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THE IMPACT OF AGE AND NATURAL DEGENERATIVE PROCESSES IN THE TESTES OF STALLION EJACULATE

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ABSTRACT

The aim of our study was to assess the age and anatomical structure of stallion testes with regard to the quality of the produced ejaculate. Stallions were divided into three age groups. The first group (n=8) consisted of individuals aged 3–5 years, the second group (n=8) included stallions aged 6–11 years, and the oldest group (n=8) comprised individuals aged 12–25 years.Our study focused on reproductive parameters such as ejaculate volume, sperm concentration, and sperm motility. Significant differences were observed in sperm concentration and ejaculate volume. Sperm concentration decreased with age, with a reduction of 44.45 million sperm when comparing the first and second age groups and a further decrease of 43.79 million sperm between the second and third groups. In contrast, ejaculate volume exhibited an increasing trend with age, showing a difference of 8.32 mL between the first and second groups and 5.1 mL between the second and third groups. Additionally, we performed a palpation examination to assess degenerative processes associated with aging. We evaluated the parenchymal tone, the positioning of the testis within the scrotum, and testis size. No significant differences were found that would indicate an association between changes in testicular tone and the examined ejaculate parameters.

Keywords: Age, palpation examination, reproductive parameters, testicular dysfunction

INTRODUCTION

Horses are incorporated into the breeding process based on different criteria than other livestock species. Their inclusion is determined by pedigree, conformation, movement mechanics, and performance rather than by andrological and reproductive indicators. Despite this, breeders and stallions are subject to strict breeding deadlines to comply with the covering season. Foals born at the beginning of the season have a significant physiological advantage over those born later during the competitive season **(Griffin *et al.* 2019)**. Apart from the mare's readiness, stallion management is equally crucial. Spermatogenesis is a highly sensitive process that can be influenced by even minor factors. The study by **Farnia *et al.* (2025)** examines how stallion management affects key reproductive parameters. The majority of breeding stallions demonstrated reduced fertility due to poor breeding decisions and inadequate nutrition. Additionally, environmental and seasonal changes were identified as major factors negatively impacting reproductive potential. Welfare plays a significant role in stallion management. To prevent injuries caused by fights, stallions are often isolated, sometimes even deprived of visual contact with other horses. Such practices frequently lead to the development of various stereotypic behaviors or inappropriate interactions with humans, as stallions are denied their natural physiological and social needs. This disruption of mental and physiological well-being may directly impact ejaculate quality **(Popescu *et al.*, 2022)**. Spermatozoa exhibit a highly specialized structure compared to other cells and display considerable morphological variability. Overall sperm quality is essential not only for the fertilization of the oocyte but also for maintaining pregnancy and ensuring proper embryonic development **(Wysokińska, 2022)**. Due to various factors, artificial insemination (AI) is increasingly preferred in equine breeding. Compared to natural mating, AI offers several advantages, the most fundamental being the evaluation and health screening of stallion ejaculates. Subfertile stallions achieve higher reproductive success, and AI enables them to maintain a concurrent sports career while participating in breeding. AI is also beneficial for mares with strong immunological responses to semen components, as insemination with a minimal dose is possible. Additionally, AI increases the number of mares that can be bred by a single stallion and eliminates the risk of sexually transmitted diseases and injuries associated with natural mating. Another significant advantage is the removal of geographical barriers, allowing for the importation of semen without transporting the mare **(Morel, 1999).** Highly fertile stallions can cover a large number of mares throughout their reproductive lifespan. However, while many stallions initially achieve high pregnancy rates, fertility inevitably declines over time. Numerous factors contribute to reduced fertility in aging stallions, including physical fitness, body condition, mating ability, and ejaculatory dysfunction **(Blanchard *et al.*, 2012).** Stallions at breeding stations should undergo reproductive examinations at least twice a year, including blood tests and hormonal profiling. Routine procedures should include palpation and ultrasonographic examination of the testes, as well as assessment of penile health. Furthermore, transrectal ultrasonography should be performed to evaluate accessory sex glands. Standard ejaculate analysis encompasses assessments of color, volume, concentration, morphology, and sperm motility **(Samper *et al.*, 2009).** Among the critical components influencing fertilization success are seminal plasma proteins **(Karastoki, Katila 2008)**. Modern research techniques, such as flow cytometry and gel electrophoresis, enable precise characterization of these proteins **(Mogielnicka-Brzozowska & Kordan, 2011).** In addition to proteins, other seminal plasma components, such as antioxidants and enzymes, serve as fertility predictors, as this fluid contains essential elements necessary for sperm survival **(Talluri *et al.*, 2017)**. During semen transportation, cooling is required to maintain a lower temperature than body heat, preventing the accumulation of waste products and free radicals that induce oxidative stress and impair sperm function. Providing an adequate energy and nutrient source for sperm is equally important **(Cuervo-Arango *et al.*, 2015)**. Lowering the storage temperature reduces microbial activity and sperm metabolism, but not to the extent that it causes cellular damage **(Wiebke *et al.*, 2022)**. In modern equine reproductive management, stallion evaluation should not be limited to a basic spermiogram analysis. In addition to standard reproductive parameters, comprehensive physical examination, biochemical assays, sperm function tests, and endocrine assessments should be considered. These diagnostic tools are crucial for identifying deviations from physiological norms and can rapidly detect degenerative disorders caused by trauma or aging-related testicular degeneration, commonly referred to as idiopathic testicular degeneration. Currently, no precise test exists to assess the genetic predisposition to fertility **(Ball, 2008)**. The SP22 protein has been proposed as a biomarker of stallion fertility, integrating into spermatozoa during spermiogenesis and playing a role in oocyte interaction. Aging-related testicular degeneration alters its expression. Additionally, monitoring anti-Müllerian hormone (AMH) levels can help identify testicular dysfunction. AMH is a biomarker of testicular damage resulting from toxic exposure, with elevated levels in stallions suffering from testicular degeneration induced by anti-spermatogenic compounds **(Waqas *et al.* 2024)**. **Blanchard *et al.* (2008)** described a suppression of estradiol and FSH levels in stallions with compromised fertility. Testicular degeneration primarily affects germ cell development during spermatogenesis, leading to an approximately 43%

reduction in sperm production. Meiosis-associated losses include a decrease in primary spermatocytes, while elongated spermatid attrition reaches approximately 35%. Idiopathic testicular degeneration or age-related testicular degeneration are terms used to describe physiologically diminished fertility in aging stallions, which can result in economic losses in the equine breeding industry **(Turner, 2019)**. This condition is typically progressive, leading to a gradual decline in the production and quality of physiologically normal, progressively motile spermatozoa. As sperm output in the ejaculate declines, pregnancy rates decrease. In breeds allowing artificial insemination, several management strategies can optimize reproductive performance in aging stallions with declining testicular function. These strategies include reducing the number of insemination doses per ejaculate, concentrating diluted ejaculates, utilizing deep horn insemination techniques, and applying sperm separation methods to enhance insemination quality in low-dose insemination programs. Intensified reproductive management can improve pregnancy rates, thereby increasing the number of foals sired by aging stallions with declining testicular function **(Blanchard *et al.*, 2012).** Intracytoplasmic sperm injection (ICSI) is another reproductive strategy. This technique is particularly beneficial for stallions that have died with only a limited supply of frozen semen available or those suffering from health conditions preventing semen collection. Epididymal spermatozoa retrieved post-castration or post-mortem can also be used for ICSI **(Ramiréz-Amagez *et al.*, 2023).** ICSI allows for sperm selection based on morphology and motility, typically performed under 200- to 400-fold magnification **(González-Castro *et al.*, 2018).** While age-related testicular degeneration is commonly observed in older stallions, young stallions can also experience temporary testicular dysfunction due to hyperthermia or anti-GnRH vaccination. This condition is reversible. Testicular degeneration is characterized by acquired atrophy of seminiferous tubules, leading to testicular shrinkage and a direct decline in ejaculate quality. In advanced cases, stallions exhibit markedly altered testicular parenchyma with a soft consistency **(Da Silva- Alvaréz *et al.*, 2022)** The initial phase of age-related testicular dysfunction in stallions is characterized by a declining sperm production rate. Reduced spermatogenic activity leads to a gradual decrease in fertility. Early in this process, pregnancy rates may remain unaffected unless the stallion is breeding a large number of mares. However, as spermatogenic function continues to deteriorate, this phase is frequently associated with a reduction in the percentage of morphologically normal and progressively motile spermatozoa in ejaculates, although significant changes in sperm DNA integrity are generally not observed. Older stallions with declining testicular function may also produce ejaculates with a low number of motile sperm that have a shortened lifespan (i.e., rapid in vitro motility loss). For stallions whose semen can be cryopreserved, freezing ejaculates while the stallion still maintains normal fertility can ensure future reproductive use when semen quality and fertility begin to decline **(Blanchard *et al.*, 2012).** Due to reduced mitochondrial activity, spermatozoa from aging stallions are likely to exhibit lower motility and increased susceptibility to oxidative stress. Aged stallion ejaculates contain higher levels of reactive oxygen species than antioxidants, leading to oxidative stress **(Darr, 2017)**. Additionally, testicular aging affects Sertoli cell populations. In young stallions, Sertoli cell numbers are lower, increase with age, stabilize, and subsequently decline between 13 and 20 years of age. Very young stallions exhibit Sertoli cell levels at approximately 1×10^9,, increasing to about 1.8×10^9, in 2- to 3-year-olds, and reaching 2.6×10^9, in 4- to 5-year-old stallions compared to their younger counterparts. This Sertoli cell population remains stable until approximately 20 years of age before declining again. The number of Sertoli cells is directly correlated with parenchymal mass and daily sperm production. The presence of Leydig cells is also an indispensable component, as they are responsible for testosterone production. They are localized near Sertoli cells. Leydig cells are rich in endoplasmic reticulum, which plays a crucial role in testosterone synthesis by converting cholesterol into pregnenolone and subsequently into testosterone. The volume of Leydig cells changes with age, and between the ages of 2–3 years and 13–20 years, it increases from approximately 24 ml per testis, while the volume of seminiferous tubules increases by approximately 55 ml. The length of the seminiferous tubules also changes depending on the stallion's age, ranging from approximately 2 km to 2.8 km. In addition to these changes, the color of the testicular parenchyma darkens, and the overall testicular weight increases by up to 96 g. The weight of a single testis in 1.5–2-year-old stallions is approximately 20–90 g, with a mixture of light and dark spots appearing on the parenchyma. These spots indicate changes in function and parenchymal composition. Lighter areas contain a higher volume of seminiferous tubules and a greater presence of primary spermatocytes, while the number of Leydig cells remains unchanged. However, there is an increase in macrophages within the darker parenchyma of the testis **(Johnson *et al.*, 1997).** A decline in fertility is a common phenomenon in older stallions and is associated with testicular degeneration. Additionally, aging leads to a reduction in the function of accessory sex glands. Given the implications of testicular degeneration, continuous monitoring and examination of older stallions are necessary. A critical aspect of breeding management involves testicular evaluation before the reproductive season. Ultrasonography is a suitable diagnostic method, capable of identifying pathological conditions or severe health issues such as abscesses or hematomas **(Turner *et al.*, 2019).** Palpation of the testes should be a routine procedure in every reproductive center. Each testis must be evaluated separately during the examination. Upon palpation, the testis should exhibit a firm, elastic consistency. A smaller or softer testis may indicate the onset of a degenerative process or another traumatic condition requiring intervention. Additionally, in cases of testicular degeneration, stallions frequently develop wrinkling of the tunica albuginea during palpation. During such an examination, in addition to assessing testicular consistency, other factors should be considered, including scrotal temperature, potential external injuries, or pathological findings. Routine examination should also be supplemented with ultrasonography, which is essential for guiding biopsy procedures by accurately determining the optimal sampling site **(Sitters, 2021).** An increase in scrotal temperature, which directly affects physiological spermiogenesis, may also result from testicular torsion. Elevated testicular temperature accelerates cellular metabolism, leading to increased oxygen consumption within the tissue. Hypoxia significantly contributes to cell death, initiating testicular degeneration **(Neto *et al.*, 2013).** Spermiogenesis is a prolonged and sensitive process that is easily disrupted by various factors. It can also be adversely affected by postoperative complications, bleeding, or inflammation, which may significantly reduce sperm count, lead to sperm agglutination, or even result in complete azoospermia **(Gehlen *et al.*, 2001).** Immune-mediated subfertility is caused by the presence of antibodies in seminal plasma. These antibodies are typically a consequence of disrupted testicular homeostasis, such as in orchitis, which directly affects testicular function. Antibodies may persist in ejaculate for up to two years after disease resolution, highlighting the importance of maintaining a thorough medical history of the stallion to rule out alternative causes or potential testicular dysfunction **(Ferrer *et al.*, 2018).** Histopathological examination of testes affected by testicular dysfunction typically reveals a reduced seminiferous tubule diameter, with immature cells occasionally present in the lumen of the seminiferous tubule or directly in the ejaculate. In severe cases, fibrotic tissue replaces normal structures, and the tubules lack spermatogenic cells. Additionally, fibrosis or calcification of the testicular parenchyma may be observed **(Turner, 2019).** No treatment exists for idiopathic testicular degeneration. Therefore, early detection is crucial, as it can extend the reproductive lifespan of breeding stallions by several years **(Waqas *et al.* 2024).**

MATERIAL AND METHODS

Semen collection and processing

The research was conducted at a reproductive center in Germany, involving a total of 24 Holstein stallions. The stallions were divided into three groups based on age: the first group consisted of stallions aged 3–5 years (1. Group - Y), the second group included those aged 6–11 years (2. Group - M), and the third group comprised stallions aged 12–25 years (3. Group - O). Each group contained eight breeding stallions. The study lasted for two months, with semen collection occurring six days per week. The stallions were housed in individual box stalls with straw bedding. Their diet consisted of high-quality meadow hay (10 kg/day), an energy-dense concentrate (3.6 kg/day), and an additional 1.5 kg of alfalfa, supplemented with 200 g/day of mineral additives. Water was available *ad libidum*. The stallions underwent a training regimen based on performance criteria appropriate for their age. Their daily routine included both training sessions and turnout time in paddocks. Semen samples were collected in a specialized collection room via stallion mounting on a phantom. The ejaculate was captured using an artificial vagina (Hannover, Minitube, Germany). The interior of the artificial vagina was fitted with a disposable plastic liner coated with sterile, neutral petroleum jelly. The vagina was filled with warm water, and pressure was adjusted by inflating air according to the individual preferences of each stallion. The ejaculate was collected into a preheated glass test tube. Immediately after collection, the ejaculate was filtered through a specialized filter (Minitube, Germany) designed to remove the gel fraction and impurities.

Evaluation of reproductive parameters

The semen volume was then measured using a preheated graduated cylinder, and sperm concentration was determined using a photometer SDM1 (Minitube, Minitube, Tiefenbach, Germany). The samples were diluted with a preheated milk-based extender (Kenny's extender) prepared before collection. Sperm motility was subsequently assessed by the microscope Hund Wetzlar H500 with 20× magnification using a Bürker chamber. To estimate degenerative processes, testicular palpation was performed while the stallions were standing. The evaluation included assessing testicular size, position within the scrotum, and parenchymal firmness for each testis individually.

Statistical analysis

GraphPad 8 (GraphPad Software Inc., San Diego, CA, USA) was used for all statistical analyses. The mean, minimum, maximum, standard deviation (SD) and coefficient of variation (CV %) calculations were part of the descriptive analysis. All collected data were exanimated using the Wilcoxon rank-sum test, also known as the Mann-Whitney U test. Furthermore, the comparison between each group of stallions was evaluated using Pearson correlation. The level of significance for the comparative analysis was set at ***($p < 0.001$); **($p < 0.01$); *($p < 0.05$).

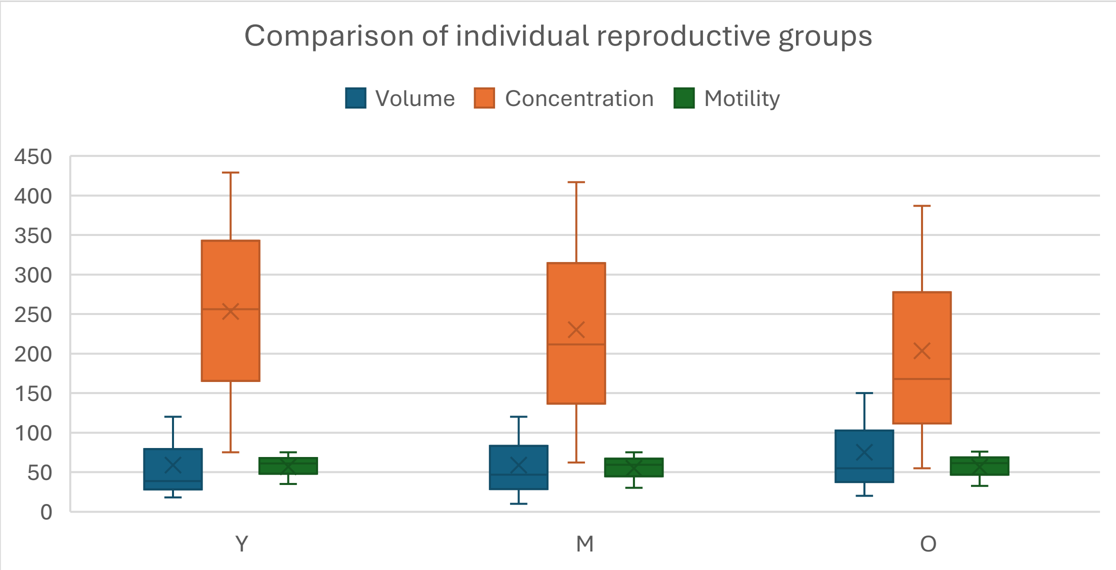
RESULTS AND DISCUSSION

The reproductive parameters of stallions are closely linked to their age. Young stallions that are just beginning their breeding careers typically do not reach optimal values in terms of ejaculate quality and quantity; however, their parameters are often still sufficient for successful fertilization. The most notable decline in sperm motility is observed between 12 and 19 years of age. With advancing age, changes occur in functional sperm parameters, including motility and the production of reactive oxygen species (ROS). Sperm motility is dependent on ATP, which is produced in the mitochondria. As stallions age, mitochondrial respiratory efficiency decreases, leading to an increase in ROS production. This results in oxidative stress and subsequent damage to DNA, proteins, and lipids. The peak mitochondrial oxygen consumption is observed between the ages of 4 and 12 years, after which it begins to decline **(Darr *et al.* 2017)**.

Table 1 Evaluation of reproductive parameters for the categories for stallions.

	Mean	Min.	Max.	SD	CV %
1. Group - Y					
Volume	38.49	18.00	120.00	20.51	53.20
Concentration	256.17	75.00	429.00	91.78	35.80
Motility	60.87	35.00	75.00	8.97	14.70
2. Group - M					
Volume	46.81	10.00	120.00	19.90	42.5O
Concentration	211.71	62.00	417.00	78.41	37.00
Motility	59.42	30.00	75.00	11.91	20.00
3.Group - O					
Volume	54.91	20.00	150.00	18.95	34.50
Concentration	167.93	55.00	387.00	62.57	37.20
Motility	61.21	32.41	76.00	6.86	11.20

Legend: Data processing was performed using the Wilcoxon rank-sum test, and statistical data were analyzed for three distinct groups(1. Group - Y: 3-5 y.o, 2. Group - M: 6-11 y.o, 3. Group -O: 12-25 y.o). For each group, the mean values, minimum (Min.) and maximum (Max.), standard deviations (SD), and coefficients of variation (CV %) are presented.



Graph 1 This graph illustrates the effect of stallion age on three key reproductive parameters: sperm motility (%), sperm concentration (million/ml), and ejaculate volume (ml). Three age groups of stallions were examined, with individual values presented as mean values. (1. Group - Y: 3-5 y.o, 2. Group - M: 6-11 y.o, 3. Group -O: 12-25 y.o)

Table 2 Statistical significance for the observed groups of stallions

Groups	Volume (p-value)	Concentration (p-value)	Motility (p-value)
Y-M	p= 0.00094***	p=0.00478**	p= 0.887
M-O	p=0.000239***	p= 0.0000073***	p= 0.634
Y-O	p= 0.0000000036***	p= 0.00000026***	p=0.887

Legend: Y-M : comparison between young age group and middle age group, M-O: comparison between middle age group and old age group, Y-O: comparison between young age group and old age group. (1. Group - Y: 3-5 y.o, 2. Group - M: 6-11 y.o, 3. Group -O: 12-25 y.o). The level of significance for the comparative analysis was set at ***($p < 0.001$); **($p < 0.01$); *($p < 0.05$).

The p-value for the comparison between the first and second groups of stallions (3–5 years old and 6–11 years old) is significantly lower than the common significance threshold, indicating a statistically significant difference in ejaculate volume and sperm concentration. A statistically significant difference in ejaculate volume also exists between the first and third groups (3–5 years old and 12–25 years old), with younger horses tending to have a lower volume compared to older horses. The test results further show a statistically significant difference in ejaculate volume between the second and third groups (6–11 years old and 12–25 years old), where middle-aged stallions tend to have a lower volume compared to older stallions. According to the table, a statistically significant difference was observed across all studied groups for the reproductive parameter of sperm concentration. Based on the results, we can confirm that sperm concentration in the ejaculate increases with the age of the stallion. For the analyzed parameter motility, no statistically significant differences were detected in any of the group comparisons. The results suggest that any differences in motility are negligible and not statistically significant (Tab.2)

Wilson *et al.* (2019) compared age categories in stallions and semen quality, showing differences in sperm concentration between 5–9-year-old stallions and 10–14-year-old stallions. The younger age group had lower ejaculate volumes but a 30% higher sperm concentration than the older stallion group. These findings align with our results, as our younger stallion group (3–5 years old) had an average ejaculate volume of 38.49 ml, while the middle-aged stallion group (6–11 years old) exhibited an 8.32 ml higher volume but 44.46 million fewer spermatozoa. Similarly, the study by **El-Sisy, Abo El-Maaty, Rawash (2016)** highlights the importance of stallion age, seminal plasma composition, and blood serum parameters in the assessment of fertility. In their research, stallions were categorized into three age groups: young group (5–10 years old), middle-aged group (11–15 years old), and old group (>16 years old). The oldest stallions exhibited the highest ejaculate volumes, which is consistent with our findings. The evaluated stallions belonged to the Arabian breed, which is known for high sperm concentration—an observation that was also confirmed in their study.

Beyond testicular dysfunction, older stallions are also susceptible to reduced ejaculate quality due to musculoskeletal disorders, which can hinder their ability to mount a phantom or a mare. This creates difficulties in semen collection, ultimately affecting reproductive parameters **(Van Beuzekom *et al.*, 2017).** According to **Benson *et al.* (1998)**, up to 50% of fertility issues in older stallions stem from musculoskeletal or neurological conditions. Conversely, younger stallions are often affected by stress hormones, which are released during intensive training or when semen collection coincides with their sports career. Corticosteroids, secreted in response to stress, significantly suppress reproductive functions in young stallions **(Rossetto *et al.* 2021).** Another important factor to consider in comparisons is breed differences. Physiological variations in ejaculate parameters exist between breeds **(Gottschalk *et al.* 2016).** These differences are also observed among individuals within the same breed, as evidenced by individual variability in ejaculate characteristics such as sperm concentration, motility, progressive motility, and DNA fragmentation. For example, Oldenburg stallions exhibited higher sperm concentrations and reduced DNA fragmentation compared to other breeds. Low DNA fragmentation was also characteristic of KWPN stallions, whereas Thoroughbreds showed the highest degree of DNA fragmentation. Hanoverian stallions stood out with a higher total sperm count and better motility compared to other breeds **(Greiser *et al.*, 2019)**. Among warmblood breeds, fertility variability was reported to reach 40–59%. The variation among stallions within the same breed accounted for 27–71% of the total variability, highlighting significant individual differences. However, these findings are not entirely consistent with those of **Gottschalk *et al.* (2016)**, who reported intra-breed differences ranging from 40–59% of total variability. They also noted the lowest sperm concentration in Dutch Warmbloods and the highest ejaculate volume in Holsteiner and Dutch Warmblood stallions, while Trakehner stallions had the lowest gel-free volume. Once again, Oldenburg stallions confirmed their reproductive quality by exhibiting high sperm motility, whereas Thoroughbreds had substantially lower motility values. The composition of seminal plasma plays a key role in the evaluation of reproductive performance. Breed-related differences are also reflected in the distinct composition of seminal plasma, the understanding of which is essential for the identification of molecules and biomarkers associated with fertility **(Bazzano *et al.* 2022).**

To accurately assess potential management adjustments aimed at enhancing the breeding success of aging stallions, it is essential to first evaluate the degree of testicular dysfunction and the overall quality of the ejaculate produced. The palpation assessment was performed with the assistance of an examiner while the stallion was standing, positioned from the left side. The parameters testicular firmness and size were evaluated using a grading scale outlined below the corresponding table. Palpation examination can detect testicular rotation of up to 180°. While the degree of rotation—whether temporary or permanent—does not necessarily correlate with clinical symptoms, in severe cases, it may lead to colic pain, swelling, tenderness, or lameness in the stallion. Some studbooks prohibit the use of stallions that are predisposed to this condition **(Sitters, 2021)**. Palpation is one of the most common diagnostic methods used to assess testicular health. Degenerative processes vary widely in severity, and their progression depends on the extent of the testicular impairment. However, it is generally recognized that if a testis appears smaller and softer, a degenerative process is occurring **(Blanchard, 2001).** The evaluation of the scrotum involves visual inspection. There should be no injuries, scars, abscesses, or signs of dermatitis. Upon palpation, the scrotal skin should be thin, smooth, and soft. The testis should be easily palpable, freely movable, and positioned horizontally. A non-physiological testicular position may have a hereditary basis, rendering such stallions unsuitable for breeding **(Crabtree, 2010)**. The epididymis is a convoluted duct consisting of three sections. It is positioned dorsolaterally to the testis, with a flattened head, which should be firmly attached to the cranial pole of the testis. The caudal tail of the epididymis is oriented posteriorly, is easily palpable, and is often visible to the naked eye. Sperm maturation occurs within the head and body of the epididymis, while the tail serves as a sperm reservoir **(Sitters, 2021).**

Table 3 Palpation assessment of stallions of the young age group 3-5 y.o.

Stallion	Testicle size		Tissue strength		Position in the scrotum	
	L	R	L	R	L	R
1.	++	++	+++	+++	+	+
2.	+++	+++	+++	+++	+	+
3.	+++	+++	+++	+++	+	+
4.	++	++	+++	+++	+	+
5.	++	++	+++	+++	+	+
6.	++	++	+++	+++	+	+
7.	++	++	+++	+++	+	+
8.	++	++	+++	+++	+	+

Legend: . (very firm parenchyma +++, firm parenchyma ++, soft parenchyma +, large testicle +++, medium-sized testicle ++, small testicle +, physiological placement +, non-physiological placement -).

(Tab. 3) shows stallions from the first group with an age range of 3-5 years. According to our results, the size of the testicles appropriate to the age of the stallions and the tone of the parenchyma can be assessed as very good. The assumption of reproductive ability is at a high level.

In the young stallion category (3–5 years old), testicular size correlated with age-related growth patterns, aligning with **Johnson et al. (1997)**, which describes the increase of Sertoli and Leydig cells leading to testicular enlargement.

Table 4 Palpation assessment of stallions of the middle age group 6-11 y.o.

Stallion	Testicle size		Tissue strength		Position in the scrotum	
	L	R	L	R	L	R
9.	+	+	+++	+++	+	+
10.	++	++	+++	+++	+	+
11.	+++	+++	+++	+++	+	+
12.	+++	+++	+++	+++	+	+
13.	+++	+++	+++	+++	+	+
14.	+++	+++	+++	+++	+	+
15.	++	++	+++	+++	+	+
16.	++	++	+++	+++	+	+

Legend: . (very firm parenchyma +++, firm parenchyma ++, soft parenchyma +, large testicle +++, medium-sized testicle ++, small testicle +, physiological placement +, non-physiological placement -).

The results of table 4 show an increase in the size of the testicles, which is a physiological phenomenon with the increasing age of the stallion. The rigidity of the parenchyma is at a very good level and there is a high predisposition to good reproductive efficiency of the stallions (Tab. 4).

Table 5 Palpation assessment of stallions of the old age group 12-25 y.o.

Stallion	Testicle size		Tissue strength		Position in the scrotum	
	L	R	L	R	L	R
17.	+++	+++	++	+++	+	+
18.	+++	+++	+++	+++	+	+
19.	+++	+++	++	++	+	+
20.	+++	+++	+++	+++	+	+
21.	++	++	+++	+++	+	+
22.	+++	+++	+++	+++	+	+
23.	+++	+++	++	++	+	+
24.	+++	+++	+++	+++	+	+

Legend: . (very firm parenchyma +++, firm parenchyma ++, soft parenchyma +, large testicle +++, medium-sized testicle ++, small testicle +, physiological placement +, non-physiological placement -).

The age range of the stallions is 12–25 years. Testicular size is at its highest levels, except for stallion No. 21, which can be explained by its age of 12 years.The parenchymal firmness examination revealed a consistency change in stallion No. 17, where the left testis exhibited a softer texture than the right testis, indicating a potential functional alteration. It is recommended that this stallion undergo more frequent monitoring, especially considering its age of 25 years, suggesting that this is a physiological age-related change. Additionally, weaker parenchymal firmness was also noted in stallions No. 19 and 23, aged 22 years and 18 years, respectively (Tab. 5).

Table 6 Average values of the total sperm number between the observed groups

Groups	Total sperm number x 10^9
Stallions 3-5 y.o	9 869
Stallions 6-11 y.o	9 306
Stallions 12-25 y.o	9 173

Sperm production is a highly sensitive function in stallions and varies individually, making it essential to consider multiple factors when changes in ejaculate quality occur within a breeding program. Fluctuations in semen quality may arise during collection, for instance, if the stallion has to wait longer than usual for the technician, or if a mare is present but not in estrus. Stallions typically produce larger ejaculate volumes when a mare in estrus is present during semen collection. Another factor affecting semen quality is the preparation of the artificial vagina (AV)—if the AV does not match the stallion's preferences, it may lead to lower semen quality. Additionally, the presence of different personnel in the collection room may influence the results. Proper sample processing is a crucial step in semen dose preparation. Ensuring optimal handling is essential for maximizing sperm survival and maintaining viability during the storage and transport of insemination doses. External factors such as the stallion's participation in competitions the day before semen collection or stressful experiences just hours before sampling may also have notable impacts on reproductive parameters.

CONCLUSION

This study focused on the basic evaluation of stallion reproductive potential by categorizing breeding stallions into three age groups. Additionally, we conducted testicular palpation examinations and compared the reproductive parameters (ejaculate volume, sperm concentration, and motility) with the findings from palpation assessments. Stallions in this group generally exhibited medium to large testicles, with six stallions having medium-sized testes and two stallions having large testes. In the middle-aged group (6–11 years old), testicular sizes ranged from small (stallion No. 9) to predominantly large, with four stallions exhibiting large testicles. The oldest age group (12–25 years old) showed a dominance of large testes, observed in seven out of eight stallions. Regarding parenchymal firmness, the first and second age groups displayed uniformly firm testicular tissue across all stallions. However, the oldest age group showed deviations in three stallions. Two stallions exhibited slightly reduced firmness, while stallion No. 17 displayed differences between the left and right testes—with the left testis classified as firm and the right testis as very firm. The remaining five stallions had very firm testicular parenchyma. Based on the findings, no significant degenerative changes were detected, as ejaculate quality remained stable. However, more frequent monitoring of testicular health is recommended, along with strict observation of sperm motility and concentration. Statistical analysis revealed significant differences in sperm concentration and ejaculate volume between the age groups. However, motility showed no significant variation and remained relatively consistent across all groups The average sperm concentration in the youngest group was 256.17 million sperm per ml, decreasing by 44.46 million sperm per mL in the middle-aged group and by 88.24 million sperm per ml in the oldest group compared to the youngest category. Conversely, ejaculate volume increased with age. The youngest stallions had an average ejaculate volume of 38.49 ml, while the middle-aged group showed an increase of 8.32 ml, and the oldest group exhibited a further increase of 16.42 ml compared to the youngest group. In breeding stallion management, appropriate handling, exercise routines, and dietary plans must be adapted to support both athletic performance and reproductive efficiency.

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