NEW VISIONS IN HEALTH SCIENCES:

CONCEPTS - THEORIES - APPLICATIONS

Editor
Assoc. Prof. Gülden AYNACI

DUVAR

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Chapter 1

Oxıdatıve Stress In Psorıasıs Pathogenesıs

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Psoriasis

Psoriasis is an inflammatory skin disease that reduces the quality of life of patients and is associated with many comorbidities, and there is no definitive treatment solution (Armstrong & Read, 2020). It affects approximately 125 million people worldwide (Korman, 2020). This disease is characterized by erythematous plaques, well-defined borders and covered with white scales, and a symmetrical distribution is observed (Griffiths & Barker, 2007).

Although they are effective in localized skin symptoms in individuals typically treated with topical agents or phototherapy, they are not sufficient to elucidate the underlying disease pathogenesis (Boehncke & Boehncke, 2014). Therefore, the focus has been on elucidating the roles of proinflammatory cytokines in order to develop targeted therapies (Baliwag, Barnes, & Johnston, 2015).

Oxidative Stress

Living things experience oxidative stress when the balance between ROS and antioxidant enzyme systems is disrupted. In order for them to continue their lives under oxidative stress, they must have antioxidants that keep ROS under control. These are elements that combat oxidation (Cabello, Lodeyro, & Zurbriggen, 2014).

Many biomarkers have been used for psoriasis pathogenesis and conflicting results have been found (Kadam, Suryakar, Ankush, Kadam, & Deshpande, 2010; Rashmi, Rao, & Basavaraj, 2009; Zhou, Mrowietz, & Rostami-Yazdi, 2009). Therefore, biomarkers with high sensitivity are needed. Oxidative stress is one of the main causes in the pathogenesis of psoriasis and is responsible for keratinocyte angiogenesis and uncontrolled transcription increase(Bito & Nishigori, 2012; West vd., 2010).

Free Radicals

The most intense free radicals formed are reactive oxygen species (ROS) and these are reactants containing unpaired electrons. These radicals are species such as superoxide anion (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) (Cabello vd., 2014).

In a healthy organism, the harmful effects of ROS are not observed due to the balance between ROS and antioxidants (McCord, 1993). Biological products such as ischemic modified albumin (IMA) and malondialdehyde (MDA) increase due to ROS (Ataş, Hacınecipoğlu, Gönül, Öztürk, & Kavutçu, 2017). Excessive increase in ROS overwhelms the antioxidant system and causes lipid peroxidation (Duthie, Robertson, Maughan, & Morrice, 1990).

The first enzyme to respond to ROS, SOD levels, will increase. As the disease severity continues to increase, the SOD enzyme begins to decrease. Although the

SOD enzyme begins to decrease, CAT and GSH-ST are not observed. Despite the increase in MDA and IMA, the increase in GSH-ST, SOD and CAT enzymes is due to the body's natural defense. According to the results of the study, the decrease in SOD and CAT enzyme activities, especially in women, is explained by the fact that the response to increased oxidative stress in women is not as strong as in men. The GSH-ST enzyme is not affected by gender, but rather acts more in detoxification and is activated in oxidation by being used as a catalyst (Ataş vd., 2017).

Antioxidants

They are divided into two groups: enzymatic and non-enzymatic antioxidants. Their locations and functions in the cell may also differ.

• Enzymatic Antioxidants

Enzymatic antioxidants are known as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and ascorbate peroxidase (APX).

SOD, an enzymatic antioxidant with three isoenzymes depending on the metal ions in its center, converts superoxide anion into hydrogen peroxide and prevents its conversion to the more toxic OH- radical. These metal ions in question are SODs containing iron, manganese, copper and zinc (Kukreja vd., 2005). SOD activities increase in living beings exposed to oxidative stress to prevent them from being affected by these stress conditions (Attia, Karray, & Lachaâl, 2009; Harinasut, Poonsopa, & Roengmongkol, 2003).

CAT, one of these antioxidants that has a protective function against stress factors; It enables the breakdown of hydrogen peroxide (H_2O_2) into H_2O and O_2. It has been determined that these enzymes provide protection under different stress conditions and at different levels (Polle vd., 1992).

Another enzymatic antioxidant, APX, which consists of five different isoforms, is more effective against H_2O_2 than CAT. Under stress conditions, this enzyme activity is increased and is thus accepted as a defense mechanism against stress (Mobin & Khan, 2007).

GPX, another enzymatic antioxidant with various isozymes and functions against stress factors, neutralizes lipid peroxides and H_2O_2 (V, V, & R, 2001). Studies show that it plays a protective role under stress conditions and the main antioxidant pathway for the removal of H_2O_2 is the GPX pathway (Baloğlu & Cetin, 2020).

• Non-Enzymatic Antioxidants

The first free amino acid to accumulate after drought stress application is proline and it is an important parameter that is measured to determine whether it is under stress under experimental conditions. The main function of this organic substance, which is a non-enzymatic antioxidant, is to protect protein and membrane structure. It also plays a

role in the regulation of cell division, signal transmission and mitochondrial functions (Öztürk, 2015).

The most abundant and most powerful non-enzymatic antioxidant in the cell, ascorbic acid (vitamin C), plays a role in preventing damage caused by reactive oxygen species (ROS) (Athar, Khan, & Ashraf, 2008). While its amount is low under normal conditions, its amount increases when the living being is exposed to oxidative stress. It ensures the elimination of hydrogen peroxide, hydroxyl and superoxide anion. According to studies, the increase in vitamin C in living beings exposed to stress is a defense mechanism against stress (Holländer-Czytko, Grabowski, Sandorf, Weckermann, & Weiler, 2005).

Carotenoids are pigments found in plants and microorganisms and have 600 different types. It has been observed that it increases with oxidative stress and this has been associated with the defense mechanism (Mittler & Zilinskas, 1992).

Tocopherols (vitamin E), which are responsible for cleaning ROS and ensuring lipid peroxidation, are found in almost all parts of the plant. They are especially dense in the chloroplast membrane. One of the tocopherols, α-tocopherols; are the strongest because they contain 3 methyl groups in their structures (Gang, Zhen-Kuan, Yong-Xiang, Li-Ye, & Hong-Bo, 2007). Studies have shown that its increase under stress applications is a defense mechanism against oxidative stress (Shao vd., 2007).

Glutathione, another non-enzymatic antioxidant, is found in almost all parts of the cell and therefore has many functions. It plays a role in many events such as signal transduction, cell death, cell differentiation, resistance to pathogens, enzymatic regulation and detoxification of xenobiotics (Rausch & Wachter, 2005).

Phenolic compounds, which are metabolite groups in plants, are also non-enzymatic antioxidants. Increases in phenolic compound levels are seen in those exposed to oxidative stress, and this is a defense mechanism (Quan, Zhang, Shi, & Li, 2008; Temple, Perrone, & Dawes, 2005). Some flavonoids, which are divided into flavonoids and phenolic acids according to their carbon atom numbers, increase under certain stress conditions (Ou, Liu 刘, & Lin, 2005; Quan vd., 2008).

Pathogenesis

The pathogenesis of the disease is complex and has not been fully elucidated. Excessive activation of immune system elements is effective in the pathogenesis of the disease and various cell types have been found to be responsible (L. Lin, Ambikairajah, & Holmes, 2002). Initially thought to be a disease of dysfunctional proliferation and differentiation of keratinocytes, neutrophils, monocytes, various cytokines, dendritic cells (DCs) and endothelial cells play a role in many stages of psoriasis (Chong, Kopecki, & Cowin, 2013). Many cytokines, cells and pathways have been identified in studies, and when the characteristic features of the disease are considered,

inflammatory cell infiltration in the dermis and epidermis, dilation of dermis vessels and keratinocyte hyperproliferation ocur (Georgescu vd., 2019).

Neutrophils, natural killer (NK) cells, myeloid and plasmacytoid dendritic cells are innate immune cells that respond rapidly to pathogens within a few hours. However, memory formation does not occur. The acquired immune response is given by T cells and memory formation is observed afterwards.

The first changes in pathogenesis are caused by Langerhans cells (LH) that capture the antigen. These received antigens are processed and then presented to Class I and II MHC molecules. T cell proliferation then occurs. Effector T cells migrate to the area where the antigen is concentrated. Activated T cells initiate inflammation by secreting cytokines that activate neutrophils, monocytes and keratinocytes (Galadari, Sharif, & Galadari, 2005).

Activated plasmacytoid dendritic cells produce the proinflammatory cytokine IFN-α, which progresses psoriasis. IFN-α activates myeloid dendritic cells along with the proinflammatory cytokines TNF-α, IFN-γ, IL-1β and IL-6. Activated myeloid dendritic cells also produce IL-12 and IL-23. Of these cytokines, IL-12 activates T helper 1 (Th1) cells, while IL-23 activates Th17 cells (Nestle, Kaplan, & Barker, 2009a). (Nestle, Kaplan, & Barker, 2009b; Swindell vd., 2013). This cycle, which is initiated once by the secretion of TNF-α from activated Th1 cells and IL-17A, IL-17F and IL-22 cytokines from Th17 cells, continues chronically. Thus, these cytokines further activate keratinocytes that produce various antimicrobial peptides, cytokines and chemokines.

Moderate to severe patients have high levels of proinflammatory cytokines not only in skin lesions but also in the blood (Arican, Aral, Sasmaz, & Ciragil, 2005). These elevations in cytokines cause inflammation that is associated with comorbidities affecting patients with psoriasis (Egeberg vd., 2016). Thus, treatments targeting proinflammatory cytokines not only improve symptoms but also reduce systemic inflammation. It is assumed that this will improve outcomes by attenuating comorbidity progression (Girolomoni vd., 2015).

Another reason responsible for its pathogenesis is the increased formation of free radicals due to oxidative stress, as well as the disruption of the balance between the antioxidant system (Briganti & Picardo, 2003; Kormeili, Lowe, & Yamauchi, 2004). When the relationship between oxidative stress and psoriasis is detailed; if oxidative stress continues for a long time, it creates abnormal amounts of free radicals. The increase in free radicals in fibroblasts, endothelial cells and keratinocyte cells causes neutrophil chemotaxis and superoxide production from accumulated neutrophils. In addition, the increase in cytokines increases SOD expression. The level of protein kinase A, which prevents free radical formation, decreases in psoriasis. This causes a

decrease in intracellular cAMP, which plays an inhibitory role in cell cycle and growth, causing hyperproliferation of keratinocytes (Zhou vd., 2009).

It has been determined that the level of MDA, the end product of oxidative stress and lipid peroxidation, is higher in psoriasis. It has also been determined that the level of MDA is correlated with the severity of the disease (Keerthana & Kumar, 2016).

The increase in CAT, SOD and GSH-ST enzymes in psoriasis patients is suppressed by the increase in MDA levels, the end product of lipid peroxidation. MDA is lower than IMA in predicting oxidative stress in psoriasis.

In addition, the level of albumin, which controls the level of heavy metals in the blood, increases in patients with psoriasis (Işik vd., 2016; Ozdemir, Kiyici, Balevi, Mevlitoğlu, & Peru, 2012).

SOD activity, which catalyzes the reduction of superoxide radical to less reactive H2O2, has generally been reported to be low according to the results of the studies (Rashmi vd., 2009; Yildirim, Inaloz, Baysal, & Delibas, 2003). This is thought to be due to antioxidant deficiency. Although Therond et al. reported that SOD values increased, they did not find a correlation with disease severity (Thérond vd., 1996).

The conversion of toxic H2O2 to H2O and O2 in the presence of CAT varies according to the study results (Polkanov, Bochkarev, Shmeleva, & Kipper, 1987; Thérond vd., 1996; Yildirim vd., 2003).

Increased GSH-ST reduces GSH with ROS. This explains the increased enzyme activity of GSH-ST against oxidation and these enzyme activities are affected for many reasons (Ataş vd., 2017).

When examined in general, enzyme activities such as SOD, CAT and GSH-ST were found to be low (X. Lin & Huang, 2016). Excessive increase in ROS causes lipid peroxidation by overcoming the antioxidant enzyme system (Duthie vd., 1990).

According to the results of the study, there was a positive correlation between disease severity and oxidative stress markers, and negative correlations between antioxidant biomarkers (Kadam vd., 2010; Nemati vd., 2014).

Result

The body tends to increase antioxidant enzyme activity to protect itself against oxidative stress. However, although the body increases antioxidant enzymes, it cannot always reduce ROS products. It can also try to keep ROS products constant in a way that is least harmful. However, the situation changes according to the severity, duration and course of the disease. Therefore, antioxidant supplementation will be beneficial in cases of psoriasis area severity index (PASI), prolonged disease duration, advanced age, female gender and high ROS levels.

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Chapter 2

Diagnosis and Treatment of Chronic Inflammatory Demyelinating Polyneuropathy

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Etiology and Pathology

Chronic inflammatory demyelinating polyneuropathy (CIDP) is an idiopathic, acquired, immune-mediated inflammatory demyelinating disease of the peripheral nervous system (1). The incidence is 1.6 per 100,000 per year (2). There is no consensus on the definition of the disease (1). T cell dysfunction and macrophage-mediated demyelination are the hallmarks of the disease (2, 3). It affects peripheral nerves from spinal roots to distal extremities. Pathological findings are segmental and multifocal demyelination starting from the nodal region. Antibodies that attack the nodal and paranodal complex have been discovered, which have been described as a small number of atypical forms whose clinical presentation differs from classical CIDP (4). Typical CIDP is characterized by progressive or recurrent symmetric proximal and distal muscle weakness lasting longer than 8 weeks, sensory involvement in at least 2 extremities, and decreased or absent deep tendon reflexes. Atypical CIDP is divided into groups such as distal, multifocal, focal, pure motor and pure sensory (5). Both genetic and environmental factors play a role in etiology. It is more common in men than in women. Immune attack occurs primarily in the proximal region of the spinal roots and peripheral nerves. The main pathological changes identified are demyelination, remyelination, edema, and onion-bulb formation (6). In histological studies performed on the sural nerves of patients affected by CIDP, onion-bulb formation provides important diagnostic information (7).

Diagnosis

CIDP is a chronic disease that lasts longer than 8 weeks (8). There are no biomarkers for diagnosis and disease monitoring. This makes diagnostic accuracy and monitoring response to treatment difficult. Decisions are made with clinical follow-up (2). In most patients it progresses slowly over eight weeks, while in a small number of patients it develops a relapsing-remitting course. Motor symptoms predominate in most patients (9). The rules for long-term CIDP follow-up have not yet been clarified.

Laboratory Examination

It should include electromyography (EMG) that is 4 extremity motor and sensory nerve measurement studies, cerebrospinal fluid (CSF) protein measurement, contrast-enhanced MRI imaging of spinal roots, brachial plexus and lumbosacral plexus, serum paraprotein examination, blood test measurements, antinuclear antibodies, thyroid function tests, extractable nuclear antigen antibodies, ganglioside antibodies, thorax CT tests (10). Hepatitis B and C viruses, Bartonella henselae, Mycoplasma pneumoniae, HIV, CMV and EBV

tests should be screened. Antibodies that target node of Ranvier proteins such as contactin-1, contactin-associated protein 1, and neurofascin 155 have been identified (6). EMG shows signs of sensorimotor demyelinating polyneuropathy, conduction block, slowing of conduction velocity, prolongation of distal motor latencies, prolongation of F wave latencies or unresponsiveness, and decreased CMAP are seen. Albuminocytological dissociation (protein elevation and normal white blood cell count) is seen in CSF examination (6, 9). Contrast enhancement of spinal roots on MRI supports the diagnosis of CIDP (11). Diagnosis is made based on many components including anamnesis, clinical examination, EMG, radiological and pathological findings, and evaluation of response to treatment.

Treatment

Clinical response and prognosis in CIDP treatment are heterogeneous. Intravenous immunoglobulin (IVIG) is the most effective and first choice method in the treatment of CIDP. Neutralization of autoantibodies and cytokines is achieved with IVIG. Two-thirds of CIDP patients respond well to treatments and require long-term treatment (4). In addition to IVIG, corticosteroids and plasma exchanges are used in treatment and are effective in 85% of cases. Treatment should be re-evaluated every 6 months to determine whether long-term treatment is needed and to discuss treatment alternatives. Response to treatment is defined as significant improvement in clinical examination. Motor symptoms generally respond very well to IVIG, while sensory symptoms respond more slowly. Adding an immunosuppressant or immunomodulatory drug may be considered, but there is insufficient evidence to recommend a specific drug (5). In the treatment of CIDP, rehabilitation is important along with drug therapy (6). The initial IVIG dose is 2 g/kg. The standard maintenance regimen is 1.0 g/kg intravenously every 3 weeks (7, 12). IVIG tapering treatments are necessary, but the risk that patients may not return to baseline levels must be taken into account. Data from clinical studies suggest that at least three IVIG treatments are necessary to detect objective response to treatment. Steroids are used less frequently than IVIG because they have more side effects (7). Immunosuppressant drugs are used in CIDP patients who are unresponsive or resistant to first-line treatments, despite the lack of evidence of their effectiveness in controlled studies. Among alternative treatments, rituximab is the most promising (13). Immunosuppression may be required with the use of agents such as azathioprine, mycophenolate mofetil, or methotrexate, especially in atypical variants of CIDP or in long-standing cases, but evidence from randomized controlled trials of these agents is insufficient (19). In pregnant women, plasma exchange is preferred (14). IVIG is not successful at the desired level in all

patients with CIDP or in all subtypes. The identification of neurofascin-155 and contactin-1 as IVIG-resistant IgG4 subtype nodopathies has demonstrated the inadequacy of IVIG in treating some forms of CIDP (8).

Rituximab (RTX)

RTX is a chimeric monoclonal antibody targeting the CD20 antigen used in the treatment of autoimmune neuropathies (15). It is also effective in CIDP with IgG4 antibodies that have distinct clinical features and are often resistant to first-line drugs (16). Over the last decade, antibodies (anti-IGg4) against proteins in the Node of Ranvier and paranodal region, including NF155, NF185, NF140, CNTN1 and Caspr1, have been detected in IVIG-resistant CIDP patients. In anti-IgG4 antibody positive CIDP patients, RTX has been observed to achieve better recovery rates compared to other treatment methods (17).

Discussion

CIDP is frequently confused with diabetic polyneuropathy, paraneoplastic polyneuropathy, vasculitic neuropathy, critical illness neuropathy, and lower motor neuron disease (10). If patients do not respond to IVIG, the diagnosis of CIDP should be reevaluated. POEMS syndrome can mimic CIDP, and an important clue is the lack of response to treatment (15). Although the etiology of CIDP is not fully understood, genetic factors are thought to play a role in predisposition to the disease. A familial risk of occurrence has been reported in CIDP patients. Several genes have been identified, such as the GA13-16 homozygous genotype of the SH2D2A gene, which has been found to be associated with the risk of CIDP, and these genes have been found to increase the risk of CIDP. The HLA-DRB1*13 allele has been found to be associated with the risk of CIDP (18). It has been suggested that diagnostic errors often result from misinterpretation of mild nerve conduction abnormalities and insignificant elevations in CSF protein (8). Another important issue is overtreatment even if the diagnosis of CIDP is correct (19). International guidelines recommend that in clinically stable patients, outcomes should be assessed every 6–12 months for the first 2–3 years of treatment and less frequently thereafter (e.g. every 1–2 years) (5). Adding an immunosuppressant to IVIG therapy may be effective in improving response rates or reducing IVIG doses (8). The diagnosis of CIDP can be made more specifically by detecting nodal and paranodal anti-IGg4 antibodies (12). More severe symptoms and recurrent disease courses may be observed in patients with positive anti-IgG4 antibodies (17).

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Chapter 3

Horse Ageing Process and Hallmarks

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1. Introduction

Ageing is a highly complex biological phenomenon. As a result, ageing is considered the most important risk factor for all age-related diseases, including infections, cancer, autoimmune disorders, and chronic inflammatory diseases (Santoro et al., 2021). Furthermore, ageing increases the risk of several chronic diseases, such as cerebrovascular and neurological disorders (Keshavarz et al., 2023).

In a study on aging, with a special emphasis on mammalian aging, nine temporal features representing common denominators of aging in different organisms have been listed. These features are; mitochondrial dysfunction, cellular senescence, stem cell exhaustion, genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, and altered intercellular communication (López-Otín et al., 2013).

Horses' health and well-being are greatly impacted by the complex biological process of ageing. Horses experience several physiological changes as they become older, which may have an impact on their lifespan and standard of living. Horse owners, carers, and veterinarians must all be aware of the signs of ageing in horses to implement management measures that improve health and reduce age-related illnesses.

When studying horse populations worldwide, it is discovered that there is a higher proportion of geriatric horses than has previously been reported. Advances in preventive health services and the increasing societal view of horses as companion animals rather than farm animals can be seen as a possible way to shape this situation. Furthermore, the provision of preventive services such as vaccination, endoparasite control and dentistry have undoubtedly been the greatest contributors to horse health and longevity in the last century. (DeNotta and McFarlane, 2023). The increase in this rate can be seen as an indication that horses' life spans are increasing because it is observed that resistance to diseases decreases with ageing in many living things. Therefore, a greater understanding of how horses age and how age affects immunity and disease susceptibility is required to develop focused preventive healthcare treatments for older horses (DeNotta and McFarlane, 2023).

The main biological indicators of ageing in horses are examined in this chapter, together with their implications for management and care and recent research findings.

2. What age is considered elderly for a horse?

At the moment, the definition of when a horse is considered geriatric or aged has evolved over the years and continues to be debated. Different criteria can be used to categorize a horse as geriatric, including chronological age, demographic age, and physiological or functional age (Timiras, 2007).

Research studies examining health and diseases in older horses have adopted various chronological age ranges—typically from 15 to over 20 years—as their inclusion criteria. This inconsistency can complicate direct comparisons of study results (Ireland, 2016). Depending on the age criterion used (e.g., chronological, demographic, physiological), a horse can be deemed old at 15 years, 20 years, or older, yet a survey indicated that horses are considered old at 20-25 years old (Herbst et al., 2023).

While multiple terms exist to describe older individuals, there is a growing recognition that these terms should reflect the significant diversity within a group that may span over 40 years in age (Kinsella and Phillips, 2005).

Given that horses are often classified as geriatric starting at age 15, yet can live beyond 40 years, it may be useful to adopt a categorization system from human geriatrics to better accommodate the considerable differences in aging rates among horses. Simply labeling all equids over 20 years as geriatric may overlook important physiological variations within this demographic (Brosnahan and Paradis, 2003b).

According to survivorship data, horses and ponies aged 15 years and older are categorized as old, while those aged 30 years and above are classified as very old. The average life expectancy in the general equine population is reported to be around 19 years. Though there are accounts of horses living into their 50s, the typical lifespan is estimated to be approximately 45 years, based on the oldest horses noted in various studies (Ireland et al., 2011; Ireland, 2016).

The average life expectancy of horses within the general equine population is estimated to be around 19 years (Mellor et al., 2001). While there are occasional reports of horses living into their 50s, research suggests that the typical maximum lifespan for horses is approximately 45 years, as indicated by the oldest individuals documented in various studies (Brosnahan and Paradis 2003a; Ireland et al., 2011; McGowan et al., 2010; Cole et al., 2005).

3. Appearance of an older horse

There are serious changes in the morphological appearance of older horses. However, most of these changes can be thought to be shaped by the emergence of certain diseases, because these diseases are among the most serious diseases for horses. Pituitary pars intermedia dysfunction, chronic weight loss, obesity,

osteoarthritis, laminitis, neoplasia, and cardiovascular dysfunction are among the more common conditions in older horses (Austin, 2023). However, even without any disease, the body can change considerably with aging.

One of the most recognisable mutant phenotypes in animals is greying with age in horses, which is a dominant inheritance trait. Horses who exhibit the greying with age phenotype have a propensity to melanoma and lose hair pigmentation while maintaining skin pigmentation. Originally fully coloured, these horses typically turn grey in their first year of life before turning white altogether. Human culture has been greatly influenced by the beauty of these white horses. White horses have frequently appeared in epics, literature, and art throughout history. This phenotype's appeal can be attributed in large part to the fact that the causative mutation solely affects hair pigmentation, leaving skin and eye pigmentation untouched (Rubin et al., 2024).

Figure 1 shows the color change in two separate horses, one that grays rapidly (top) and the other that grays slowly (bottom). As observed in the horses, the gray color changes considerably with age (Rubin et al., 2024).

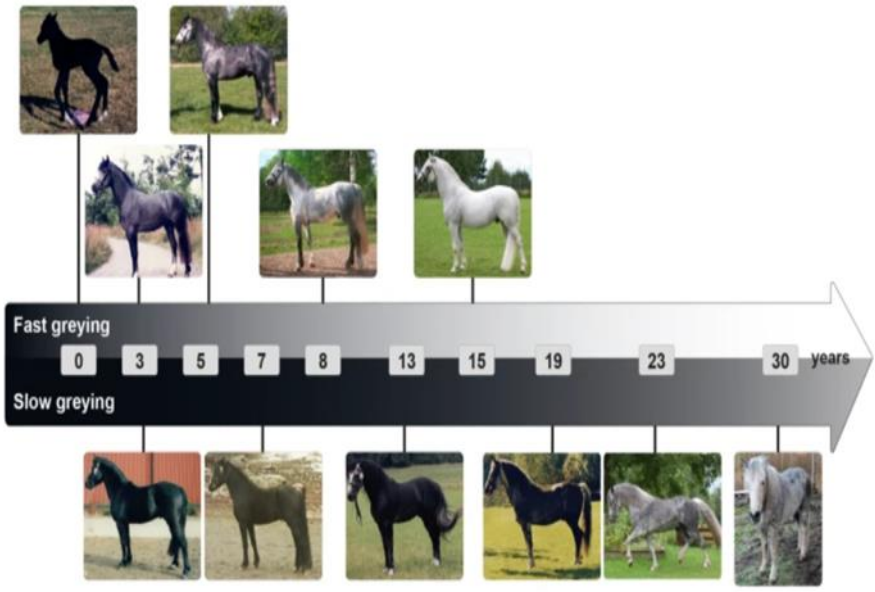

Figure 1. The gray color changes (Rubin et al., 2024)

There are some external signs of aging to look for in an older horse (Figure 2.). Some of these signs are;

- Drooping lips,
- Empty eyes,
- A sagging back and loss of muscle mass,
- Loss of strength in tendons and ligaments – especially around the heel joint,
- A dull coat with gray hairs around the mouth and eyes,
- Movements are stiff, slow, and more arrhythmic (The Horse Portal, 2024).

Figure 2. Older horse (The Horse Portal, 2024)

The structure and shape of the teeth and jaws of horses change considerably as they age. For example, after the age of 10, a Galvayne's Groove (tooth groove) is formed in the horse's teeth, as shown in Figure 3. It starts in the middle of the tooth's outer surface in a child aged ten. In 15 years, the groove runs halfway down the tooth; in 20 years, it runs the whole length of the tooth; in 25 years, the upper half of the groove disappears, leaving only the bottom half visible; and in

30 years, the groove is gone entirely. Furthermore, Figure 4 shows that older horses' mouths have longer teeth and a more angular appearance (Hoopes, 2024).

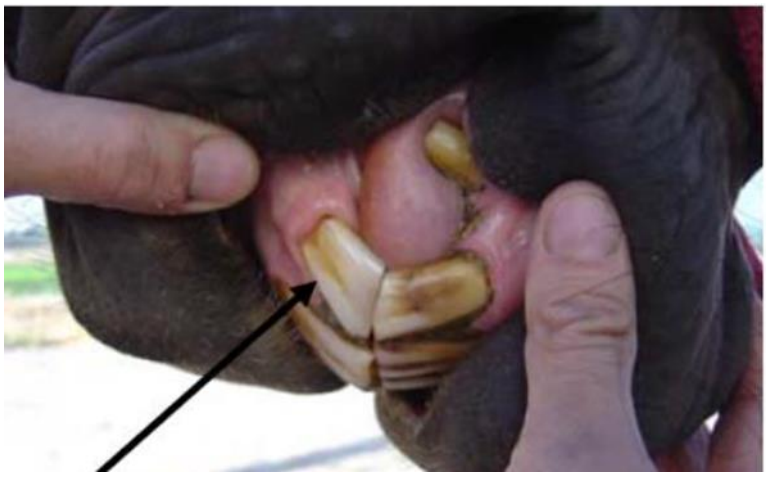

Figure 3. Galvayne's Groove (Hoopes, 2024)

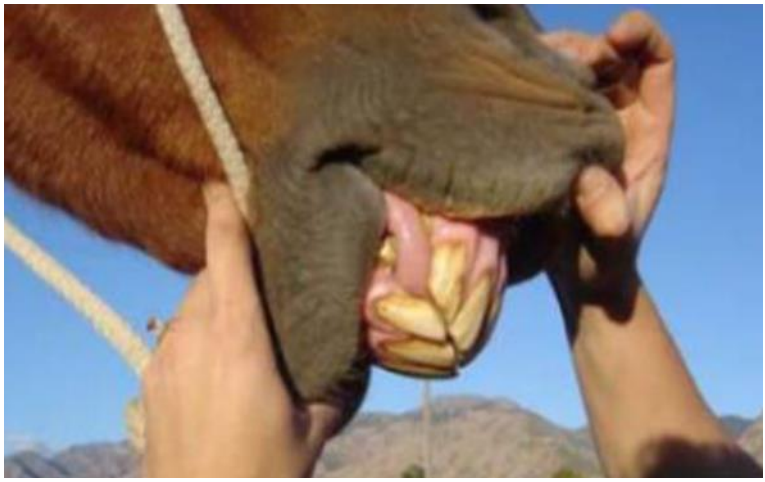

Figure 4. Older horse profile (Hoopes, 2024)

4. Senescence of Cells

One of the hallmarks of ageing is cellular senescence, which is defined by an irreversible stop in cell division. Similar to other animals, horses may experience a variety of adverse effects, such as tissue dysfunction and heightened vulnerability to illnesses, as a result of the buildup of senescent cells in different tissues. According to research, senescent cells contribute to "inflammaging," a chronic inflammatory state, by producing a range of pro-inflammatory cytokines

and chemokines (Kirkland and Tchkonia, 2017). Age-related conditions including osteoarthritis and metabolic syndrome can worsen as a result of this persistent inflammatory state (Baker et al., 2011).

Numerous stresses, such as oxidative stress, DNA damage, and telomere shortening, can cause cellular senescence. In ageing horses, the buildup of senescent cells has been connected to a reduction in organ function and general health. Senescent cell removal techniques, including senolytic treatments, have the potential to prolong life by reducing age-related dysfunctions (Zhu et al., 2015).

5. Shortening of Telomeres

The length of telomeres, which shield chromosomes from deterioration during cell division, is a key indicator of biological age. Telomere shortening in horses is associated with cellular ageing and elevated oxidative stress (Dantzer et al., 2008). Shorter telomeres have been linked in studies to a lower lifespan and a higher prevalence of age-related disorders (Eisenberg et al., 2017). Thus, telomere length could be a useful diagnostic for determining an older horse's health.

6. Failure of Mitochondria

Age causes a reduction in the function of mitochondria, which are necessary for the synthesis of ATP. In ageing horses, mitochondrial dysfunction causes oxidative stress and lower energy levels, which have a major effect on general health (Dzięgielewska and Dunislawska, 2022). According to research, improving mitochondrial function with dietary supplements like antioxidants and certain fatty acids can enhance energy metabolism and slow down the ageing process (Burtscher et al., 2024).

7. Ageing Immune System

The aging-related deterioration of immunological function is known as immunosenescence. According to Franceschi et al. (2018), this loss in older horses shows up as decreased antibody production and a decreased response to vaccines, making them more vulnerable to illnesses. Maintaining the health of elderly horses requires specialised vaccination schedules and routine veterinarian examinations. The management of coexisting medical conditions and nutritional support are two possible strategies to improve immune function (Ireland, 2016).

8. Modifications to Musculoskeletal Conditions

In horses, ageing has a major effect on the musculoskeletal system. Joint deterioration and discomfort are hallmarks of osteoarthritis, which is more common as people age (Van Weeren and Back, 2016). Furthermore, sarcopenia, or age-related muscular atrophy, results in a reduction in strength and mobility. Regular exercise to support joint health, dietary supplements high in glucosamine, chondroitin sulphate, omega-3, fatty acids, and veterinary treatments to reduce inflammation and pain are all important management measures (Jerosch, 2011).

9. Deterioration of Cognitive Function

As to some researchers, cognitive dysfunction or age-related behavioural changes in horses have not been recorded systematically; however, some indicate that cognitive impairment may occur. Senior horses with this condition usually exhibit abnormal behavioural changes and memory loss, such as confusion and disorientation in a familiar environment, inability to be led, and failure to recognise a familiar object; however, the relationship between these signs and age-related neurologic lesions has yet to be established (Youssef et al., 2016). To manage cognitive decline and improve older horses' quality of life, early detection and intervention—such as mental stimulation and environmental enrichment—are essential (Krebs et al., 2018).

10. Needs for Nutrition and Housed

Horses are housed in a wide range of facilities, and the characteristics of these facilities may have a big impact on disease risk factors and management strategies. While few elderly horses are stabled year-round, the bulk of them get regular field turnout for at least some portion of the year (Brosnahan and Paradis 2003a, Ireland et al., 2011, McGowan et al., 2010). The length of field turnout varies significantly from season to season (Ireland et al., 2011).

As horses age, their nutritional needs alter, requiring dietary modifications. Softer, easier-to-digest meals are crucial since dental issues can hinder chewing (Defilippis, 2006). To promote general health and treat aging-related disorders, nutritional strategies should concentrate on supplying enough protein, fibre, vitamins, and minerals (Fekete et al., 2022).

11. Conclusion

For efficient administration and care, it is crucial to comprehend the signs of ageing in horses. Horse owners and veterinarians can improve the quality of life for older horses by proactively addressing the physiological changes brought on

by ageing. Further horse geriatrics research will shed more light on the ageing process and offer insightful information for creating better care procedures. Ageing horses can be kept healthy, active, and comfortable into old age with a comprehensive program that includes regular veterinarian treatment, proper nutrition, and customised management techniques. In this respect, if a horse is considered to be older, almost all of its management needs to be modified, mostly regarding diet and exercise.

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Chapter 4

How Much Fluid, Electrolyte, and Mineral Loss Occurs When A Horse Sweats, and What Can Be Done Afterward?

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1. Introduction

When in their thermoneutral zone, which is between 5 and 25 °C, healthy adult horses can regulate their body temperature by accumulating and dissipating heat in a manner that keeps it between 37.5 and 38.5 °C. Horses may, however, suffer from heat stress in certain situations, such as during strenuous exercise in hot or hot-humid circumstances, where the body heat accumulation surpasses the dissipation (Kang et al., 2023). In horses, prolonged or extreme heat stress can cause heat stroke, anhidrosis, or brain damage. The ideal physiological system that plays a role in discharging or balancing this heat in horses is the sweating system. In other words, horses can maintain their body temperature through thermoregulation systems that allow sweat to evaporate. However, under hot and humid conditions, this heat distribution is limited (Wendt et al., 2007; Cheuvront and Haymes, 2001; Holcomb et al., 2014).

Sweating has been identified as a component that helps preserve the water balance during and after physical activity in hot weather (Jenkinson eet al., 2006). Horses use sweating as their primary means of releasing heat to resist thermal stress and maintain body temperature (Hodgson et al., 1994; Honstein and Monty, 1977). However, high ambient humidity jeopardizes the efficient operation of this mechanism (Foreman 1996). Therefore, management of this sweating period in horses is important for the sustainable welfare of the animals.

There are many reasons for sweating in horses, such as effort, anxiety or stress, infections, hormonal disorders and skin diseases (Hohl, 2024).

The exercising muscles of horses produce heat through metabolic reactions. The heat produced must be dissipated to prevent overheating. Therefore, the horse sweats and dissipates this heat by evaporative cooling (Auwerda, 2024).

Horse sweat has the same function as human sweating: it evaporatively regulates body temperature (Jenkinson et al., 2006). Sweating is a natural and basic process, but sweating too much or too little can cause problems. These include dehydration, electrolyte imbalance, skin irritation from excessive sweating, and overheating from lack of sweat.

Apocrine and eccrine sweat glands are found in horses, who are said to produce the most sweat of all domesticated animals. The apocrine glands are the main sweat glands. When your horse is exercising hard or is out in the heat, these are the ones that cause them to perspire. Whereas eccrine glands are limited to the sole of the foot, apocrine glands are present throughout the body of your horse (Eldrege, 2015). The apocrine glands, which emit sweat with a higher concentration of electrolytes and less protein than the eccrine glands in humans, are primarily responsible for producing perspiration in horses. For this reason, horses that work hard have a higher electrolyte requirement (McCutcheon and

Geor, 1998). A group of researchers investigating the number of sweat glands in horses found the sweat glands to be 3.10 ± 0.24 per mm2, which is higher than cattle, buffalo, goats, pigs and dogs (Raghav et al., 2022).

Foaming is observed in some horses during sweating. This is especially seen in times of stress or excessive effort, on a white sweat. The reason for this foaming is a protein called latherin. Since this protein has a soap-like structure, it creates a foam layer on the skin (McDonald et al., 2009).

2. Amount of fluid loss due to sweating

The amount a horse sweats varies depending on the environmental conditions, the type of work being done, and the fitness of the horse. Horses can lose 5 to 7 liters of sweat per hour while galloping for an hour in moderate temperatures. However, as temperature and humidity increase, these sweating rates approach 10 to 12 liters per hour (Auwerda, 2024). Furthermore, sweating rates are highly correlated with increasing ambient temperature and maximum skin temperature (Bullard et al., 1970).

Some researchers have stated that horses lose heat mostly through sweating when exercising in hot environments and try to reduce their body temperature by producing 10-15 liters of sweat per hour during moderate exercise in hot environments (Demirtaş, 2017).

It has been observed that horses (500-600 kg in weight) lose between 7-10 litres of sweat even during their free time, the same amount they lose during moderate exercise (Vervuert, 2024).

Jenkinson et al. (2006) showed the temperature and sweat loss according to the hourly intervals in their pattern (Figure 1). When the hours here were examined, a relatively slow rise to the plateau level was observed in the first 2 to 4 hours and then a gradual decrease in output was observed.

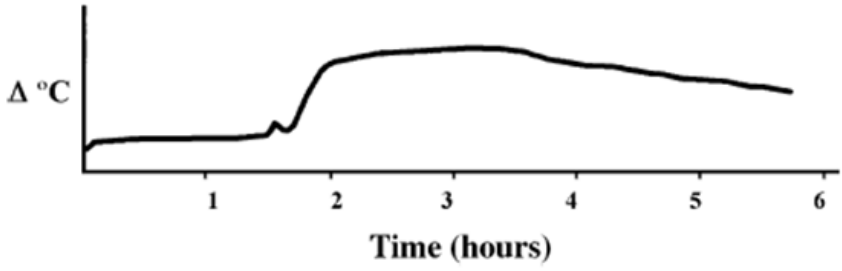

Figure 1. Pattern of cutaneous water loss from a horse (Jenkinson et al., 2006)

The plateau sweat outputs of the horses resting at different ambient temperatures obtained by the researchers are shown in Table 1.

Table 1. Sweat outputs of resting horses at different temperatures

Temperature (°C)	**Sweat rate (g m^{-2} h^{-1})**	**Reference**
23.4	55-75	Honstein and Monty, (1977)
24	15-30	Nakayama et al., (1957)
25	61-93	Johnson and Creed, (1982)
40	100	Allen and Bligh, (1969)
40	133-157	Montgomery et al., (1982)
40-43	267	Honstein and Monty, (1977)
45	306-1210	Johnson and Creed (1982)

In general, the increase in ambient temperature values has increased the amount of sweat coming out of the horses' bodies. In aphakic increases in ambient temperature, that is, increases above 40 ° C, the rate of sweating is quite high. For this reason, the general health of horses should be constantly monitored at these temperatures and horses should be provided with as much fluid as possible in extremely hot weather.

Some researchers (McCutcheon et al., 1999) who wanted to calculate the amount of sweat loss in horses calculated the ratio of the skin surface to the whole body when calculating the loss on the skin surface and looked at the total sweat loss from the skin on this ratio. The following formula was used to find the total calculation of the skin surface.

Total body surface area = 1.09 + 0.008 x Body Weight (kg) (Hodgson et al., 1993)

3. Sweating-related electrolyte and mineral loss

Decreases in sodium, potassium, and chloride levels in living beings' bodies and the resulting deficiencies can disrupt the animals' balance. Under physiological conditions, fluids and electrolytes in the body are in perfect balance. Even small deviations from this balance can lead to serious disorders that can eventually lead to death (Özkan et al., 2016).

Prolonged sweating causes horses to lose a significant amount of water and electrolytes. Replacing electrolytes and water is necessary to maintain hydration

and prevent dehydration. In equestrian sports, dehydration is a regular occurrence that can be prevented to promote equine welfare, better performance, and increased safety for both horses and riders. Significant dehydration happens after an hour or longer of physical activity or transportation. One efficient way to replenish water and electrolytes lost via perspiration is through oral electrolyte supplementation. The stomach and small intestine act as a holding tank for the water and electrolytes that are taken one to two hours before physical activity and transportation (Lindinger, 2022).

Low water intake by horses can lead to dehydration and in severe cases colic, leading to fatigue and muscle weakness. In severe electrolyte deficiencies, the horse can collapse and die if left untreated (Auwerda, 2024).

Sweat released from the body contains water, protein and minerals. Horses that sweat during exercise lose significant amounts of water and electrolytes such as sodium, potassium and chloride (Vervuert, 2024). Sodium, chloride, and potassium—collectively referred to as electrolytes—are the main minerals. Electrolytes assist keep the body's acid-base balance, facilitate muscular contractions, activate neurone function, and maintain fluid balance and circulatory function. Deficits in electrolytes can cause or exacerbate symptoms such as a horse eating less and drinking less water (Auwerda, 2024).

Since horse sweat is hypertonic compared to body fluids, water and large amounts of sodium, chlorine, potassium, magnesium and calcium are lost with sweat (Demirtaş, 2017).

Equine sweat is rich in electrolytes, with a mean Na of 120 mEq/l, K of 50 mEq/l, and Cl of 180 mmol/l, in addition to having lower quantities of calcium and magnesium (Ecker and Lindinger, 1995; McCutcheon et al., 1995). Apocrine sweat glands are found mostly in equine skin (Jenkinson et al., 2006), and they appear to release K and Cl but resorb a little quantity of Na (Gottlieb-Vedi et al., 1996; Snow et al., 1982; Wilson et al., 1988). According to Waller and Lindinger (2005), the concentrations of Na, K, and Cl in extracellular fluid are 140, 4, and 100 mEq/l, respectively.

When examining the sweat produced by horses and the resulting electrolyte loss, the highest amounts of sodium and Cl are lost, followed by K, calcium and magnesium. Electrolytes are not stored in the body and therefore must be provided by horses' diets (Auwerda, 2024).

Horse sweat is an isotonic to slightly hypertonic secretion that mostly consists of potassium, sodium, and chloride ions. Horse sweat's ionic composition is mostly determined by this ratio, which makes it susceptible to influences from the environment and level of exertion. Elevations in salt content are a result of increased sweating rate. Long-term exercise-related sweat fluid losses result in

severe ionic shortages, which alter the ion composition of skeletal muscle and raise the risk of muscular dysfunction. Additionally, increased perspiration fluid losses might lead to severe dehydration (McCutcheon and Geor, 1998).

Following an investigation by certain researchers (Kingston et al., 1997) into a study where horses engaged in low-intensity exercise for longer than three hours;

- Horses experienced hypohydration of approximately 6% despite voluntarily drinking water during two rest phases of the exercise protocol;
- Sweat rate and the ionic composition of sweat did not change during the three phases of exercise;
- Sweat Na and Cl were positively correlated with Sweat rate;
- Plasma osmolality remained unchanged despite a decrease in plasma volume of approximately 21% and a reduction in total body water of approximately 8.5%; and
- Approximately 95% of the metabolic heat load was dissipated during exercise, of which approximately 70% was lost through sweat evaporation and approximately 23% via the respiratory tract (Kingston et al., 1997).

Researchers also have estimated that during low-intensity activity, horses lose between 10% and 30% of their body heat through respiration (Heilemann et al., 1990; Hodgson et al., 1993).

A study by Kingston et al. (1997) found that whereas McConaghy et al. (1995) showed variations in sweat Na and Cl of up to 200 mM during 30 minutes of low-intensity exercise, this degree of fluctuation did not appear to differ between persons or over time.

Legume hay, grass hay and pasture grasses consumed by horses are generally rich in K, low in Na and variable in Cl concentrations. Whole grains such as corn, oats and barley are low in all electrolytes. Therefore, horses should be provided with NaCl supplements in their diets and, if necessary, they should be provided with sufficient electrolyte intake. The daily NaCl requirement for horses is, in simple terms, two tablespoons per day. However, as sweating increases due to heat, humidity, exercise or a combination of both, horses may need more salt. Therefore, this rate can be as little as four tablespoons of NaCl per day. However, this amount of salt should be offered to horses in divided doses between meals (Auwerda, 2024).

White salt blocks can provide enough NaCl for maintenance and light work if the horse consumes enough regularly. If you think a horse is not getting enough from the block then you can mix some salt into the feed (Auwerda, 2024).

Another study investigating salt intake in horses found that salt intake increased when horses exercised, and therefore it was important to ensure that horses reached their salt intake (Back and Houpt, 2023).

4. Sweating less or more

Horses should be well hydrated when they begin a race or competition. Horses can be effectively hydrated before competition and rehydrated after exercise by using electrolyte pastes and having unrestricted access to water (Auwerda, 2024).

When concerns about the amount of sweating in horses are examined, in most cases, little or no sweating is feared. Because sweating little or sweating much less than the amount that should be sweated can be a symptom of anhidrosis, an undesirable disease in horses.

Signs of poor thermoregulation from improper sweating in inappropriate environments are characteristic of anhidrosis in horses. Anhidrosis is a frequent condition in horses in hot, humid environments that affects horses of all ages, breeds, coat colours, sexes, and activities. Despite the significant emotional and financial toll that anhidrosis takes, its aetiology and causes are mostly unknown, and there is currently no proven cure for the condition (MacKay et al., 2015).

The skin, especially under the saddle pad, will be slightly wet and shiny on a horse who sweats often. The perspiration will move to his neck, chest, and legs if he is exercising a little more. A healthy horse will still have a decent hunger and thirst even if he is exhausted and perspiring heavily (Bennarroch, 2024).

The best way to determine if a horse is sweating excessively is to compare it to other horses in the same condition. If a horse is sweating excessively while other horses in the same condition are not sweating, this may be a sign of a problem. If a horse is sweating excessively, consider the following:;

- Has the horse had too much exercise for its fitness level?
- Did the horse make a journey that would expose it to climate change?
- Has the horse experienced something that caused stress or fear?

This has been shown to increase abnormal levels of sweating in horses, and if the horse is sweating excessively, exercise should be stopped and signs of dangerous heat stress should be evaluated (Bennarroch, 2024).

The temperature and relative humidity of the environment in which horses are located greatly affect the amount of sweating and the respiratory system of horses. For example, in cold conditions, the respiratory rate decreases and deepens to reduce heat loss through respiration and maintain gas exchange in the lungs (Mejdell et al., 2020) and thus the amount of sweat released decreases.

Because evaporation increases in low relative humidity due to the difference in vapour pressure between the atmosphere and the body surface, the efficiency of heat dissipation through evaporation is highly dependent on relative humidity (Geor and McCutcheon, 1998; McCutcheon and Geor, 2014; Girard et al., 2008). Sweat evaporation is negatively impacted by hot and humid weather, and gradually the horse loses its ability to sweat. The amount of heat lost by sweat evaporating from the animal's skin is only 5–10% of the total heat loss (Guthrie and Lund, 1998; McCutcheon and Geor, 2014), however, in this way the animal sheds a certain amount of sweat.

Horse sweat contains a protein called latherin, which causes a foamy bubble to form on the skin. This stops sweat from pouring off the coat, increasing evaporation (Eckersall et al., 1982; Hodgson, 2014) and thus ensures that the horse's sweat is removed.

As seen in research, ambient weather conditions, exercise and psychological conditions are directly effective in the amount of sweat in horses.

In another study where researchers examined the total amount of sweat and fluid loss in horses, they observed that the loss on day 0 was greater than the loss on day 21 (McCutcheon et al., 1999). In other words, this situation can be said that the sweat or fluid losses of a horse at the beginning of the training or working period may decrease over time as the horse gets used to training or working.

5. What to do after sweating

It's critical to allow your horse to fully cool down after a strenuous session in which they perspired. In order to guarantee that horses sweat appropriately, the following crucial factors should be taken into account:

- Give the horse enough time to thoroughly sweat off allow ample time: The duration of the cooling process varies based on the response of each individual and the intensity of the exercise. Hold off until the sweat has totally evaporated and the body temperature has returned to normal.
- Allow the horse to gradually cool down following an exercise session: Take a slow stroll, ideally a walk, to relax the circulatory system and cool the body gradually. A sudden stop or a cold water immersion should be avoided as these actions can tense your muscles.
- Shade: During the cooling phase, give the horse shade or a well-ventilated place. This promotes quicker cooling.
- Use sweat rugs: They can use a sweat-off blanket to assist cool off if necessary. These blankets aid in the efficient evaporation of perspiration by distributing it uniformly and absorbing it. Make sure the blanket is permeable, though, so the horse doesn't get too hot.

- Check the horse's condition again when it has cooled down and attained its typical body temperature. Look out for any signs of dehydration, stiff muscles, or other anomalies. They ought to speak with a veterinarian if they have any worries (Hohl, 2024).

If the horse needs electrolytes, this can be done with electrolyte supplements. Electrolyte supplements are available in many forms (granules, liquid, paste, etc.). A good electrolyte supplement should have the main components Na, Cl and K, which mimic the electrolytes found in sweat. The purpose of using electrolytes is to replace electrolyte losses in the horse's sweat and help promote rehydration (Auwerda, 2024).

Giving electrolytes one hour prior to activity gives muscles and other soft tissues a supply of water and ions, which may enhance cellular function and thermoregulation during exercise (Waller and Lindinger, 2021).

Effective electrolyte supplements are designed to restore the ions lost via perspiration; failure to do so may result in electrolyte imbalance. To aid stomach emptying and intestine absorption, enough water and electrolytes must be consumed to keep solution osmolality below that of bodily fluids. The electrolyte supplement should taste great, and horses should be educated to take it willingly before and during transportation, as well as before and after exercise (Lindinger, 2022).

However, before giving electrolytes, wait for the horse to drink water. Never give electrolytes directly to a horse with fluid loss. This can worsen the horse's fluid loss. To assess whether the horse is dehydrated; Take a pinch of skin from the shoulder point and release it. If there is no fluid loss, the skin should flatten immediately (0-1 seconds), but if it takes 2-3 seconds, the horse is dehydrated. In addition, the following important points should be considered in the dehydration or compensation of horses;

- Electrolytes should be given according to the amount of work the horse does,
- Electrolytes should be taken not only on the day of the competition but also during training,
- Electrolyte supplements with high sugar content should be avoided,
- Clean and fresh water should be made freely available,
- A salt lick bowl should be available to the horse at all times (Auwerda, 2024).

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Chapter6

Testicular Torsion in Children

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Introduction

Testicular torsion is extremely rare in the neonatal age group. The reported torsion rate in this age group in the literature is approximately 6 per 100,000. While testicular torsion in older children occurs due to torsion of intravaginal vessels, perinatal and neonatal torsion occurs extravaginally due to lack of testicular fixation or excessive mobility. (Ghosh et al. 2022). The rate of testicular torsion in children up to 18 years of age has been reported to be between 3.8 and 6.6 per hundred thousand (Mäkelä et al. 2007, Zhao et al. 2011). It is accepted in many countries that the incidence of testicular torsion peaks epidemiologically at the average age of 12 years, which is the years of entering adolescence, and within the first year after birth (Lee et al. 2014, Zhao et al. 2011).

Thirty-two to forty-two percent of patients who undergo surgical exploration due to torsion undergo orchiectomy, and studies have shown that asymmetric healthy solitary testes could also be affected and could face poor semen quality in the future (Dogra et al. 2004, Taskinen et al. 2008, Zhao et al. 2011).

Physiopathology in testicular torsion

As a result of ischemia in the testicular tissue that progresses directly proportional to the severity of torsion, excessive secretion of reactive substances in vascular endothelial cells and nitric oxide derivatives released to a lesser extent occur with ischemia-reperfusion injury in the stages up to gangrene. The entity that names the chain of biochemical events that occur as testicular compartment syndrome also covers the entire event (Cowled et al. 2020, Karagüzel et al. 2014, Kutikov et al. 2008).

Although antioxidant mechanisms are activated to eliminate reactive oxygen radicals (ROS), the balance in favor of ROS is disrupted and the increase in intracellular glutathione increases ischemia. Glutathione peroxidase and reductase antioxidant enzymes are insufficient to eliminate glutathione, as a result, the antioxidant defense mechanism cannot cope with oxidative stress (Aitken, & Roman. 2008, Çay et al. 2006, Payabvash et al. 2007). An experimental study has shown that circulating macrophages and mast cells in the normal testicle contralateral to the torsioned testicle induce germ cell apoptosis via TNF-alpha, and the seminiferous tubules suffer focal damage. In other words, the contralateral healthy testicle can be affected by this ischemic event. This process could be evident that the antioxidative tolerance limit of the humoral and cellular immune system has been exceeded (Rodriguez et al. 2006). Although it has been tried to prove that the use of antioxidant substances in coping with oxidative stress in testicular torsion can provide healing, the

reliability or healing role of these substances in practical clinical applications remains a mystery.

Clinical approach to testicular torsion

The fact that boys and their parents present to pediatric emergency services rather than outpatient clinics shows that scrotal pain is an emergency rather than an elective disease. In clinical practice, clinicians should be more careful in managing patients with acute scrotum, which shows the importance of this issue. The loss of the reproductive organ can cause legal negativity that cannot be tolerated by misdiagnosis. In a patient presenting with scrotal pain and swelling, testicular torsion should always be the first diagnosis that comes to mind. However, since nausea, vomiting, and abdominal pain may also be present in these patients, the patient's concealment of scrotal pain and the parent's focus on abdominal pain may delay diagnosis. Especially introverted adolescent boys may direct the practitioner to their abdominal pain, nausea, and vomiting; thus, delaying diagnosis. In our clinical experience, we have encountered such cases, although few.

While diagnosis is made with history and physical examination in most patients, today the gold standard diagnostic method with Doppler ultrasonography makes the practitioner's job easier (Bowlin et al. 2017).

Although the application of Barbosa et al., who proposed a scoring system that includes the parameters of scrotal or abdominal pain, scrotal swelling, nausea, and vomiting, is known, the fact that Doppler ultrasonography supports the diagnosis has limited the use of this scoring system in practical applications (Barbosa et al. 2012).

Although testicular torsion is the most important genitourinary emergency in boys, it should be considered in the differential diagnosis with epididymo-orchitis, infected hydrocele, torsion of the testicular appendix, and torsion of the epididymal appendix. Scrotal ultrasound is again the most important diagnostic tool in making this distinction (Manohar et al. 2018).

The hospital admission of a child with testicular torsion within the first four hours after the pain and other symptoms is a critical period in preventing testicular ischemic events. Studies and clinical experiences have provided a common consensus that patients presenting eight hours or later after the pain are exposed to testicular ischemia (Bowlin et al. 2017, Zhao et al. 2011).

Testicular torsion or testicular ischemia may also be encountered as a complication in undescended testes or incarcerated indirect inguinal hernia. According to our clinical experience, in these patients who have been exposed to ischemia for more than a day or in patients with only testicular torsion, we

believe that in cases where even a small amount of bleeding is detected through a small incision after opening the tunica vaginalis, it would be more appropriate to follow up the patient by fixing the testicle to the inner surface of the scrotum after detorsion (Ozdamar, & Karakus 2017). During surgery, opening the tunica albugunia with a small incision the absence of blood flow to the testis with warm compression for 5-10 minutes, and the absence of change in the necrotic color of the testicle makes orchiectomy inevitable. A previous study reported that 76% of children with testicles exposed to ischemia, especially for more than 24 hours, lost their testicles with orchiectomy. In other words, only detorsion was performed on the testicles of 24% of the patients. This information shows us that not every patient who was delayed underwent orchiectomy (Bayne et al. 2017, Ozdamar, & Karakus 2017). It is understood from the literature that urinalysis, urine culture, and tests for sexually transmitted infections do not change patient management in patients with testicular torsion (Frohlich et al. 2017).

In patients presenting to emergency services, when surgery is not immediately possible, manual detorsion should be attempted and then surgical intervention should be performed. Such an approach will relieve the patient's pain and may increase the opportunity for testicular salvage (Sharp et al. 2013).

Conclusion

Testicular torsion requires clinicians to approach with urgent management, as it will cause negative situations that will affect fertility in children in their later lives. Otherwise, a legal process is inevitable. The practitioners' approach to saving the testicle is the most important treatment element.

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Chapter 6

Gender-Determinated Sperm Use and Fertility

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INTRODUCTION

Studies on sex separation have begun to be conducted especially to prevent hereditary diseases that occur due to sex in humans. These studies have focused on which factors may affect sex (Hylan, 2007). Many techniques such as albumin and percoll gradient separations, cephadex columns, swim-up and flow cytometry have been used to separate sperm carrying X and Y chromosomes (Johnson et al., 1989).

In dairy farms, it is generally desired for the offspring to be female, while in periods when meat prices increase and the demand for male offspring increases, it is desired for the offspring to be male. From this perspective, it is of great importance for breeders to be able to adjust the sex of the offspring to be born as desired and to increase the number of offspring with the desired sex (Demiral et al., 2007). Thus, it will be possible to obtain breeding females in dairy farms and to increase the number of male animals that will meet the meat requirement in meat farms in a short time.

After the sperm separation process, 90% of the sperm of the desired gender can be obtained (DeJarnette et al., 2008; Garner and Seidel, 2003). Therefore, as a result of insemination with sexed sperm, the sex of the calf to be born will be determined by more than 90% (Seidel, 2007).

It has been reported that sexed sperm is used more in heifers (DeJarnette, Nebel, & Marshall, 2009; Healy, House, & Thomson, 2013). However, when sexed sperm and conventional sperm are compared in terms of some parameters such as birth weight, neonatal mortality, and survival rate of the calves, there is no difference (Tubman et al., 2004).

There is a difference in pregnancy rates in conventional or sexed sperm depending on whether the animal is a cow or a heifer. For example, while the pregnancy rate in cows varies between 35% and 50% in insemination with conventional sperm (Seidel and Schenk, 2008), this rate varies between 65% and 75% in heifers (Andersson et al., 2006; Sales et al., 2011).

When compared in terms of pregnancy rates, the pregnancy rate in cows with conventional sperm is between 35% and 50% (Seidel and Schenk, 2008) and between 65% and 75% in heifers (Andersson et al., 2006; Karakaya et al., 2014; Sales et al., 2011). In addition, the decrease in pregnancy rates in the use of sex-determined sperm varies in cows and heifers. For example, while the pregnancy rate decreases by 20% to 30% when using sexed semen in cows, this decrease is less in heifers, at 10% to 20% (DeJarnette et al., 2009; Seidel and Schenk, 2008).

HISTORY OF SPERM WITH DETERMINED GENDER

Studies on determining gender date back to before Christ (460-377) and were first conducted by the Greek philosopher Democritus. Democritus suggested that male offspring are born from the right testicle and female offspring from the left testicle. Another claim regarding the birth of male and female offspring is that the uterus has an effect on determining gender. According to this claim, it has been suggested that male offspring are born from the right uterine horn and female offspring are born from the left uterine horn. Hippocrates also suggested that male offspring are born from strong sperm and female offspring are born from weak sperm. Later, in 1677, Anton Van Leeuwehoek defined sperm without gender distinction (Klinc and Rath, 2006).

Separating X and Y chromosome sperms before fertilization is the most effective way to determine the sex of the offspring. For this purpose, separation processes were carried out using many different features of sperm (speed, density, immunological). However, they were not very successful (Klinc, & Rath, 2006).

Garner's project in 1982, which was about separating sperm containing X and Y chromosomes, was supported by the US Department of Agriculture. Within the scope of the project, different DNA contents were detected in many animal sperm (cattle, pig, rabbit) using the flow cytometry method, but unfortunately these separated sperms died (Garner & Seidel, 2008).

The first separation of sperm as sex was made by Guyer in 1910. However, Johnson and his friends made the separation of sperm as sex based on the DNA differences of X and Y chromosomes in 1989. Johnson and his friends made this separation process with the flow cytometry technique. In fact, the flow cytometry technique has emerged as the most reliable technique in recent years (Blondin et al., 2009; Van Munster et al., 1999; Welch and Johnson, 1999). Although the number of separated spermatozoa obtained in the relevant technique is low, abnormal morphology and dead spermatozoa are also separated (Seidel and Garner, 2002; Seidel, 2007). While the flow cytometry technique could initially separate 400,000 sperm per hour and needed 25 hours to produce just one straw (Loggan, 2019), in 2008, the sensors and software programs were changed, and both the speed of separation of sperm and the reliability of the device were increased. In fact, a straw could be produced in a time of 9 minutes (Garner & Seidel, 2008).

ADVANTAGES OF USING GENDER-DETERMINED SPERM

Using gender-determined sperm will provide maximum benefit in terms of biosecurity by allowing females with good breeding value to remain in the farm (De Vries et al., 2008) and will not require the purchase of new breeding females from outside the farm (Holden and Butler, 2018; Seidel, 2003). Another important advantage of using gender-determined sperm is that it accelerates genetic progress (Chebel et al., 2010). In fact, it has been reported that there is a 15% increase in the rate of genetic progress with the use of gender-determined sperm (De Vries et al., 2008). In fact, the use of gender-determined sperm will increase the rate of genetic progress in our country's animal husbandry by easily providing both the breeding stock needed in dairy farms and the availability of suitable animals for fattening.

It is possible to obtain offspring of the desired sex at rates of more than 90% with sex-determined sperm (De Vries et al., 2008).

DISADVANTAGES OF USING GENDER-DETERMINED SPERM

The most serious problem with using gender-determined sperm is that the pregnancy rate is lower than conventional sperm. If a comparison is made, the pregnancy rate in gender-determined sperm is 75-80% of the pregnancy rate in conventional sperm (Vishwanath and Moreno, 2018). There are many reasons why the pregnancy rate is lower in gender-determined sperm. In particular, the low sperm count in gender-determined sperm and the decrease in sperm motility after thawing are the leading causes of low pregnancy rates (Bodmer et al., 2005; Karakaya et al., 2014). In addition, it has been reported that sperm are exposed to certain stress factors (chemical and physical) during the sperm separation stage, shortening their lifespan (Maxwell et al., 2004). It has also been suggested that some changes occur in sperm functions which are similar to capacitation during the separation process, and that capacitation begins and even accelerates after these changes (Vazquez et al., 2003).

It has been reported that some solutions used in the separation process cause a decrease in sperm movements. It has also been reported that the chromatin stabilization of sperm is damaged as a result of the separation process and that this situation is caused by the pressure and ultraviolet radiation applied during the process. In addition, there is information that capacitation and acrosome reactions accelerate during freezing and thawing, reducing the life span of sperm (Garner, 2006). In summary, the life span and fertilization ability of sperm of known sex in the female genital tract decreases. Therefore, the pregnancy rate will decrease compared to conventional sperm (Mocé et al., 2006). In addition to all these difficulties experienced in the separation process,

the fact that only 30% of the sperm of the desired sex can be obtained from the processed ejaculate constitutes the biggest handicap in the use of sexed sperm (Sharpe & Evans, 2009). In addition, although it has been reported that sperms are damaged by the sperm separation process, it has been reported that there are no anomalies in calves born after insemination using sexed sperm (Seidel & Garner, 2002).

Another difficulty in the use of sexed sperm is the high cost of the device used in sperm separation and the need for experts who can perform this process (Garner, & Seidel, 2008). Despite all these difficulties listed, the critical issue in improving the pregnancy rate is the insemination time. Because the morning and evening rule in insemination with conventional sperm is not the appropriate insemination time for sexed sperm, and it has been reported that the pregnancy rate can be increased by slightly delaying the insemination time. For example, in a study conducted on Holstein heifers, it was reported that there was a 10% increase in pregnancy rate as a result of insemination performed 20 hours after the onset of estrus and up to the 24th hour.

FACTORS AFFECTING PREGNANCY IN GENDER-DETERMINED SPERM

1. Sperm Separation Method

The separation process causes some damage to the sperm. Due to this damage, pregnancy rates decrease. Thus, the pregnancy rate in gender-determinated sperm is 60-90% of the pregnancy rate in conventional sperm (Healy et al., 2013).

2. Sperm Concentration

While conventional sperm straws contain approximately $20x10^6$ sperm, gender-determinated sperm straws contain $2.1x10^6$ sperm. When this information is taken into consideration, it is seen that the rate in gender-determinated sperm straws is much lower than in conventional sperm straws. From this point of view, the low pregnancy rate is attributed to the low sperm concentration in gender-determinated sperm straws.

3. Fertility Differences Between Bulls

The fact that pregnancy rates are different when different bulls are used has been attributed to the difference in the fertility characteristics of the bulls. In fact, pregnancy rates have also been different when different bulls are used in studies. This shows that the sperm of each bull is not affected by the separation process to the same extent. In fact, while the sperm of some bulls is affected by

the sperm separation process, the sperm of some bulls is not affected by this process (Macedo et al., 2013). For example, in a study conducted on meat heifers, the sexed sperm of 3 different bulls was used and the pregnancy rates obtained were 42.6%, 44.4% and 54.9% (Colazo et al., 2018). From this point of view, it will be possible to make profitable breeding as a result of inseminations with high fertility bulls in the use of sexed sperm or conventional sperm.

4. Insemination by Observing Estrus After Estrus Monitoring

In the use of sex-determined sperm, when the estrus is monitored and the animal showing estrus is inseminated, the insemination time is adjusted and the insemination is performed by observing estrus before insemination, a higher pregnancy rate is achieved (Meyer et al., 2012). It has been reported that a good pregnancy rate is achieved after the use of sex-determined sperm in heifers after observing estrus. It has also been reported that pregnancy rates are good after the use of sex-determined sperm in the first inseminations of cows after observing estrus and following estrus (DeJarnette et al., 2009; Norman et al., 2010). In fact, it has been reported that there is a 43% pregnancy difference between the pregnancy rate in heifers inseminated by observing estrus and using sex-determined sperm after estrus monitoring and insemination without observing estrus (Meyer et al., 2012).

5. Different Insemination Protocols

Studies have reported that different pregnancy rates are obtained using different insemination protocols when using sexed sperm. For example, while a pregnancy rate of 27.9% is obtained using the Ovsynch protocol, this rate is determined as 35.5% in the Presynch-Ovsynch protocol. Again, the pregnancy rate obtained in the Double-Ovsynch protocol is reported to be 47.6% (Karakaya et al., 2019).

6. Number of Inseminations

Although the pregnancy rate is quite good in the first insemination when using sexed sperm, it has been reported that the pregnancy rate decreases in subsequent inseminations (Macedo et al., 2013). For example, in inseminations performed on jersey heifers using sexed sperm, the pregnancy rate in the first insemination was determined to be 55%. However, the pregnancy rate obtained in the same study was determined to be 35% after the third insemination. Based on this, it is possible to say that the pregnancy rate decreases as the number of inseminations increases in the use of sexed sperm.

7. Time of Insemination

When inseminating with sexed sperm, it has been reported that pregnancy rates can be better if the insemination is done not at the time of insemination as with conventional sperm, but rather if the insemination is done a little later and closer to ovulation (Sales et al., 2011). For example, in one study, the pregnancy rate obtained when heifers were inseminated with sexed sperm 18-24 hours after the onset of estrus was 55%. When the insemination time was 0-12 hours after estrus, the pregnancy rate obtained was determined to be 25% (Schenk et al., 2009).

8. Follicle Diameter Measured at the Time of Insemination

In the use of sex-determined sperm, the follicle diameter measured at the time of insemination affects the pregnancy rate. For example, in timed inseminations using sex-determined sperm, if the follicle diameter measured at the time of insemination is more than 8 mm, pregnancy is achieved at a rate of 57%. However, if the follicle diameter measured at the time of insemination is less than 8 mm, the pregnancy rate has been reported to be 31% (Sa Filho et al., 2012). Despite these claims, there are also studies reporting that the pregnancy rate is not affected by the follicle diameter at the time of insemination (Hillegass et al., 2008).

9-Heat Stress and Season

It has been reported that heat stress also has an effect on the pregnancy rate when sex-determined sperm is used. This effect is negative and it has been reported in studies that it causes a decrease in pregnancy rate of 10-30% (De Rensis and Scaramuzzi, 2003). In a study conducted on Holstein heifers, the pregnancy rate obtained in the spring season was 53.9%, while the pregnancy rate in the fall season was 50.8%. It was announced that the pregnancy rate obtained in the winter season was 50.7% (Cerchiaro et al., 2007). In another study in which insemination was performed using sexed sperm, the pregnancy rate in the warm season was 33%, while the pregnancy rate detected in the cool period was 64% (Donovan et al., 2003). In studies conducted on cows, the effect of heat also caused a decrease in the pregnancy rate. Because the pregnancy rate obtained in the cool period was 21%, and in the hot period it was 16% (Mellado et al., 2014).

10- Differences in Experience Among Inseminators

The difference in experience between the physicians or technicians who will perform the insemination in preparing the straw and performing the

insemination may have affected the result to a very small extent (DeJarnette et al., 2011; López-Gatius, 2012).

COMPARISON OF PREGNANCY RATES OF GENDERED SPERM WITH CONVENTIONAL SPERM

In many reported studies, it has been reported that the pregnancy rate obtained after insemination with gendered sperm is lower than the pregnancy rate obtained after insemination with conventional sperm. For example, in a study on Holstein heifers, the pregnancy rate obtained with gendered sperm was 45%, while the pregnancy rate obtained with conventional sperm was 56% (DeJarnette et al., 2009). In a study on beef cows, while the pregnancy rate obtained with conventional sperm was 56%, the pregnancy rate obtained with gendered sperm was 49% (Crites et al., 2018). In another study, the pregnancy rate obtained with the gendered sperm/conventional sperm ratio was reported as 40%/56%. Despite all these results, it has been reported that pregnancy rates in beef heifers are the same in both conventional sperm and sexed sperm (Thomas et al., 2017).

CONCLUSION

With the use of sexed sperm, it is possible to obtain female calves that will meet the breeding needs in dairy farms and male calves that will meet the meat needs in meat farms. The fact that the pregnancy rates obtained with the use of sexed sperm are lower than the pregnancy rates obtained with conventional sperm is a major disadvantage. However, the use of sexed sperm in the first inseminations of heifers and cows after estrus is observed by following estrus, creates an improvement in pregnancy rates. In addition, insemination with sexed sperm at a time closer to ovulation rather than at the standard insemination time creates an improvement in pregnancy rates.

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