et al., 1987; Giguere & Polkes, 2005; Hurcombe et al., 2012).

1. Zinc sulphate test: A solution containing ZnSO₄ causes globulin in the foal's serum to become insoluble. The turbidity of this reaction determines the concentration. A spectrophotometer reads the turbidity (Crisman & Scarratt, 2008; Tizard, 2013d; Baird et al., 1987; Giguere & Polkes, 2005).
2. Glutaraldehyde test: A coagulation reaction when foals serum IgG is added to a glutaraldehyde solution. The IgG concentration in the serum determines the speed of the reaction and thus the amount of IgG present. For example a strong concentration of serum IgG (>800mg/dl) can cause coagulation in 5-10 minutes whereas a weak concentration (>400mg/dl) can take up to 1 hour. When comparing against SRID the sensitivity for concentrations 400mg/dl and 800mg/dl were 95 and 100% respectively and the specificity for the same concentrations were 80 and 59%, respectively. Thus, the poor specificity at 800mg/dl means that the foal may have adequate passive transfer but be diagnosed with FPT (Crisman & Scarratt, 2008; Baird et al., 1987).
3. Latex agglutination test: latex particles covered with anti-equine antibody in the presence of foal serum IgG agglutinate. This test can be used on whole blood and serum and it takes 10 minutes to conclude. This test, however favoured, is relatively insensitive with 72% at concentrations less than 800mg/dl IgG and 53% at concentrations less than 400mg/dl. This is, therefore, not very reliable test (Crisman & Scarratt, 2008; Tizard, 2013d; Baird et al., 1987).
4. ELISA test: This is a quantitative and semi-quantitative membrane filter system. By using ELISA dipsticks the colour intensity reaction on the test filter is compared to colour calibration spots. This test can be used on whole blood, serum and plasma and when comparing to SRID its sensitivity at IgG concentrations less than

400mg/dl and 800mg/dl were 90 and 95% respectively and specificity were 79 and 52% respectively. This means best results are seen when concentrations are very low and/or high in the serum. Thus, there is potential discrepancy whereby a foal can have adequate passive transfer but react as FPT with this test (Crisman & Scarratt, 2008; Tizard, 2013d).

5. Total plasma protein (TPP): This test, using refractometry, is a rapid and economical but inaccurate when determining IgG levels in foal serum. Foals immunoglobulins are varied in TPP unlike calves (Baird et al., 1987; Hurcombe et al., 2012). Increases in other solids such as lipids, glucose, urea etc... can all influence the result. Therefore instead of measuring TPP, the serum total protein (sTP) or rather serum immunoglobulin-G (sIgG) is measured by a biochemical analyser and found to be more accurate (Baird et al., 1987; Hurcombe et al., 2012).

Treatment and prognosis:

Treatment depends on age, time and clinical signs. A newborn foal should be fed within the first 2 hours of life if it has not already been seen suckling the mare. Any fresh or thawed colostrum available should be checked for sufficient levels of IgG. It is possible to check the levels using a colostrometer (hydrometer) which measures the specific gravity of the colostrum (Crisman & Scarratt, 2008; Tizard, 2013d, Giguere & Polkes, 2005; McKinnon et al., 2011d). The ideal range for optimal protection in colostrum is 1.060-1.085 which is equivalent to 3000-8500mg/dl IgG concentration. Manual feeding of 1-2 litres per 45kg foal separated into 200-400ml doses every 1 hour is ideal (Tizard, 2013d, Giguere & Polkes, 2005). If mares' colostrum is not available then bovine colostrum can be used. Bovine colostrum IgG's have a half life of 7-10 days in comparison to 26 days in equine. However, it has been studied that IgG levels in foals' serum after ingesting bovine colostrum did not differ from those that had ingested mares' colostrum (Giguere & Polkes, 2005; McKinnon et al., 2011d). If colostrum is not available or the foal is old enough whereby intestinal absorption has ceased then oral and plasma immunoglobulin substitutes are an alternative form of protection. Though, oral equine serum IgG products are not recommended as they fail to raise the foals IgG serum concentration to protective levels. Studies have proven that they are not absorbed by the intestine and serum IgG concentration levels are by no means high enough, to provide protection, after administration (Tizard, 2013d, Giguere & Polkes, 2005; Hammer et al., 2000; Hammer et al., 2001). Consequently IV plasma immunoglobulin substitutes are chosen over oral

substitutes. Concentrations available usually range between 1500-2000mg/dl in a 1 litre bag. In order to raise the IgG concentration to 400-800mg/dl then approximately 25000mg/dl should be administered which means one foal might need about 1-3 litres to reach a protective level. With IV plasma infusion careful administration is important. The foal should be monitored for signs of tachycardia, hyperthermia and any other adverse reactions (Tizard, 2013d, Giguere & Polkes, 2005; McKinnon et al., 2011d; Hammer et al., 2001).

Prevention:

Good management is vital for prevention. Constant inspection of the mare before birth and, the foal, after birth can prevent this failure of passive transfer. A stress free and clean environment can furthermore decrease predisposition to infection and also reduce the virulence load in the surroundings. Measuring colostrums' specific gravity should be routinely practiced and if a foal cannot suckle then manual feeding ought to be carried out. If one is unsure whether or not a foal received maternal protection then plasma infusions are advised as a preventative measure.

5.2 Septicaemia

This is the most frequent disease affecting neonatal foals. Mortality rates for sepsis can range from 25-45% (Hackett et al., 2014) and the most common pathogenic invaders are Gram-negative enteric bacteria; E-coli, Actinobacillus, Enterococcus and Salmonella, Gram-positives; Clostridium, Streptococcus and Staphylococcus, fungi; Candida albicans and viruses; Rota, Corona, EHV1 (McKinnon et al., 2011e; Hackett et al., 2014; Stewart et al., 2002)

Pathogenesis:

The main predisposing factors which cause septicaemia to occur are FPT, dystocia, placentitis, stress, wounds, dirty teats, unhygienic environment and failure to disinfect the navel (Hackett et al., 2014; McKinnon et al., 2011e; Stewart et al., 2002). After predisposition bacteria for example can invade and cause a transient bacteraemia. If the foal cannot shift this invasion or if an uncontrolled inflammatory response is activated then infection is called sepsis (Hackett et al., 2014). Invading pathogens can set up focal infections in different body system causing inflammatory responses and depending on the

site cause enterocolitis, pneumonia, arthritis, omphalophlebitis etc...(McKinnon et al., 2011e; Taylor, 2015). The neonatal response to infectious agents and even non infectious ones is called systemic inflammatory response syndrome (SIRS). It is initiated by either over-activation or immune-suppression of the immune system. SIRS causes massive cytokine response which then leads to clinical signs. (McKinnon et al., 2011e; Taylor, 2015; Dunkel & Corley, 2015)^{28,30,31}

Clinical signs:

Typical clinical signs for septicaemia are tachycardia, tachypnoea, pyrexia, hypo/hyperthermia, depression, lethargy, recumbency etc. Depending on severity, degree and site of infection diarrhoea, coughing, nasal discharge, lameness, nervous problems, cold extremities, decreased urine output and uveitis frequently occur (McKinnon et al., 2011e; Stewart et al., 2002; Dunkel & Corley, 2015). In case of diarrhoea, the main bacteria to think of are Enterobacteriaceae, Actinobacillus, Clostridium, Salmonella and Rotavirus. Diarrhoea affects ~20-40% of neonatal foals (McKinnon et al., 2011e). They're usually found in foals less than one month of age. In Actinobacillus case the foal is typically sick from birth with recumbency and diarrhoea. Neonates with enteric Gram-negative infections tend not to be ill at birth (Stewart et al., 2002).

Pneumonia affects ~ 50% of infected foals. Infection is acquired through the blood stream, inhalation or per-os. Orthopaedic osteomyelitis and synovitis has been found in 12 and 33% of foals respectively with typical clinical signs such as swollen joints with lameness and pain upon palpation. Meningitis although rare presents clinical signs such as seizures, depression and nystagmus and opisthotonus. Infection in the blood stream can also affect coagulation and circulation and cause haemorrhages, thrombosis and disseminated intravascular coagulopathy (McKinnon et al., 2011e).

Diagnosis:

A common but rather unreliable method of diagnosis is the culturing of bacteria from blood samples taken from the affected animal. Blood cultures can fail up to 40% of the time so repeated culturing is necessary (Hackett et al., 2014). Such a high failure rate is possibly due to the fact that there might not be a strong concentration of pathogen in the bloodstream, the foal's immunity may be defending itself and previous antibiotics may inhibit true results (Stewart et al., 2002). Also, culturing can be a long process ~48 hours which is not very practical (McKinnon et al., 2011e). Blood biochemistry is the main

diagnostic test, it is the most reliable and results can be achieved within minutes. Typical blood results in a septic patients show lower total white blood cell counts, leukopenia and neutropenia with a degenerative left shift, hypoglycaemia, high fibrinogen and high levels of acute phase proteins such as serum amyloid A (McKinnon et al., 2011e; Taylor, 2015).

Treatment and Prognosis:

Supportive care; oxygen, heat, parental and enteral nutrition, fluids for electrolyte, acid base balance and rehydration and antimicrobial broad-spectrum antibiotics for infection are important treatment measures. (McKinnon et al., 2011e). The survival rate depends on severity of septicaemia but it is usually 45-60% (Taylor, 2015).

Prevention:

Prevention is imperative, since FPT is the main cause of septicaemia one should make sure that the foal gets sufficient quantities of colostrum after birth, that the environment and mares teats are disinfected and cleaned respectively (to decrease the load and virulence of pathogens in the foals surroundings) and that correct disinfection (chlorhexidine and alcohol wash) of the foals umbilical stump is adequately done and immediately after birth. Antibiotic preventative therapy directly after birth has not proven efficient in preventing infection (McKinnon et al., 2011e).

6. Primary (Inherited) Immune-deficiencies in Foals.

6.1 Severe Combined Immunodeficiency Disorder (SCID)

SCID is a primary immune-deficient disorder affecting horses, humans, dogs and mice. It is an inherited autosomal recessive trait. Its occurrence is rare, nevertheless in certain breeds such as Arabian horses and Jack Russell terriers it should be a cause for concern and included in ones differential diagnosis for infectious disease (Crisman & Scarratt, 2008; McKinnon et al., 2011f; McGuire & Poppie, 1973; Ding et al., 2002; Reed et al., 2010). A study performed in the USA in 2002 revealed that 8% of the Arabian horse populations are carriers for this trait (McKinnon et al., 2011f; Reed et al., 2010).

Background:

SCID is caused by a mutation or a defect on the chromosome encoding DNA Protein Kinase catalytic subunit (DNA-PK_{cs}). This mutation/defect is a 5 nucleotide deletion in DNA-PK_{cs} gene which prevents the formation of enzyme DNA protein kinase (DNA-PK) and thus failure of V(D)J recombination (Crisman & Scarratt, 2008; McKinnon et al., 2011f; Ding . et al., 2002; Reed et al., 2010; Wiler et al., 1995). DNA-PK_{cs} is a DNA repair factor the components of which process non homologous DNA end joining (NHEJ) and resolve the DNA double strand breaking (DSB). NHEJ and DSB are responsible for genomic stability and integrity of chromosomes respectively (Ding et al., 2002; Wiler et al., 1995). This repair process occurs alongside V (D) J recombination of immunoglobulins and T cell receptor gene segments in developing lymphocytes (Ding . et al., 2002). Therefore the defect in DNA-PK causes an unstable and faulty V (D) J recombination site (Crisman & Scarratt, 2008; McKinnon et al., 2011f; Ding et al., 2002; Reed et al., 2010; Wiler et al., 1995). This recombination site called V (D) J recombination is essential for the assembly of unique antigenic receptor genes in immature lymphocytes. Its process involves immunoglobulin variable, diverse and joining regions which rearrange themselves several times forming billions of random coding sequences of immunoglobulin and lymphocyte antigen receptor variable regions. It is this assortment that provides the body with a specific immune system response (Crisman & Scarratt, 2008; McKinnon et al., 2011f; Ding et al., 2002; Reed et al., 2010; Wiler et al., 1995). Thus absence of DNA-PK

and V (D) J recombination as a result of defective expression of DNA-PK_{cs} results in the lack of lymphoid precursor immune tissues and cells. This lack of precursor cell development means that in accordance with the humoral system affected foals are agammaglobulinaemic with nonexistent germinal centres and lymphopenia. The cellular immune system, furthermore, lacks thymic tissue and thymic dependent lymphocytes in the spleen and consequently T lymphocytes do not mature and depletion is imminent (Crisman & Scarratt, 2008; McGuire & Poppie, 1973). Accordingly lymphocytes are unable to express antigenic specific receptors on their surfaces and therefore antigenic specific immunity is absent (Crisman & Scarratt, 2008; McKinnon et al., 2011f; Ding et al., 2002; Reed et al., 2010; Wiler et al., 1995). Neutrophils, monocytes and NK cells, however, are still functional in SCID foals but unfortunately these cells are not enough to defend the body against infectious microbiological agents (McKinnon et al., 2011f).

Diagnosis:

Since SCID is an inherited recessive trait, foals have to be homozygous for the trait to present clinical characteristics. Heterozygous carriers for the trait are physiologically normal and healthy and thus this inherent deficiency can go unnoticed (Crisman & Scarratt, 2008; McKinnon et al., 2011f; McGuire & Poppie, 1973; Ding. et al., 2002; Reed et al., 2010; Wiler et al., 1995). However in saying that, one study has revealed that there is a correlation between increased tumour incidences and carrier animals. Out of 295 Arabian horses with sarcoid tumours 13.9 % were found to be heterozygous carriers of the SCID mutant gene and out of 102 sarcoids tested 18.6 % carried the mutant allele (Ding . et al., 2002).

Diagnosis is based on the clinical signs; it is very typical that after maternal protection wanes the foal becomes persistently susceptible to infections and unresponsive to antibiotic treatment. The usually age for sepsis is 3-4 weeks of age, this timeframe can vary depending on environmental conditions and the quality and quantity of maternal passive immunity. In and around this time however maternal protection decreases and endogenous immunoglobulin production has not yet reached protective levels. Death becomes a definite outcome at roughly 5-6 months of age (Crisman & Scarratt, 2008; McKinnon et al., 2011f; McGuire & Poppie, 1973; Reed et al., 2010). The fact remains, the environment is full of microorganisms (opportunistic, pathogenic etc...) and because the foal, as a result of a defective immune system, cannot mount any specific immune response, death becomes inevitable. A noteworthy clinical sign apart from persistent

infection/sepsis is the overall under development, weakness and lack of weight (McKinnon et al., 2011f; Reed et al., 2010).

In order to diagnose the disorder before death one should test the foals pre-suckle serum directly after birth. Pre-suckle serum in immune normal foals contain small quantities of IgM. Endogenous production of this immunoglobulin starts to develop in the foetus at approximately 180-240 days of gestation. Therefore, at birth there are small quantities found in pre-suckle serum. After acquiring maternal antibodies, through colostrum ingestion, testing serum levels in the foal is not practical. However, IgM can be used as a diagnostic test again after the foal reaches 3-4 weeks of age. At this time maternal IgM decreases due to its complete metabolization by the foal and its short life compared to other immunoglobulins. Another reliable diagnostic parameter to look out for is persistent lymphopenia (<500-1000/uL) in haematology samples. A persistently low lymphocyte level is a key feature for SCID diagnosis. Once off low levels can be due to a number of other reasons like sepsis, FPT etc., so care must be taken when concluding those readings. There are also commercially genetic tests and laboratory tests (PCR) which diagnose homozygous affected, normal and heterozygous carriers. These tests require blood or buccal swab samples (Crisman & Scarratt, 2008; McKinnon et al., 2011f; McGuire & Poppie, 1973; Reed et al., 2010).

Treatment and Prognosis:

Treatment with antibiotics, plasma infusions and isolation is not practical. Homozygous foals do not respond positively to treatment and in-fact it just prolongs the disease course. Death is to be expected by 5 months of age even with treatment. Bone marrow (BM) transplant, however not practiced, is a possibly option but it has to be carried out with histocompatible full siblings (Crisman & Scarratt, 2008; McKinnon et al., 2011f; Wiler et al., 1995).

Prevention:

In Arab horse breeding studs, genetic testing should be a regular procedure to inhibit heterozygous carriers from mating. Two heterozygous for the mentioned trait produce a 25% chance each for a homozygous affected and homozygous normal offspring and a 50 % chance for a heterozygous carrier offspring. Mating a heterozygous carrier and a homozygous normal horse produce a 50% chance for normal and 50% chance for carrier

offspring. Mating heterozygous horses is not advised (Crisman & Scarratt, 2008; McKinnon et al., 2011f; Reed et al., 2010).

6.2 Fell Pony Immunodeficiency

This immunodeficiency affects only Fell and Dales pony breeds. These ponies are an uncommon breed, native to the UK, England. Due to their low numbers breeders inbred these ponies in order to save and increase the breed population. As a consequence of this inbreeding an inherent immunodeficiency, first described in 1998, appeared. This immunodeficiency known as Fell pony syndrome (FPS) or Foal immunodeficiency syndrome (FIS) is a lethal inherited autosomal recessive trait which causes B cell lymphocytopenia, severe anaemia and ganglionopathy (Crisman & Scarratt, 2008; Fox-Clipsham, 2011; Tallmadge, 2012; Scholes, 1998; Thomas, 2005) Each year approximately 10% of Fell ponies and 1% of Dales die from this disease (Fox-Clipsham, 2011).

Background:

This unique immunodeficiency came into recognition because of its lethal nature in this pony breed. Foals were born healthy but at around 2-4 weeks of age presented non-specific signs such as diarrhoea, coughing and pale mucous membranes, symptoms which did not respond to treatment. By 3-5 months of age these affected foals died. Due to the fact that these problems were only associated with the Fell pony breed and that clinical and pathological examination showed similarities to other primary immunodeficiency disorders extensive study of this breeds genetic makeup was carried out. Studies have mapped affected and healthy Fell pony foals' genes and came to conclude that there are genetic mutations/defects associated with the cause of this disease (Crisman & Scarratt, 2008; Fox-Clipsham, 2011; Tallmadge, 2012; Scholes, 1998; Thomas, 2005). One such genetic mutation was found on chromosome ECA 26 (Fox-Clipsham, 2011; Tallmadge, 2012). Two studies have established, that a mutation/alteration and/or amino acid substitution on, co-transporter gene SLC5A to be a predictor of the disease. This gene regulates sodium and myoinositol co-transport in the cell membranes and osmotic stress responses essential for lymphoid tissue during embryonic development. The defect of this gene results in erythropoietin failure, a compromised immune system and ganglionopathy causing severe anaemia, B lymphocytopenia and peripheral nerve development problems respectively. It

should not be presumed, however, that SCL5A3 is the main cause of thus mentioned developmental failures (Fox-Clipsham, 2011; Tallmadge, 2012).

Another important finding is based on the fact that FPS foals are not sick at birth and haematocrit and antibody levels match healthy foals. The reason being, the foetal liver (in utero) has greater haematopoietic properties than the bone marrow and hence explains why B cells and erythrocytes can be detected in the peripheral blood in the just born foals and why they do not present with clinical signs. After birth, however, the bone marrow becomes the main haematopoietic organ (Tallmadge, 2012). In FPS foals B lymphocyte and erythrocyte levels fall dramatically by 4-6 weeks of age (coherent with the onset of clinical signs) and therefore this disease has been traced to the haematopoietic cells in the bone marrow (Tallmadge, 2012).

Studies have also found, through gene mapping, low PAX5 gene expression in the bone marrow and spleen of affected foals. PAX5 is responsible for haematopoiesis and B lymphocyte function, signalling and survival in peripheral tissues. From this we can assume that there is more than one genetic problem causing this serious immunodeficiency disorder (Tallmadge, 2012).

Diagnosis:

The main characteristic when diagnosing this immunodeficiency is the breed and the age of the animal. Fell and Dales ponies aged less than 5 months are the main attributes for this disorder suitably called Fell pony syndrome (FPS). Another indication of FPS, when clinical signs occurring at 2-3 weeks of age include diarrhoea, inability to suck, coughing, anaemia etc., but more importantly when the foals do not respond to treatment. By 3-5 months the affected foal inevitably succumbs to death (Crisman & Scarratt, 2008; Fox-Clipsham, 2011; Tallmadge, 2012; Scholes, 1998; Thomas, 2005).

Diagnosing FPS on a cellular level is carried out by testing serum immunoglobulin concentrations, complete blood counts, peripheral blood lymphocyte phenotyping and cytology bone marrow aspirations. B lymphocyte deficiency in these foals can be observed through immune-histochemistry of the lymph-nodes, bone marrow and thymus. One may also notice small scarce erythroid precursor cells (in bone marrow), thymus, lymphoid organs, germinal centres and plasma cells while T lymphocytes on the other hand do not underprovided in FPS affected foals (Crisman & Scarratt, 2008; Tallmadge, 2012; Scholes, 1998; Thomas, 2005).

Total immunoglobulin levels help with diagnosis also, by using single radio immunodiffusion (SRID) and ELISA test kits information about the lack of endogenous immunoglobulin level can be determined. These tests reveal reductions and absence in serum IgM levels by 3-4 weeks of age. Maternal IgM antibodies do not interfere with diagnosis, as they are not present at that time, due to their fast metabolization and short half life in the foal. Therefore this immunoglobulin isotype is a good diagnostic for the lack of endogenous production. IgG can still be detected at thus mentioned age so this isotype finding is not entertained, these levels reflect maternal levels rather than the foals (Crisman & Scarratt, 2008; Fox-Clipsham, 2011; Tallmadge, 2012; Scholes, 1998; Thomas, 2005).

Blood counts expose a non regenerative non haemolytic anaemia in the affected (Fox-Clipsham, 2011). Post-mortem diagnostic exam results in small and underdeveloped thymus and secondary lymphoid organs (Crisman & Scarratt, 2008; Tallmadge, 2012; Scholes, 1998; Thomas, 2005). Cytology of the bone marrow reveals erythroid hypoplasia and myeloid dysplasia by week 5 of life and genetic testing of mRNA expressions involving B lymphocytes and erythrocyte functions can be performed using RT-PCR (Tallmadge, 2012).

Treatment and Prognosis:

Treatment can be provided with supportive and antibiotics therapy. One should understand that this disorder cannot be treated successfully and as the foal ages and infection persists clinical signs become progressively worse and euthanasia is the only option. This disorder is 100% lethal (Crisman & Scarratt, 2008; Fox-Clipsham, 2011; Tallmadge, 2012; Scholes, 1998; Thomas, 2005).

Prevention:

Through genetic screening, for the mutated/defective SCL5A3, one can breed their ponies selectively and single out those, carrying defective alleles, from breeding and thus help accumulate the population of Fell and Dale's ponies (Fox-Clipsham, 2011; Tallmadge, 2012).

6.3 Selective IgM deficiency

This deficiency affects immunoglobulin IgM concentration levels without concern to other parts of the immune system or immunoglobulin's such as IgA, IgG, IgG(T) etc... It is most frequently seen in Arabian and Quarter horses. The age group most typically affected are, foals between 2-8 months of age and adult horses between 2-5 years of age. A genetic origin, as the cause, has been contemplated but yet to be identified, therefore one cannot exactly categorize it as a primary immune-deficiency (Giguere & Polkes, 2005; McKinnon et al., 2011g; Gardiner et al., 1992).

Background:

IgM is the first immunoglobulin to react to foreign antigen invasion. After antigenic exposure isotype switching occurs and other immunoglobulins take over and increase, whereas IgM does not (Perkins et al., 2003). This antibody acts, in ensuing immune development, by not only neutralizing and opsonising pathogenic microorganisms but also promoting pathogen recognition to APC's (Kaveri, 2012). During B cell ontogeny it is the foremost immunoglobulin recognised and secreted during antigen specific immune response. In summary there are two types of IgM; natural (first defence before immune exposure) and immune (response to antigenic stimuli) (Kaveri, 2012).

Interest in this immunoglobulin has arose in adult horses, with neoplasias' such as lymphosarcoma and other immune conditions, typically showing defective IgM levels in their serum (Giguere & Polkes, 2005; Perkins et al., 2003). I will discuss the suspected link with lymphosarcomas further in the diagnosis section, but essentially this immunoglobulin deficiency is difficult to understand. It is variable in its cause of neoplasia and foetal disease. In some cases tumours form and death occurs and in others there is increased susceptibility to infections but there have been cases where the affected animals have recovered (Giguere & Polkes, 2005). IgMs' selective immunodeficiency mechanism is therefore undetermined and even though a hereditary basis is suspected it has not been fully proven (Giguere & Polkes, 2005; McKinnon et al., 2011g; Gardiner et al., 1992; Perkins et al., 2003; Louis & Gupta, 2014). In humans, researchers have linked IgM immune deficiency to a chromosomal (22q11.2) deletion whereby IgM was scarce but other parts of the immune system, the innate immunity and T cell populations, stayed intact. Relative to this finding they mention that full pathogenesis is unknown because

other factors like defective B or plasma cells, decreased T helper activity or increased isotype specific suppression can all attribute to the IgM deficiency (Louis & Gupta, 2014).

Diagnosis:

Because there are variable reasons for this deficiency diagnostic focus has been placed on the levels of IgM in the serum, the clinical signs, the breed and the age of the horses.

Young foals 3-4 weeks old show typical signs such as enteritis, pneumonia, arthritis and decreased body development. However, typical these clinical signs are, they are not definite enough to diagnose this deficiency. The foals' failure to respond to treatment is more significant, however. As with the other immune deficiencies mentioned, normal foals endogenous production should start to rise by 4-5 weeks. This does not occur in affected foals and mentioned clinical signs can be seen any time below 10 months of age (Giguere & Polkes, 2005; McKinnon et al., 2011g; Gardiner et al., 1992). In order to determine this disease from the other immunodeficiencies immunoglobulin testing can be performed with ELISA and SRID kits. The resulting parameter for this deficiency should be that only IgM is deficient but other immunoglobulin such as IgG are still present (Giguere & Polkes, 2005; McKinnon et al., 2011g; Gardiner et al., 1992; Perkins et al., 2003). Older horses presenting with lymphosarcoma can be diagnosed in the same way. In normal horses immunoglobulin IgM concentration levels are 120 +/- 30 and horses less than 60mg/dl are classified as immune deficient. In a study some healthy horses, however, had IgM concentrations <60mg/dl. Since then classification of this deficiency transpired to IgM levels less than 23mg/dl (Giguere & Polkes, 2005; Perkins et al., 2003).

The sensitivity and specificity when testing IgM for lymphoma with concentrations less than 60mg/dl were 50 and 35% respectively. Therefore when lymphoma is present, the type of lymphoma can make diagnosis difficult thus the reason for poor specificity and sensitivity when using IgM as a diagnostic agent and thus IgM should not be a sole diagnosis for lymphoma (Giguere & Polkes, 2005; Perkins et al., 2003).

Selective IgM deficiency is difficult to presume a cause for. It can be affected by many factors. These factors suggest an inability/inaccuracy when quantifying total IgM; such factors corrupting total levels are because of IgMs' ability to bind to complements, its translocation to mucosal surfaces, isotype switching, its fast catabolism from serum and also its short half life (Perkins et al., 2003).

Lymphocyte phenotypes and blood lymphocyte counts are usually physiologically normal in foals and adults with selective IgM deficiency unless they are suffering from an underlying condition (Giguere & Polkes, 2005).

Treatment and Prognosis:

Treating clinical signs caused by septicaemia, which occur secondary to this immunodeficiency, with antimicrobials and supportive care is basic procedure. In some cases foals can live beyond one year but in the majority of cases those presenting recurrent infections die. Adults with selective IgM deficiency classically present with lymphoma and IgM concentration levels less than 60mg/dl. Those without lymphoma have been known to recover successfully (Giguere & Polkes, 2005; McKinnon et al., 2011g). Needless to say, since this is an immunodeficient disorder, prognosis is poor (McKinnon et al., 2011g). Plasma infusions do not provide much help although there are new products containing pooled 12% IgM which can be given as a treatment to those with sepsis and as a supplement therapy for other causes of infection, due to lack of B cells in association with immunoglobulin deficiency (Kaveri et al., 2012).

Prevention:

There is no tested preventative measure because this immunodeficiency has not been traced to a specific gene. It has only been assumed that it is inherited. Also, there are several different factors that decrease IgM in serum and leave other immunoglobulin without harm.

6.4 Agammaglobulinaemia

This is a rare immunodeficiency which predominantly affects Thoroughbred, Quarter and Standard bred horses. In a 2,516 horses study only 0.16% of these were diagnosed with agammaglobulinaemia (Perryman et al., 1983). It was also noted that only male horses were affected and thus it was suspected that this immunodeficiency resembled human X-linked agammaglobulinaemia. X-linked denotes only males inherit the defective gene and agammaglobulinaemia indicates the absence of immunoglobulins. Not much genetic study has been performed on horses, nevertheless when comparing it to the human equivalent then one would say it is also inherited (McKinnon, 2011a; Perryman et al., 1983; Banks et al., 1976).

Background:

Pathogenesis is based on the fact that immunoglobulins, which make up the humoral immunity, are absent or scarce. Studies have revealed normal T lymphocyte levels with low to nonexistent immunoglobulin concentrations in affected horses (McKinnon, 2011a; Perryman et al., 1983; Banks et al., 1976). Upon further investigation, one such study revealed that B lymphocytes established in the circulation but they didn't bear surface receptors for immunoglobulins. Electron microscope examination found that these affected lymphocytes had a non villous surface whereas healthy lymphocytes had (Banks et al., 1976). Other studies have elected B lymphocyte and plasma cell absence as the cause of agammaglobulinaemia (McKinnon, 2011a; Perryman et al., 1983) and even during experimental antigenic exposure these animals were unable to produce antibodies (Banks et al., 1976). Consequently, both of these findings lead to immunoglobulin production failure and inability to differentiate into antibody producing cells.

The inherent nature of this deficiency is based on human studies. In humans there is a chromosomal mutation of the BTK gene (Bruton tyrosine kinase) on an X chromosome which, when functionally normal, acts by signalling and transcribing immunoglobulins in response to B cell receptor engagement. Its dysfunction thus prevents B cell differentiation (McKinnon, 2011a; Perryman et al., 1983; Banks et al., 1976). The possible reason as to why the inherent nature of this deficiency has not been studied more in horses is perhaps because it is extremely rare and also its diagnostic findings resemble humans so much so that there is no need to examine it.

Diagnosis:

Based on ELISA immunoglobulin and lymphocyte testing one can diagnose agammaglobulinaemia by the lack or absence of B lymphocytes, immunoglobulins and plasma cells and normal T lymphocyte concentrations. The consequences of these parameters mean that horses, show clinical signs later in life, generally from 2-6 months. The late onset is down to their normal T lymphocyte cellular immunity. However, recurrent infections are common (McKinnon, 2011a; Perryman et al., 1983; Banks et al., 1976).

Characteristically absent immunoglobulins recognised are IgM and IgA. IgGs are severely decreased if not absent (Perryman et al., 1983). The lack of these important

immunoglobulins is down to the defective B lymphocyte. It is thought that T cells have some suppressive effect on B lymphocytes (Perryman et al., 1983) thus causing the deficiency but it is more probable that B lymphocytes own defective nature causes the problem. Following pathological and histopathological examinations it was also recognized that plasma cells, germinal centers and primary follicles in the lymph nodes and/or spleen were missing in these horses (McKinnon, 2011a; Perryman et al., 1983; Banks et al., 1976). Therefore it is a serious immunodeficiency and the only factor separating it from SCID is the fact that T lymphocytes have normal concentrations and functional abilities.

Treatment and Prognosis:

These horses also have better longevity when compared to SCID horses and with proper medication and management affected animals can quite frequently live beyond 1.5 years of age (McKinnon, 2011a; Perryman et al., 1983). Nonetheless these animals still present an inability to produce an adequate immunity response and in consequence infection susceptibility and recurrence persists.

7. Immune Mediated Disorders

7.1 Neonatal Isoerythrolysis (NI)

This disease is an immune mediated haemolytic anaemia. It occurs in post-partum neonatal foals ~24 hours after ingesting mares' colostrum. Immune mediated meaning, due to incompatibilities between the mare and foals erythrocytes, the mare produces alloantibodies in her colostrum against antigens on her foals' erythrocytes. When the foal ingests this colostrum after birth the alloantibodies attack its red blood cells and causes haemolysis and agglutination resulting in anaemia, jaundice and eventually death (Giguere & Polkes, 2005; Finding & McSloy, 2011; Johnson, 2006).

Pathogenesis:

There are 7 blood groups in horses and each one contains different antigen sites called factors. There can be greater than 30 antigenic factors among the different blood groups. In the case of neonatal isoerythrolysis the most common blood group and factor involved is Aa and Qa. In order for neonatal isoerythrolysis to occur a mare must lack Aa and Qa alloantigens (negative) and her foal must contain (positive) these antigens on their erythrocytes. The foals' positive status is inherited from the stallion. The mare requires exposure to these antigens before it can produce antibodies against it. This exposure can occur by blood transfusion and placenta haemorrhage after first pregnancy. It is quite typical that NI is not recognised until the second pregnancy. Exposure after first parturition sensitizes her to these foreign antigens and prepares her for subsequent gestations. The first pregnancy is not affected because there is a lack of time between initial exposure (placental haemorrhage) and antibody development. The foal can only be affected if it ingests colostrum (full of alloantigens) as there is no attack intra-utero because of the special characteristics of the equine epitheliochorial type placenta (Giguere & Polkes, 2005; Finding & McSloy, 2011; Johnson, 2006).

About 14% of foals have incompatibilities with their dams. There are however differences among breeds as to the production of alloantibodies against foals antigenic erythrocytes. Mares that are Aa negative have a high incidence of producing alloantibodies against Aa positive foals but Qa negative mares don't always produce Qa antibodies against Qa positive foals (Giguere & Polkes, 2005). Approximately 95% of thoroughbreds

have factor Aa (Johnson, 2006) and thus the inherent chance of a foal becoming Aa positive to a negative mare is 85% (Giguere & Polkes, 2005).

Because there are so many different antigenic factors and blood groups in horses not all antigens and antibodies are created the same and they don't always produce a response, for example there is an anti Ca group that is relatively common but doesn't cause disease in the neonatal foal. It has been found to actually prevent the mare immunizing against erythrocyte antigens (Giguere & Polkes, 2005; Johnson, 2006).

Clinical signs:

Clinical signs are frequently seen within the first 8 hours-5 days after birth. The foal become lethargic, depressed with tachypnoea- cardia, dyspnoea, anaemic, hypoxic and pyrexia. Its mucous membranes and conjunctiva turn yellow (icterus/jaundice), characteristic for NI, which is a consequence of pigment build up in the liver due to erythrocyte haemolysis. One may notice discoloured urine resulting from haemoglobinuria. Sepsis is frequent due to its compromised state. The haemolysis and icterus together can cause hepatic failure and to effect nervous signs (Giguere & Polkes, 2005; Finding & McSloy, 2011; Johnson, 2006).

Diagnosis:

Blood sample analysis can diagnose NI. Haemolysis of erythrocytes causes plasma to turn pink and the pack cell volume to decrease. Another indicator is the increased level of hepatic enzymes (AST, ALP, SD) and total and direct bilirubin which all result from hepatocellular damage as a result of iron and bilirubin build up, toxicosis, anaemia and hypoxia (Giguere & Polkes, 2005; Finding & McSloy,2011; Johnson, 2006).

Treatment and Prognosis:

IV fluids are typically administered to correct any acid-base and electrolyte abnormalities and to decrease the concentration of circulating haemoglobin. Colloid fluids treat any underlying hypoproteinaemia. Supportive therapy; rest and heat are also required. If the bloods' packed cell volume (PCV) is low then a blood transfusion may be necessary. The donors' blood must be negative for antigenic Aa and Qa. The mares own blood is ideal but it must be washed, prior to transfusion, to remove antibodies (Giguere & Polkes, 2005; Finding & McSloy, 2011; Johnson, 2006).

IV polymerized bovine haemoglobin can be used if donor blood is not available straight away or if washing and packing has to be completed. This substitute provides foals with an oxygen supplement for up to 18 hours (Perkins & Divers, 2001). Care must be taken when carrying out blood transfusions because adverse reactions like tachypnoea, tachycardia and weakness can occur. It should be administered slowly at 20ml/kg BW to avoid distressing the body.

In acute cases foals can die even before clinical signs present. If the foal survives for several days and has only mild signs then prognosis is good.

Prevention:

NI is prevented by stopping the foal from ingesting its mother's colostrum. The foal should not be allowed to suckle from its dam for approximately 36 hours or until colostrum turns to milk. The foal should be bottle fed stored colostrum from a safe source or given IV plasma transfusions. Blood typing mares and stallions can also be performed to prevent the disease (Giguere & Polkes, 2005; Finding & McSloy, 2011; Johnson, 2006). Preventative methods using diagnostic tests can be carried out. Jaundice agglutination test (mixes foals blood with mares' colostrum, after centrifugation agglutination (positive) can be seen at the bottom of the tube (Giguere & Polkes, 2005; Finding & McSloy, 2011; Johnson, 2006). Coombs test (mixes foals RBC (washed) with anti-horse antibodies; agglutination confirms presence of anti-RBC antibodies (Finding & McSloy, 2011). Another method called Direct Immunofluorescence (DIF); a flow cytometry test which determines the class of antibodies bound to erythrocytes. Sensitivity between Coombs and DIF in dogs was found to be 58 and 100% respectively, however specificity was 100 and 87.5% respectively. DIT was more valuable when detecting low values and responses to therapy (Wilkerson, 2000).

7.2 Immune-Mediated Thrombocytopenia (IMT)

IMT is a rare disorder which occurs sporadically in horses. Many different factors can cause IMT such as inherited allo-immune thrombocytopenia, equine infectious anaemia (EIA) to mention a few. The basis behind this disorder is that antibodies mediated toward platelets can cause their destruction and thus cause the animal to become thrombocytopenic (McKinnon et al., 2011g; Clabough et al., 1991; Buechner-Maxwell et al., 2008)

Pathogenesis:

In essence thrombocytopenia occurs when platelet levels are inadequate in the body. This can be due to decreased production, increased destruction and consumption (McKinnon et al., 2011g; Clabough et al., 1991; Buechner-Maxwell et al., 2008). With immune mediated destruction such as allo-immune thrombocytopenia the foal inherits paternal antigens on their platelets which differ to its dams. When the foal ingests its mares' colostrum antibodies in the colostrum attack the foals paternally inherited platelet antigens and thus leads to their destruction and overall absence in the foal.

One such study found that one foals' full sibling had the same thrombocytopenia resulting from maternal antibody ingestion. Because this disorder is extremely rare in horses not much study had been found but based on its effects and diagnosis it is very similar to neonatal isoerythrolysis (McKinnon et al., 2011g; Buechner-Maxwell et al., 2008).

The mechanism behind immune thrombocytopenia in relation to infections equine infectious anaemia is that virus specific antibodies have epitopes on normal thrombocytes and thus immunoglobulins bind platelets and form immune-complexes. The bound platelets cause decreased platelet levels in the blood. Immune-complexes cause further problems for the affected equine by depositing in renal glomeruli, vessels and other areas of the body (Clabough et al., 1991)

Diagnosis:

In the case of allo-immune thrombocytopenia clinical signs are lethargy, increase bleeding from injection sites, haematomas, skin lesions, mucous membrane ulcers, slow capillary refill time and sepsis. Diagnose is established by using flow cytometry and direct fluorescence methods to determine platelet bound antibodies. These tests should be

performed early, in disease course, before the mononuclear system can clear the formed complexes from the circulation. Other diagnosis can be through indirect measuring of maternal antibodies binding with foals platelets. Haematological values showing low to absent thrombocytes levels in the blood, levels below 100,000/uL, are consistent with thrombocytopenia. Pathological studies of thrombocytopenic horses show enlarged liver and spleen due to their increased efforts to clear the formed immune-complexes from the body (McKinnon et al., 2011g; Buechner-Maxwell et al., 2008).

With EIA signs include severe anaemia, fever, glomerulonephritis and immune suppression due to the persistence of the virus and consequential thrombocytopenia. Diagnosis by bone marrow analysis, flow cytometry and direct fluorescence indicate immune mediated thrombocytopenia and the use of Western blot detects the virus specific antibodies in the serum. This viral infection resembles other immune mediated thrombocytopenias in regards to its clinical signs, diagnosis and pathology (Clabough et al., 1991)

Treatment and Prognosis:

Supportive, antimicrobial therapy and plasma infusions from a suitable donor are usually carried out. Corticosteroids can be administered to help stabilize the blood platelet levels and to increase their production however sometimes this medication is not found to be effective (McKinnon et al., 2001g; Buechner-Maxwell et al., 2008).

Prevention:

In the case of allo-immune thrombocytopenia which is similar in a way to NI, preventing foals from ingesting colostrum for the first few days after birth is important in preventing this disorder. Antimicrobial treatment against possible infections is recommended. Plasma or platelet infusions are always beneficial as a preventative measure also (McKinnon et al., 2001g)

8. Conclusion.

From this theoretical review I can conclude that the basic understanding of the development and activation of the immune system is important because neonatal foals lack a functional adaptive immunity for a short period in their early life. The neonatal foal does develop an immune system but it is generally deficient in many cells at birth that are required to initiate the adaptive immunity. It is born into an environment full of microbes which challenge its defence. This exposure is important for its immune development but additional protection is ultimately required while its defence is being stimulated and developing. The downfall in neonatal foal immune protection is caused by the lack of maternal antibody transfer intra-uterine. The foal is therefore born immune deficient and its only means of defence is by maternal antibody- and lymphocyte-supplement through breast-feeding. For many reasons this passive transfer can fail leaving the foal vulnerable and susceptible to infectious agents. This process is called failure of passive transfer (FPT) and is the most common secondary immunodeficiency in neonatal foals. This deficiency leads to septicaemia which is the biggest cause of death in thus affected foals.

SCID and Fell pony syndrome are primary immunodeficiencies and are caused by genetic defects of the immune system. These disorders cause more serious immunodeficiency diseases, which are life-threatening. SCID is the most studied primary immunodeficiency because interest lies in its correlation with the human equivalent. These two immunodeficiencies are frequently seen in certain breeds of horses, SCID in Arabs and Fell pony syndrome in Fell and Dales ponies. Because these breeds are relatively rare it is very possible inbreeding caused these disorders. Agammaglobulinaemia and IgG selective immunodeficiency disorders are less common or tend not to have a specific genetic cause because other factors can be involved in their defective processes.

Immune mediated diseases such as neonatal isoerythrolysis and immune mediated thrombocytopenia affects the foal after ingesting colostrum. Instead of maternal antibodies in the colostrum protecting the foals they instead attack the foals cells causing problems.

Of these disorders mentioned the primary immune disorders are the most uncommon and the most deadly. Immune mediated disorders have better prognosis and once correct preventative measures, such as inhibiting the foal from ingesting its mares' colostrum thus eliminates these disorders occurring.

The most frequent immune disorder, the FPT can be treated successfully if caught early and depending on the case these foals can have a positive outcome. Treatment is

always recommended for secondary immunodeficiencies but once a primary immune disorder is diagnosed then death is inevitable and euthanasia is the humane option.

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