Fakulta zemědělská a technologická
Faculty of Agriculture and Technology
Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

FAKULTA ZEMĚDĚLSKÁ A TECHNOLOGICKÁ

Disertační práce

Využití výpočetních metod v chovu koní

Mgr. Veronika Čoudková

ČESKÉ BUDĚJOVICE

2022

Vypracovala:

Mgr. Veronika Čoudková

Studijní program: Zootechnika

Studijní obor: Speciální zootechnika

Školitel:

prof. Ing. Jindřich Čítek, CSc.

Katedra zootechnických věd

Fakulta zemědělská a technologická

Jihočeská univerzita v Českých Budějovicích

PROHLÁŠENÍ

Prohlašuji, že svoji disertační práci jsem vypracovala samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47 b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v nezkrácené podobě elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

V Českých Budějovicích dne 13.6. 2022 ………………………

Mgr. Veronika Čoudková

PODĚKOVÁNÍ

Děkuji doc. Ing. Miroslavovi Maršálkovi, CSc. a prof. Ing. Jindřichovi Čítkovi, CSc.za odborné připomínky, pomoc a ochotu při vypracování této disertační práce. Dále bych ráda poděkovala rodičům, manželovi a dětem za podporu a toleranci v dobách studia.

Souhrn

Disertační práce se zabývá možnostmi využití výpočetních metod v chovu koní. Předkládaná práce je rozdělena do dvou částí. Tou první je literární rešerše a druhá je rozdělena do čtyř podkapitol, které obsahují originální publikace a výsledky vlastní výzkumné práce.

První studie byla zaměřena na vytvoření vzorce pro odhad hmotnosti teplokrevných koní s využitím tělesných rozměrů. V rámci práce bylo změřeno a zváženo 524 teplokrevných koní ve věku od 97 do 1290 dnů a byli rozděleni do 4 věkových kategorií. Výsledný vzorec byl prokázán jako nejvhodnější pro teplokrevné koně v porovnání se vzorci dalších autorů (p-hodnota < 0,001). Byly v něm využity 4 parametry, které nejtěsněji korelovaly s hmotností koní, a to délka těla (DT), obvod hrudníku (OH), výška hrudní kosti (VHK) a přední šířka pánve (PŠP), která se měří mezi zevními hrboly kyčelními. Z těchto čtyř parametrů byl vytvořen vzorec

$$pomocná\ hodnota = \frac{[(2*VHK*OH)*2*DT^2*PŠP]}{10\ 000\ 000}\ .$$

$$hmotnost = koeficient\_1 * pomocná\ hodnota + koeficient\_2$$

Hodnoty koeficientu pro koně ve věku do 365 dnů: koeficient_1 = 4,46; koeficient_2 = 91,12; pro věk od 365 do 650 dnů: koeficient_1 = 3,83; koeficient_2 = 135,87; pro věk od 651 do 950 dnů: koeficient_1 = 3,60; koeficient_2 = 168,09; pro věk nad 950 dnů: koeficient_1 = 3,23; koeficient_2 = 221,55.

Druhá studie se zabývala aktualizací růstové křivky teplokrevných hřebců, která je využívána v České republice při jejich třídění. Celkem bylo provedeno 7488 měření u 117 teplokrevných hřebců ve věku od 7 do 36 měsíců. K sestavení růstového modelu byla zvolena Brodyho funkce, ze které vychází aktuální používaná růstová křivka. Jako nejvhodnější pro chovatele se ukázal percentilový graf s pásmem standardu mezi 25. a 75. percentilem, který byl sestaven pro kohoutkovou výšku měřenou páskou (KVP) a hůlkou (KVH), obvod hrudníku (OH) a obvod holeně (Ohol).

Třetí práce hodnotila vztah mezi parametry motility spermií a morfometrií hlavičky. Celkem bylo odebráno 35 ejakulátů od 10 klinicky zdravých hřebců a s pomocí počítačem řízené analýzy (Sperm Class Analyzer; SCA, MICROPTIC SL) byl hodnocen objem ejakulátu (ml), koncentrace spermií (M/ml), pohyblivost spermií kinematickými parametry a morfometrie spermií. Mezi hřebci byly signifikantní rozdíly ve všech hodnocených parametrech spermií (p-hodnota < 0,05). Mezi parametry plocha hlavičky a rychlost (přímočará rychlost spermií, průměrná rychlost a koeficient kolísání) byla prokázána pozitivní korelace. Prodloužení hlavičky spermií pozitivně korelovalo s celkovou motilitou. Více protáhlé spermie vykazovaly nižší frekvenci průsečíků křivočaré dráhy s průměrnou dráhou.

Čtvrtá studie hodnotila vliv použitého ředidla na chlazenou dávku spermatu teplokrevných hřebců. Celkem bylo odebráno 21 ejakulátů od 11 hřebců. K ředění byla využita ředidla Kenney, EquiPLus a INRA 96. Hodnoceno bylo sedm parametrů – vitalita spermií (VIT, %), celková motilita (MOT, %), progresivní motilita (PMOT), počet progresivních spermií v mililitru (prog M/ml, milion/ml), průměrná rychlost (VAP, μm/s), křivočará rychlost (VCL, μm/s) a přímočará rychlost spermií (VSL, μm/s). Byl prokázán významný vliv doby skladování spermatu (p-hodnota < 0,001) na všechny hodnocené parametry, kdy byl zaznamenán největší pokles kvality spermatu během prvních 24 hodin skladování. Vliv ředidla byl signifikantní (p- hodnota < 0,05) v případě VIT, MOT, PMOT, VCL, VSL a VAP. Průměrný ochranný účinek ředidla INRA 96 byl významně lepší po celou dobu skladování u všech sledovaných parametrů než u ředidla Kenney (p-hodnota < 0,01) a než u EquiPlus (p-hodnota< 0,05) u hodnot PMOT, VCL a VAP.

Klíčová slova: český teplokrevník, odhad hmotnosti, růstové křivky, kvalita spermatu

Summary

Ph.D. thesis is focused on the possibilities of using mathematical methods in horse breeding. The presented work is divided into two parts. The first is a literature review and the second is divided into four subchapters, which contain original publications and the results of my own research work.

The first study focused on creating a formula for estimating the weight of warm-blooded horses using body parameters. In the work, 524 warm-blooded horses aged from 97 to 1290 days were measured and weighed and were divided into 4 age categories. The resulting formula was proven to be the most suitable for warm-blooded horses compared to the formulas of other authors (p-value <0.001). It used 4 parameters that most closely correlated with the weight of the horses, namely body length (BL), girth (G), sternum height (SH) and anterior pelvic width (APW), which is measured between the external hip bumps. A formula was created from these four parameters

$$value = \frac{[(2 * SH * G) * 2 * BL^2 * APW]}{10\ 000\ 000}$$

$$weight = coeffcient\_1 * value + coefficient\_2$$

Coefficient values for horses up to 365 days of age: coefficient_1 = 4.46; coefficient_2 = 91.12; for ages 365 to 650 days: coefficient_1 = 3.83; coefficient_2 = 135.87; for ages 651 to 950 days: coefficient_1 = 3.60; coefficient_2 = 168.09; for age over 950 days: coefficient_1 = 3.23; coefficient_2 = 221.55.

The second study focused on the update of the growth curve of warm-blooded stallions, which is used in the Czech Republic for their classification. A total of 7488 measurements were performed on 117 warm-blooded stallions aged 7 to 36 months. To established a growth model, the Brody function was used, from which the currently used growth curve is based. A percentile graph with a standard between the 25th and 75th percentiles, which was compiled for height at the withers measured by tape (HWt) and by stick (HWs), girth (G) and cannon circumference (CC), proved to be the most suitable for breeders.

The third work evaluated the relationship between sperm motility parameters and head morphometry. A total of 35 ejaculates were collected from 10 clinical healthy stallions and ejaculate volume (ml), sperm concentration (M/ml), sperm motility by kinematic parameters and sperm morphometry were evaluated using computer-assisted analysis (Sperm Class Analyzer; SCA, MICROPTIC SL). There were significant differences between stallions in all evaluated sperm parameters (p-value < 0.05). A positive correlation was demonstrated between the parameters of the head area and velocity (direct sperm velocity, average velocity and fluctuations). Elongation of sperm head positively correlated with overall motility. The more elongated sperm the lower the frequency of the intersections of the curvilinear path with the average path was observed.

The fourth study evaluated the effect of the diluent used on the cooled shipped semen of warm-blooded stallions. A total of 21 ejaculates were collected from 11 stallions. Kenney, EquiPLus and INRA 96 diluents were used for dilution. Seven parameters were evaluated - sperm vitality (VIT,%), total motility (MOT,%), progressive motility (PMOT), number of progressive sperm in milliliters (prog M/ml, million/ml), average path velocity (VAP, μm/s), curvilinear velocity (VCL, μm/s) and rectilinear sperm velocity (VSL, μm/s). A significant effect of sperm storage time (p-value 0 < 0.001) was demonstrated on all evaluated parameters, when the greatest decrease in sperm quality was recorded during the first 24 hours of storage. The effect of diluent was significant (p-value 0 < 0.05) for VIT, MOT, PMOT, VCL, VSL and VAP. The average protective effect of INRA 96 was significantly better during the whole storage period for all monitored parameters than for Kenney (p-value <0.01) and than for EquiPlus (p-value <0.05) for PMOT, VCL and VAP values.

Key words: Czech warmblood, weight estimation, growth curves, sperm quality

Obsah

1 Úvod 10
2 Literární přehled 11
2.1 Základní výpočty v chovu koní 11
2.1.1 Měření koní 11
2.1.2 Hodnocení kopyt 14
2.1.3 Hodnocení laterality u koní 15
2.2 Odhady hodnot 16
2.2.1 Odhad hmotnosti koní 16
2.2.2 Růstové křivky 18
2.2.3 Odhad plemenné hodnoty 22
2.3 Moderní technologie ve výzkumu koní 24
2.3.1 Hodnocení spermatu 26
2.4 Statistické metody 27
3 Hypotézy a cíle práce 33
4 Experimentální část a výsledky 34
4.1 Odhad tělesné hmotnosti hřebců z lineárních měření teplokrevných koní 34
4.2 Růstové křivky vybraných parametrů hřebců českého teplokrevníka 35
4.3 Počítačem řízená analýza morfometrie hlavičky a kinetických parametrů spermatu teplokrevných hřebců 36
4.4 Vliv ředidla na chlazené sperma teplokrevných hřebců 37
5 Přílohy 38
6 Závěr 75
7 Seznam literatury 76

1 Úvod

Cílem vědy v oblasti chovu koní je hlavně podněcovat a podporovat využití objektivního výzkumu a pokročilé praxe, které v konečném důsledku zlepší welfare koní v jejich spojení s člověkem. Moderní výpočetní technika a specializovaný software neustále zvyšuje možnosti hodnocení dat, ale nezbytným předpokladem je znalost výpočetních metod. Mezi ty lze zařadit řešení lineárních a nelineárních rovnic, interpolace a aproximace funkcí, numerické derivace a integrování nebo řešení počátečních úloh pro diferenciální rovnice. Pro správnou volbu numerické metody při řešení konkrétních úloh je důležité znát konvergenční teorii a odhady chyb. Další oblastí jsou statistické metody, konkrétně jejich aplikace, zaznamenávání dat o jevech a jejich zpracování, tedy jejich třídění a interpretování. Statistika úzce souvisí s informačními technologiemi, mezi které lze zařadit informatiku a výpočetní techniku.

Konkrétní využití výpočetních metod v chovu koní je v řadě oblastí. Nejjednodušší výpočty se týkají oblasti stáda nebo například výpočtu krmných dávek, hodnocení kvality spermatu. Složitější výpočty souvisí s odhadem funkcí, například růstová funkce, nebo s vytvářením vzorců pro odhady hodnot. Další oblastí je genetika, kde se jedná například o odhad genetických parametrů a následně plemenné hodnoty na základě zvolených ukazatelů. A neopomenutelnou oblastí je statistika, která má svůj význam hlavně při vědecké práci. Umožňuje hodnotit informace o celku i o jeho částech a dává důležité informace, které lze využít pro další rozhodovací procesy v běžné chovatelské praxi.

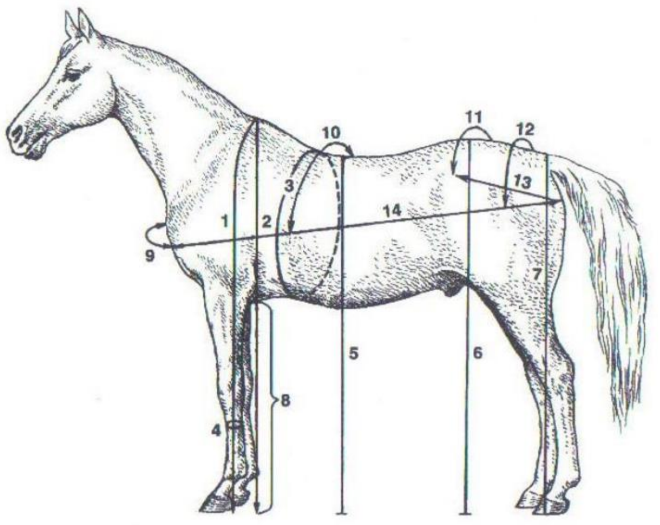
Nelze opomenout fakt, že matematika má stále větší význam nejen v chovu koní. Výsledky nejsou ovlivněny názorem nebo interpretací. Poskytuje tak přesnou formulaci myšlenek a umožňuje určit příčinu a důsledek. V neposlední řadě je součástí velkého množství aplikací, které v současné době chytrých telefonů do značné míry vylepšují a zjednodušují práci chovatelům koní.

2 Literární přehled

2.1 Základní výpočty v chovu koní

2.1.1 Měření koní

Mezi základní výpočty se řadí hlavně využívání existujících vzorců, do kterých lze dosadit získané hodnoty. Koně jsou během života měřeni z důvodu posuzování jejich růstu a pro posouzení, zda splňují chovný cíl daného plemene. COSTA *et al.* (2016) uvádí, že tělesné míry a úhly koně definují jeho možnosti pohybu. Jedná se tak o významné údaje z hlediska potenciálu a výkonu koně. Základní míry jsou pro plemeno stanoveny v Řádu plemenné knihy. Existují však i plemena, jako například anglický plnokrevník, u kterých není měření součástí plemenného standardu. Míry zjišťované u českého teplokrevníka lze vidět na obrázku 1.



Obrázek 1: Schéma měření tělesných rozměrů: (1) kohoutková výška pásková (KVP), (2) kohoutková výška hůlková (KVH), (3) obvod hrudníku (OH), (4) obvod holeně (Ohol), (5) výška hřbetu, (6) výška kříže, (7) výška kořene ohonu, (8) výška kosti hrudní od země, (9) šířka prsou v ramenních kloubech, (10) šířka hrudníku za lopatkou, (11) šířka pánve v kyčelních hrbolech, (12) šířka pánve v kyčelních kloubech, (13) délka pánve, (14) šikmá délka těla (ŠDT)

Z naměřených hodnot lze počítat hipometrické indexy, které vyjadřují jednotlivé absolutní rozměry v základních mírách, které jsou s nimi v bližším anatomickém nebo fyzikálním vztahu. Jsou zaměřené na význam jednotlivých poměrů tělesných tvarů. Následující indexy patří mezi ty, které se nejběžněji využívají v praxi (SOBOTKOVÁ *et al.*, 2006).

$$Index\ formátu\ těla = \frac{šikmá\ délka\ těla}{kohoutková\ výška\ hůlková} \times 100$$

$$Index\ kompaktnosti = \frac{obvod\ hrudníku}{šikmá\ délka\ těla} \times 100$$

$$Index\ mohutnosti = \frac{obvod\ hrudníku}{kohoutková\ výška\ hůlková} \times 100$$

$$Index\ kostnatosti = \frac{obvod\ holeně}{kohoutková\ výška\ hůlková} \times 100$$

$$Index\ přestavěnosti = \frac{výška\ v\ kříži}{kohoutková\ výška\ hůlková} \times 100$$

$$relativní\ hloubka\ hrudníku = \frac{hloubka\ hrudi}{kohoutková\ výška\ hůlková} \times 100$$

$$Index\ délky\ pánve = \frac{délka\ pánve}{šířka\ pánve} \times 100$$

$$Index\ síly\ kostry = \frac{obvod\ holeně}{kohoutková\ výška\ hůlková} \times 100$$

$$InIndex\ šířky\ v\ prsou = \frac{šířka\ v\ prsou}{šikmá\ délka\ těla} \times 100$$

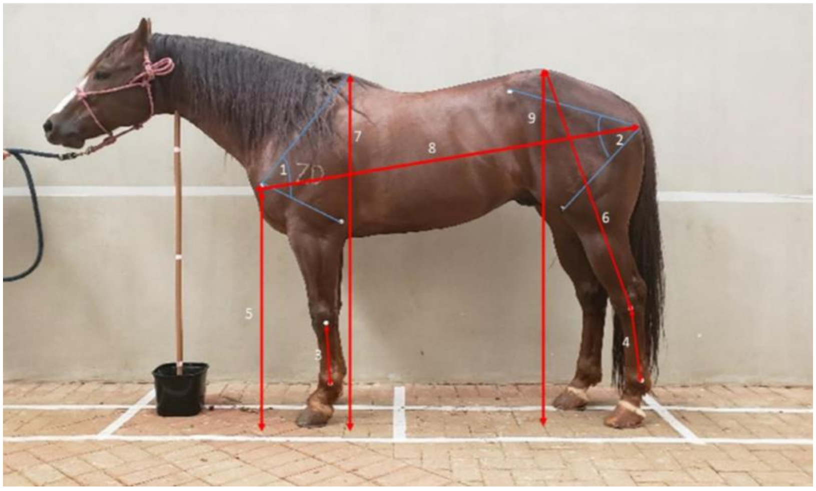
$$Index\ tvaru\ hrudníku = \frac{hlouba\ hrudníku}{délka\ hrudníku} \times 100$$

$$Index\ délky\ hrusníku = \frac{délka\ hrudníku}{kohoutková\ výška\ hůlková} \times 100$$

DUŠEK *et al.* (2007) vytvořili s cílem vyšší objektivity při hodnocení tělesné stavby a posouzení harmoničnosti pomocné kritérium. Základem je tělesný standard a na základě variability stanovena rozptylová pásma. Decilová síť tělesné stavby umožňuje bodově ohodnotit měřený tělesný parametr od jedné do deseti. Jedinec obdrží celkový počet bodů a po vydělení počtem sledovaných znaků průměrnou hodnotu (x_P). Od této hodnoty se následně odečítají jednotlivé body (zanedbávají se znaménka) a dělí se průměrem. Výsledná hodnota udává stupeň souměrnosti.

$$D_p = \frac{\sum_{i=1}^{n} \left| x_i - \bar{x}_p \right|}{n}$$

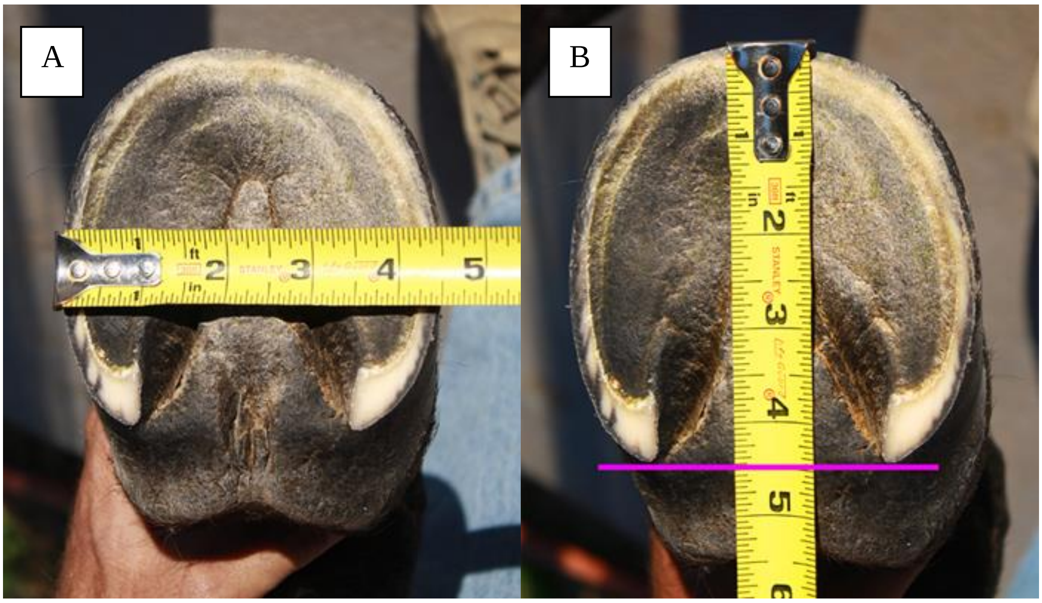
FREITAG *et al.* (2021) stanovili metodiku pro získávání úhlů a tělesných měr koní s využitím digitálního goniometru. Pro hodnocení byla použita fotografie levého profilu zvířete ze vzdálenosti 3 m a s využitím 8 značek k usnadnění vizualizace (obrázek 2). Fotografie byly následně analyzovány s využitím softwaru ImageJ. Jedná se o přesnou metodu, která vede k získání tělesných měr a úhlů.



Obrázek 2: Zaúhlené (modré linky) a lineární (červené linie) proměnné použité pro hodnocení metodu obrazové analýzy tělesného uspořádání: (1) úhel rameno-humerus, (2) úhel kyčel-stehenní kloub, (3) délka holeně přední končetiny, (4) délka holeně zadní končetiny, (5) výška ramene, (6) délka hlezno-záď, (7) kohoutková výška, (8) délka těla, (9) výška zádě (FREITAG *et al.*, 2021).

2.1.2 Hodnocení kopyt

Kopyto je svojí stavbou a funkčností velmi důmyslný orgán, který představuje nejdistálnější část končetiny. Je součástí společného tělního pokryvu a jako takový má všechny vlastnosti kůže. Zároveň je také nedílnou součástí svalového a kosterního systému koňského těla (Vinčálek, 2015). Rovnováha kopyta ovlivňuje rozložení sil a pohyb končetin (Lochner *et al.*, 1980; Riemersma *et al.*, 1996) a odchylky u těchto jednotlivých složek pohybu mohou vést ke zranění končetin (Moyer *et* Anderson, 1975; Pool *et* Meagher, 1990).



Obrázek 3: Měření lateromediální šířky kopyta (A) a předozadní šířky kopyta (B) (Parés *et* Casanova, 2011)

Parés *et* Casanova (2011) vytvořili vzorec pro odhad plochy kopyta u katalánských koní. Znalost tohoto parametru má význam hlavně v oblasti veterinářství a podkovářství, kdy lze řešit deformity kopyta nebo nerovnoměrné zatížení kopyt. Autoři vyslovili hypotézu, že plochu došlapové části kopyta lze extrapolovat z jednoduchých lineárních měření. Vzorec vychází ze dvou naměřených parametrů, kterými jsou lateromediální šířka kopyta a předozadní šířka kopyta (obrázek 3). Hodnota směrodatné odchylky chyby je pro tento vzorec 5,15 a koeficient determinace 0,9384.

$$plocha\ kopyta = (1{,}229\ \times lateromediální\ šířka)^2 \times předozadní\ šířka^{0{,}772}$$

Také KAUFFMANN *et* CLINE (2020) uvádí, že malá kopyta v poměru k velikosti koně jsou náchylnější k laminitidě a podotrochlóze. Důvodem je příliš velký tlak na určitou plochu a u větších kopyt působí méně kilogramů na centimetr čtverečný kopytních struktur. Ideální jsou zdravá velká kopyta, u nichž se na nesení hmotnosti podílejí všechny k tomu určené části, díky čemuž na ně působí nejmenší tlak.

2.1.3 Hodnocení laterality u koní

Žádný tvor není dokonale symetrický. Asymetričnost, tedy upřednostňování pravé nebo levé strany, se nazývá lateralita. U lidí se jedná o důkladně prozkoumanou oblast, zatímco u koní méně, i když se jedná o jev, který ovlivňuje práci s koňmi.

V posledních dvou desetiletích toto téma získává na popularitě a mnoho prací se zabývá studiem laterality u koní. Existuje řada metodik, kterými lze lateralitu hodnotit. Řadí se mezi ně testování motorické a senzorické laterality, posuzování čelních chlupových vírů nebo olfaktorické testy. Při testování se zaznamenává využívání pravé a levé strany a následně se lateralita hodnotí kvalitativně s využitím vzorců. V těchto vzorcích písmeno „L“ označuje míru levostranné preference a „R“ míru využívání pravé strany.

Nejpoužívanějším vzorcem je dle SOVÁKA (1985) „index laterality“ (LI), který se někdy označuje jako Cuffův vzorec. Počítá se jako LI = [(R - L)/(R + L)] a pozitivní výsledné hodnoty značí pravostrannou preferenci a záporné hodnoty levostrannou preferenci (BRODER *et* ANGELONI, 2014). Výsledek takto vypočteného LI je z důvodu lepší interpretace násobit číslem 100 a obecně se pohybuje mezi hodnotami -1 (- 100 %) a 1 (100 %). Hranice intervalu pro určení preference je ±25 % a je nejsilnějším ukazatelem směru asymetrie (BATT *et al.*, 2007). Čím více se podle SCHNEIDERA *et al.* (2013) absolutní hodnota výsledného čísla blíží nule, tím více je jedinec nevyhraněný. Tedy nula značí ambilaterálnost a v intervalu od nuly do sta roste preference pravé strany a od nuly do záporných hodnot roste preference levé strany. Absolutní hodnota výpočtu LI udává pouze sílu laterality, ale ne její směr.

Další využívanou rovnici indexu laterality uvádějí SOVRANO *et* ANDREW (2006) a zaměřuje se na směrové preference, nebo na jednu ze stran. Při použití vzorce LI = [(R/L + R) * 100] je hraniční hodnotou k určení preference pravé nebo levé strany 50 %. Pravostrannou preferenci naznačují hodnoty vyšší než 50 %, a čím je číslo větší než 50 %, tím je preference pravé strany silnější. Hodnota pod 50 % označuje levostrannou preferenci.

Příkladem další používané míry lateralizace je absolutní index laterality (ALI). Většinou je využíván k hodnocení individuální asymetrie nebo k měření síly laterality bez ohledu na její směr. Výpočet ALI je absolutní hodnota LI: |LI|. V případě využití vzorce LI = (R/L + R) je následně dle BARNARDA *at al*. (2016) ALI vypočteno jako ALI = |LI – 0,5|. Nulové hodnoty představují jedince, kteří vykazují bez preference, a výsledek 50 (popřípadě 0,5) označuje zcela lateralizovaného jedince.

Dalším způsobem hodnocení laterality je kvocient pravorukosti (Dexterity Quotient, DQ), který vyjadřuje procentuální zastoupení pravostranných reakcí z celkového počtu všech reakcí jedince. Výpočet je podle vzorce DQ = (P+A/2) / (P+L+A), kde P, L a A označují pravostranné, levostranné a ambilaterální reakce.

K určení síly, směru či závislosti na jiných faktorech lze využít statistické vzorce a výpočty. Pro zjištění vlivu pohlaví nebo věku na lateralitu na pohlaví je v některých výzkumech použita analýza rozptylu ANOVA (WELLS, 2003, QUARANTA *et al*., 2004).

Z přehledu výpočtů vyplývá, že existuje řada způsobů hodnocení laterality u koní, a navíc jeden způsob výpočtu může mít jiné hranice intervalů, pro určení laterality. Z tohoto důvodu je velmi složité porovnávat výsledky různých autorů, protože pak vzniká velmi rozdílné zastoupení ambilaterálních koní ve výsledcích. Faktem však zůstává, že lateralita je běžným jevem u koní a má velký dopad na jejich praktické využití i zdravotní stav.

2.2 Odhady hodnot

2.2.1 Odhad hmotnosti koní

Chovatelé nemívají vybavení potřebné k vážení koní a zároveň nebývají schopni přesně odhadnout tělesnou hmotnost koně. Jedná se však o důležitý parametr, který charakterizuje tělesný vývoj a fyziologické funkce organismu (DUŠEK *et al*., 2007). Jedná se také o důležitý ukazatel zdraví. Pokud chovatel zná hmotnost koně a zda aktuálně hubne nebo přibírá, může odhalit případné zdravotní problémy. Nízkou tělesnou hmotnost mohou způsobovat nemoci, stáří, parazité, nadměrné využívání koně, nevyvážená krmná dávka, omezený přístup ke stádu (způsoben např. hierarchií ve stádě) nebo genetika (SENDEL, 2010). Znalost tělesné hmotnosti koně má význam v mnoha oblastech jejich chovu. Jedná se například o sestavování krmné dávky nebo podávání léčiv (WAGNER *et* TYLR, 2011). Léky a přípravky na odčervení jsou toxické a měly by být podávány v přesném dávkování doporučeném výrobcem s ohledem na hmotnost zvířete (MILNER *et* HEWIT, 1969; CARROL *et* HUGTINGTON, 1988). SENDEL (2010) uvádí, že nesprávný odhad tělesné hmotnosti může vést k předávkování nebo poddávkování zvířete. Předávkování léčivy může vyústit v otravu a koliku nebo jiné závažné zdravotní komplikace až po úhyn zvířete. Naopak poddávkování nevede k požadovaným účinkům léku a v případě antibiotik nebo odčervovacích prostředků může vést k rezistenci. Řada prací se zaměřila na vytvoření vzorce pro odhad tělesné hmotnosti s využitím tělesných rozměrů dospělých koní (WAGNER *et* TYLER, 2011; MILNER *et* HEWITHALL, 1969; CARROL et HUNGTINGTON, 1988; HOFFMANN *et al.*, 2013; SHAKER *et al.*, 1999; GIBBS *et* HOUSEHOLDER, 2003; HALL, 1971; MARTISON *et al.*, 2014; NEDER *et al.*, 2009; CATALANO *et al.*, 2015). Přehled vzorců je uveden v tabulce 1.

Tabulka 1: Rovnice pro odhad tělesné hmotnosti (TH) dospělých koní (kg) s využitím obvodu hrudníku, výšky a délky těla (cm).

Zdroj	Rovnice
SHAKER *et al.* (1999)	$TH = kohoutková\ výška \times výška\ zádě \times délka\ těla \times 0{,}0001$
CARROL *et* HUNGTINGTON (1988)	$TH = \frac{obvod\ hrudníku^2 \times délka\ těla}{11{,}877}$
HALL (1971)	$TH \frac{obvod\ hrudníku^2 \times délka\ těla}{11{,}880}$
MARCENAC *et al.* (1964)	$TH = \left(\frac{obvod\ hrudníku}{100}\right)^3 \times 80$
BROWN *et al.* (2003)	$TH = \frac{obvod\ hrudníku^2 \times délka\ těla}{8{,}717}$
MILNER *et* HEWIT (1969)	$TH = \frac{(obvod\ hrudníku \times 0{,}39)^{1{,}97} \times (délka\ těla \times 0{,}39)^{1{,}05}}{228{,}1} \times 0{,}45$

2.2.2 Růstové křivky

Podle DUŠKA *et al.* (2007) se koně řadí mezi zvířata s rychlým růstem. Koně plemene anglický plnokrevník dosahují 46 % z tělesné hmotnosti v dospělosti a 83 % z kohoutkové výšky v dospělosti již v 6 měsících věku. ŁUSZCZYŃSKI *et al.* (2019) uvádí, že kohoutková výška hříbat při porodu je rovna více než 60 % výšky dospělého koně, což je vyšší proporcionální podíl v porovnání s dalšími hospodářskými zvířaty. VALETTE *et al.* (2008) upozorňují na to, že celkové hodnocení mladých koní je poměrně složité, vzhledem k tomu, že je jejich růst asymetrický. DUŠEK (1998) popisuje růstové změny u mladých koní, kdy u hříbat lze pozorovat rámec stojícího obdélníku, protože kohoutková výška je větší než délka zvířete. Mezi 12. a 18. měsícem věku se formát mění na čtvercový tvar. Během dalšího růstu se délka těla navyšuje rychleji než kohoutková výška a rámec těla nabývá tvaru ležícího obdélníka na delší straně.

Použití matematických růstových modelů poskytuje efektivní způsob, jak shrnout informace obsažené v datech do několika parametrů s biologickým významem (FITZHUG, 1976). SILVA *et al.* (2011) uvádí, že nelineární funkce jsou snadno biologicky interpretovatelné a lze je snadno vzájemně porovnávat. Pro stanovení růstových křivek se využívají speciální růstové modely. Základním modelem pro vyjádření růstové křivky je Richardsova funkce (RICHARDS, 1959). Jedná se o nelineární čtyřparametrovou funkci. Další růstové funkce lze z této odvodit jako speciální případy. V tabulce 2 jsou uvedeny nejčastěji používané modely růstu. Patří mezi ně modely Gompertze, Brodyho, Logistický, Weibullův, Richardsův a von Bartalanffyho. Uvedené modely se od Richardsova liší tím, že vzhledem k asymptotě mají fixní polohu inflexního bodu. U Bertalanffyho funkce dochází k inflexi přibližně v 1/3 asymptoty (u_1 = 0,296), u logistické funkce v ½ asymptoty (u_1 = 0,5) a u Gompertzovy funkce přibližně v 1/3 (u_1 = 0,368). Brodyho funkce inflexní bod nemá. NATH *et* MOORE (1992) doplňují růstovou funkci o využití první derivace (absolutní a relativní rychlost růstu) a druhé či třetí derivace, kterými lze stanovit délku exponenciální a lineární fáze růstu. Také FITZHUGH (1976) ve své práci uvádí odvozené parametry od růstové křivky. Uvedeny jsou vzorce pro Richardsův model a Brodyho model.

- Stupeň dospělosti vyjadřuje podíl velikosti dosažené ve věku t z velikosti v dospělosti A

$$u_t = \frac{y_t}{A} = (1 \pm be^{-kt})^M$$
$$u_t = 1 - be^{-kt}$$

- Věk potřebný k dosažení určitého stupně dospělosti (tu) pro Brodyho funkci:

$$t_u = -\frac{1}{k} ln \left| \frac{1 - u_t}{b} \right|$$

- Okamžitá absolutní intenzita růstu – dy/dt je první derivací růstové funkce

$$\frac{dy}{dt} = \pm MkAbe^{-kt}(1 \pm be^{-kt})^{-1}$$
$$\frac{dy}{dt} = kAbe^{-kt}$$

- Okamžitá relativní rychlost růstu: y^{-1} dy/dt

- Okamžitá absolutní intenzita dospívání: A^{-1} dy/dt

- Absolutní intenzita růstu:

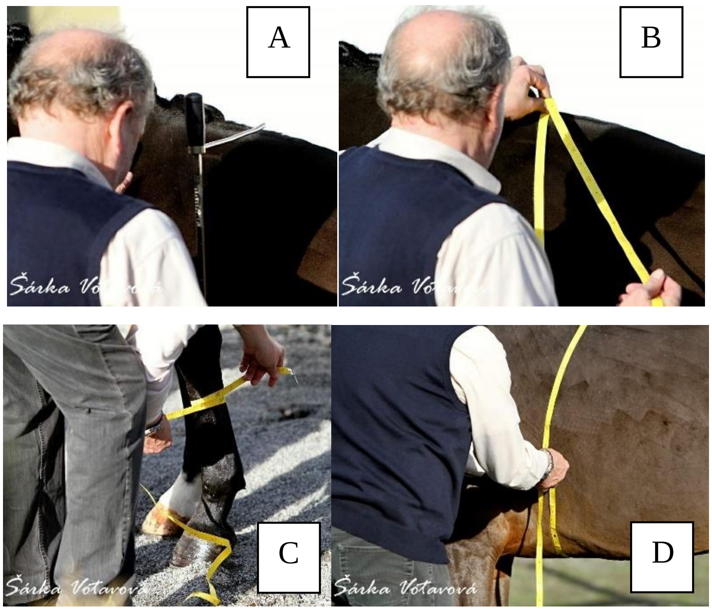
$$\frac{0{,}5AMk}{2M - 1}$$

Čtyři poslední parametry jsou vtaženy k inflexnímu bodu, nejsou tedy využitelné v případě Brodyho funkce.

Podle JAKUBCE *et al.* (1998) by měla růstová funkce splňovat několik požadavků. Mezi ně patří odrážet biologickou podstatu růstu, umožnit biologickou interpretaci parametrů funkce, umožnit dobré přizpůsobení funkce empiricky zjištěným hodnotám a umožnit snadnou matematicko-statistickou interpretaci.

U koní se často využívá Brodyho funkce. Dle VESELÉ (2003) může být důvodem pro výběr této funkce skutečnost, že tříparametrové funkce s inflexním bodem (Bertalanffyho, logistická, Gompertzova) a především čtyřparametrová Richardsova funkce jsou poměrně náročné na kvalitu vstupních údajů. Vyžadují rovnoměrné pokrytí celého růstového období, včetně údajů z dospělosti. V případě méně kvalitních údajů může být flexibilita těchto funkcí spíše na obtíž.

Rychlost růstu je jedním z důležitých parametrů, který se bere v úvahu při vytváření krmné dávky pro koně. Proto je velmi důležité, aby byly známy přesné růstové křivky pro každé plemeno (FRADINHO *et al.*, 2016). PIMENTEL *et al.* (2017). Dle DUŠKA *et al.* (1998) je srovnání rozměrů jednotlivých hříbat se standardem v různém věku důležité zvláště při posuzování celého ročníku hříbat, ve kterém jsou mezi jedinci velké rozdíly ve věku. Dále je hodnocení růstu součástí výběru hřebců do chovu, kdy přesný postup upravují řády chovatelských svazů a zákony. Dle Dobeše (1977) je nutné, aby pro posuzování růstu a vývinu hříbat jednotlivých plemen byly vypracovány standardy alespoň pro nejzákladnější tělesné rozměry (obrázek 4). Pro praxi jsou významné standardy v grafické interpretaci. Jsou v nich uvedeny průměrné vývojové křivky, doplněné rozptylovými pásmy, aby tak podle polohy naměřených hodnot u jednotlivých hříbat bylo možné vyhodnotit jejich růstovou dynamiku.



Obrázek 4: Měření kohoutkové výšky hůlkou (A), páskou (B), obvodu holeně (B) a obvodu hrudníku (C) (schct.cz).

Tabulka 2: Rovnice a parametry ve speciálních případech Richarsovy růstové funkce

Model	Rovnice pro y_t	M	u_1	Asymptota	dy/dt	A^{-1} dy/dt	y^{-1} dy/dt
Brody	$A(1-be^{-kt})$	1	…	A	$kA(1-u)$	$k(1-u)$	$k(u^{-1}-1)$
Bertalanffyho	$A(1-be^{-kt})^3$	3	8/27	A	$3ky(u^{-\frac{1}{3}}-1)$	$3ku(u^{-\frac{1}{3}}-1)$	$3k(u^{-\frac{1}{3}}-1)$
Logistický	$A(1+be^{-kt})^{-1}$	-1	0,5	A	$ky(1-u)$	$ku(1-u)$	$k(1-u)$
Gompertzův	$A\exp(-be^{-kt})$	$M\to\infty$	e^{-1}	A	$ky\ln(e^{-1})$	$ku\ln(e^{-1})$	$k\ln(u^{-1})$
Richardsův	$A(1\pm be^{-kt})^M$	variabilní	$[(M-1)/M]^M$	A	$Mky\,(u^{-\frac{1}{M}}-1)$	$Mku(u^{-\frac{1}{M}}-1)$	$Mk(u^{-\frac{1}{M}}-1)$

Biologická interpretace parametrů (FITZHUG, 1976; RICHARDS, 1959, STAINAR et al., 2005)

A - *asymptotická hodnota pozorovaného biometrického znaku (pro t → ∞), obecně vysvětlovaná jako průměrná hodnota v dospělosti.*

b - *integrační konstanta, časový parametr měřítka, definuje stupeň zralosti v době narození t = 0 (1-b)*

k - *index dospívání, který vyjadřuje relativní růstovou rychlost, kterou je dosaženo asymptotické hodnoty (vyšší hodnoty značí raně dospívající jedince a malé hodnoty označují pozdně dospívající jedince)*

M - *tvarový parametr určující polohu inflexního bodu křivky, který stanovuje stupeň dospělosti v inflexním bodě.*

u_t – *stupeň dospělosti ve věku t*

2.2.3 Odhad plemenné hodnoty

HAJIČ *et al.* (1995) definuje plemennou hodnotu jako číselné vyjádření vlastností a znaků, které jedinec determinuje u svého potomstva a je možné ji odhadovat na základě fenotypového projevu většího množství potomstva. Plemenná hodnota představuje odchylku od průměru populace. V Evropě je běžnější vyjádření této hodnoty jako relativní plemenná hodnota. Vlastní vyjádření relativní plemenné hodnoty jedince vychází z průměru populace daného plemene a proměnlivosti dané vlastnosti uvnitř plemene, která je vyjádřena směrodatnou odchylkou. Ve světě se pro objektivní odhad plemenné hodnoty používá metoda BLUP (Nejlepší lineární nestranná předpověď) ve formě AM (Animal model). Tato metoda využívá pro odhad plemenné hodnoty matici příbuznosti, takže jsou do výpočtu zahrnuty dostupné údaje o předcích sledovaného jedince nebo o jeho bočním příbuzenstvu (MARŠÁLEK, 2008). Metody odhadu plemenné hodnoty se volí tak, aby byly vzájemně porovnatelné a pro chovatele známé a srozumitelné (PŘIBYL *et al.*, 2008). Před zavedením odhadu plemenné hodnoty uvádí VOSTRÝ *et al.* (2011), že byla využívaná selekce zvířat do chovu na základě fenotypového projevu. DUŠEK (2007) uvádí, že při nezávislosti selektovaných znaků lze intenzitu selekce při výběru podle jednoho až více znaků počítat podle Lushe ze vztahu

$$k = \sqrt[m]{n}$$

kde k je podíl jedinců vybraných tak, aby všichni z nich měli vyjádřenou každou z m vlastností odpovídající selekčnímu limitu; m = počet nezávislých vlastností, n = počet jedinců vybraných podle vlastností.

URBAN (2011) uvádí, že plemenná hodnota uvádí hodnotu genů, které jsou předány jeho potomkům. Také je to pravděpodobnost, že potomstvo bude vykazovat dobré genetické založení. TAVERNIER (1992) ve své práci zmiňuje široké využití plemenných hodnot sportovních a dostihových koní na základě veřejných záznamů jejich výkonů. V České republice se tomuto tématu věnuje tým z VÚŽV Praha, který vydal dvě certifikované metodiky. Spolehlivost předpovědi plemenných hodnot pro skokovou výkonnost u teplokrevných koní (NOVOTNÁ *et al.,* 2018) obsahuje postup předpovědi spolehlivosti, který lze využívat v chovatelském postupu.

Plemenné hodnoty jsou předpovídány podle následující modelové rovnice:

$$y = pohl + vek + sout + jezd + prost + jed + e$$

kde: *y* – vyhodnocované vlastnosti

pohl – pohlaví koně (fixní efekt, ve třídách)

vek – věk koně (fixní efekt, ve třídách)

sout – soutěž (fixní efekt)

jezd - jezdec (náhodný efekt)

prost – trvalé prostředí jedince (náhodný efekt)

jed – efekt jedince (náhodný efekt) e – reziduum

Další certifikovanou metodou je Spolehlivost předpovědi plemenných hodnot pro znaky zevnějšku hodnocených lineárním popisem u teplokrevných plemen koní (SVITÁKOVÁ *et al.*, 2018). V tomto případě jsou plemenné hodnoty předpovídány podle následující modelové rovnice:

$$y = pohl + vek + mist + hodn * rok + jed + e$$

kde: *y* – vyhodnocované vlastnosti

pohl – pohlaví koně (fixní efekt, ve třídách)

vek – věk koně (fixní efekt, ve třídách)

mist – místo hodnocení – fixní efekt

*hodn*rok* - interakce efektů hodnotitel a rok hodnocení – fixní efekt

jed – efekt jedince – náhodný efekt

e – reziduum

PŘYBIL (1997) tvrdí, že spolehlivost plemenné hodnoty závisí na dědivosti a především množství informací o daném zvířeti, u plemeníků také na počtu potomků s užitkovostí, kteří se potkávají s vrstevníky, tzn. efektivním počtem potomků. DUŠEK *et al.* (2007) uvádí výpočet dědivosti (heritability) jako podíl podmíněné dědičnosti σ^2_G z celkové fenotypové proměnlivosti σ^2_P.

$$h^2 = \frac{\sigma_G^2}{\sigma_P^2}$$

Koeficient dědivosti h^2 se pohybuje v rozsahu od nuly do jedné. Hodnoty blízko nule ukazují na nízkou dědivost vlastnosti, hodnoty blízké jedné znamenají dědivost vysokou. Obecně h^2 v rozsahu 0,41 až 0,6 charakterizuje střední dědivost. Znalost dědivosti je důležitá, protože podmiňuje ostrost selekce a její modifikaci při důrazu na konkrétní vlastnosti (DUŠEK *et al.*, 2007).

2.3 Moderní technologie ve výzkumu koní

Historicky jezdectví postrádalo podporu vědecky měřených údajů, které by mohly poskytnout objektivní důkazy o tom, zda mají konkrétní postupy pozitivní nebo negativní vliv na pohodu koní (WARAN *et* RANDLE, 2017). V posledních letech prošla věda v oblasti chovu koní velkým růstem. Přínosem je aplikace technologií z jiných oborů, které umožňují hodnotit činnosti s koňmi (MCLEAN *et* MCGREEVY, 2010). HOLMES *et* JEFFCOTT (2010) upozorňují, že je důležité dobré využití technologie po pečlivém zvážení problémů, které ji mohou doprovázet. RANDLE *et* BUTTON (2008) uvádějí řadu technologií, jako například zařízení pro záznam napětí a tlaku, elektromyografie, monitory srdeční frekvence a teploty (s využitím infračervené termografie), akcelerometry, analýzu chůze a citlivosti. Desítky let je využíván videozáznam nebo data loggery, ale ty vstoupily do nové éry s většinou bezplatnou dostupností aplikací v chytrých telefonech, které umožňují manipulaci s daty v reálném čase a okamžitý záznam dat (RANDLE *et* BUTTON, 2008).

V oblasti studia welfare se využívají fyziologická měření jako přímé nebo nepřímé ukazatele fyzického nebo psychického stavu jedince a jeho výkonu (RANDLE *et al.*, 2015). Řadí se sem hodnocení tepové frekvence, kdy se jako efektivní a neinvazivní metoda ukázalo využití monitorů srdečních tepů (HINCHCLIFF *et al.*, 2008). Novější studie hodnotí variabilitu srdeční frekvence namísto dříve využívané srdeční frekvence (VON BORELL *et al.*, 2007). Celkově variabilita srdečního tepu odráží velikost mezidobých intervalů a nejčastěji kvantifikovanou proměnnou je vzdálenost mezi R-vlnami (GEHRKE *et al.*, 2011).

Nově vznikající technologií je povrchová telemetrie, která je neinvazivním nástrojem, který dokáže kvantifikovat nástup svalové aktivity, kontrakce a zkoumat vztahy mezi definovanými událostmi a svalovým výkonem. Dále lze monitorovat svalovou zátěž a adaptaci v průběhu času (HUG *et al.*, 2010).

Infračervená termografie je zobrazovací technika, která vytváří mapy změn teploty povrchu. Ve veterinární medicíně se využívá možnost identifikování zánětlivých, vaskulárních nebo neurologických poruch (SOROKO *et* HOWELL, 2016).

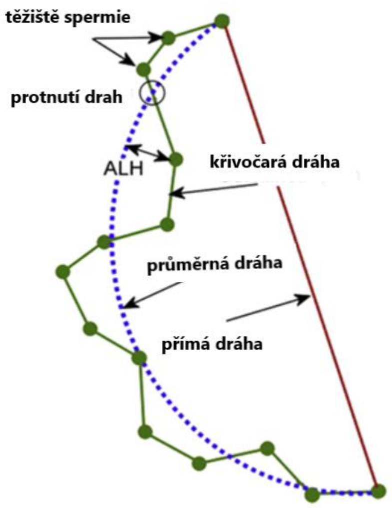
Pro výkon je důležité, aby se kůň mohl pohybovat bez omezení. Proto je velká pozornost věnována lokomoci koní (BACK *et* CLAYTON, 2013). Výzkumy jsou zaměřeny na odchylky, které mohou být ovlivněny jak faktory vnitřními (např. zdravotní komplikace), tak vnějšími (např. typ povrchu nebo schopnosti jezdce).

Akcelerometr je elektormechanický snímač pohybu, který se používá k měření sil zrychlení (MUTOH *et al.*, 2016). Dalším snímačem pohybu je gyroskop, který zaznamenává rotaci. Ty jsou často součástí produktů, které provádějí inerciální snímání, tj. umožňují současný záznam relativní polohy, orientace a rychlosti pohybujícího se předmětu (inerciální senzor), který může být připevněn ke koni nebo jezdci. GANDI *et al.* (2014) hodnotily pohybové vzorce jezdců při jízdě, konkrétně rotaci kyčlí. MUTOH *et al.* (2016) prokázali účinek hipoterapie na pacienty s dětskou mozkovou obrnou. Konkrétním příkladem je dlouhodobé zlepšení chůze po absolvování terapie. Také podle CASKOVÉ (2021) přinesl nástup výpočetní techniky výrazné zkvalitnění biomechanické analýzy, za pomoci softwarového zpracování dat ze zobrazovacích technik a simulačních nástrojů – techniky např. 3D kinematografická analýza - se pohled především na biomechaniku pohybu koně a člověka mění.

Využití technologií se ukázalo jako užitečné i při získávání údajů o chování, konkrétně vzdálené záznamy. Výhodou je, že je odstraněna subjektivita při sběru dat a lze umožnit identifikaci složek individuálního chování, které by vzhledem k omezeným možnostem lidského oka nebylo možné. Zejména v podmínkách, kdy je sledováno více subjektů najednou (RANDLE *et al.*, 2015). Již po desetiletí se zaznamenávalo kategorizované chování do etogramů, pomocí kterých lze při observačním výzkumu poskytovat objektivní výstupy (MCDONNELL, 2002). Chování je tradičně zaznamenáváno do etogramů ručně, v posledních letech s využitím videozáznamů (ABBEY *et* RANDLE, 2016). V nedávné době se vyskytla nenákladná možnost využití licencovaného softwaru v mobilních aplikacích. Limitem je však omezený počet kategorií chování, který je považován za atraktivní a poskytují tak pouze zjednodušený pohled na chování. NORTH *et al.* (2015) navrhli vývoj počítačem podporovaného nástroje pro automatickou identifikaci chování koní (HABIT). Cílem je vyhnout se antropomorfizaci při interpretaci interakcí člověka a koně. Existují však otázky, zda lze pomocí hardwaru a softwaru detekovat více projevů, které tvoří jediné klasifikované chování.

2.3.1 Hodnocení spermatu

Významnou kapitolu tvoří hodnocení kvality spermatu hřebců, které je užitečným nástrojem při odhadu plodnosti hřebce a má velký význam při zvyšování efektivnosti reprodukce koní (COLENBRANDER *et al.*, 2003, RODRIGUEZ *et al.*, 2001). Z toho důvodu mají tyto hodnoty velký ekonomický význam v chovu koní. Počítačem řízená analýza spermií (Computer Assisted Sperm Analysis – CASA) se díky vyšší míře objektivity a možnosti vyhodnocení tisíců trajektorií v jeden okamžik postupně stává standardem pro hodnocení spermií a jejich kinetických parametrů (CONTRI *et al.*, 2013). CASA je založena na postupném zachycení snímků z mikroskopu a jejich následné digitalizaci. Pohyblivé spermie pozorované na těchto snímcích jsou následně identifikovány na následujících snímcích, což umožňuje stanovení jejich trajektorií. Ty jsou matematicky zpracovány a lze je vyjádřit v číslech. Výsledky tohoto zpracování se odrážejí v řadě parametrů, které přesně definují pohyb pro každou jednotlivou spermii (QUINTERO-MORENO, 2003). Při hodnocení kvality spermatu jsou nejdůležitějšími parametry koncentrace, motilita a morfologie spermií (VANTMAN *et al.*, 1988, NEUWINGER *et al.*, 1990). Konkrétně hodnocení motility je jedním z nejvyužívanějších parametrů kvality spermatu (VĚŽNÍK *et al.*, 2004, CONTRI *et al.*, 2010). Mezi další se řadí hodnocení rychlosti spermií. Obrázek 3 znázorňuje terminologii systému CASA hodnotící rychlost spermií. Zelené body znázorňují polohu těžiště spermie a jejich spojení poskytuje obraz aktuální trajektorie spermie, která se nazývá křivočará dráha (curvilinear path). Časově zprůměrovaná rychlost podél této trajektorie se nazývá VCL (mm/s). Modře označená je vypočtená průměrná dráha (average path) a časově zprůměrovaná rychlost podél této dráhy se nazývá VAP (mm/s). Pro každé umístění těžiště existuje odchylka od průměrné dráhy, která se nazývá ALH (mm). Dále existují body, kde křivočará dráha protíná průměrnou dráhu a jejich počet se označuje BCF (počet za sekundu). Koeficient kolísání (WOB) se počítá jako podíl VAP a VPL (%) (AMANN *et* WABERSKI, 2014).



Obrázek 5: Hodnocení pohybu spermií systémem CASA

Automatická morfometrie vylepšila hodnocení morfologie spermií s přesným a opakovaným měřením rozměrů hlavičky u různých druhů, včetně hřebců (SOLER *et al.*, 2003, HIDALGO *et al.*, 2005). Navíc lze tuto analýzu použít k prokázání jemných rozdílů v rozměrech hlaviček spermií u plodných a neplodných hřebců (HIDALGO *et al.*, 2008). Postup hodnocení morfometrie hlavičky může zahrnovat měření jedné stovky spermií ze vzorku, kdy lze zaznamenat následující parametry hlavičky: délku, šířku, plochu a obvod. Z nich lze vypočítat následující parametry tvaru: elipticita = délka/šířka, drsnost = 4π*plocha/obvod^2, prodloužení = (délka-šířka/délka+šířka) a pravidelnost = (π*déla*šířka)/(4*plocha).

2.4 Statistické metody

V řadě oborů se vydělil samostatný směr zkoumání založený na analýze dat. Statistická analýza dat nabývá na stále větším významu a stává se často jedním ze základních přístupů v řadě přírodovědných, technických a sociálních věd (MELOUN et MILITKÝ, 1993). V průběhu 20. století se využití statistických analýz naměřených nebo zjištěných dat stalo téměř povinností při publikování výsledků nejrůznějších typů studií. Statistických metod je ovšem celá řada a souvisí to jak s charakterem sledovaných jevů a odvozených proměnných, tak s upořádáním datových souborů i počtem proměnných. Pro popis těchto dvou vztahů je pak základem statistický model struktury dat a z něj vyplývající vhodná metoda statistické analýza (HENDL, 2015). Pro různé typy znaků se používají různé číselné charakteristiky. Podle rostoucího stupně kvantifikace rozlišujeme čtyři typy znaků: nominální, ordinální, intervalové a poměrové (BUDÍKOVÁ *et al.*, 2010).

Chov koní je v dnešní době spojen hlavně s jezdectvím. Těžké pracovní využití koní je již minulostí a chov za účelem produkce masa nemá v České republice tradici. Jezdectví zahrnuje řadu činností, při kterých je kůň úzce propojen s člověkem. Mezi celosvětově rozšířené disciplíny lze řadit parkur, drezuru, vozatajství nebo westernové ježdění. Dalším využitím koní jsou rehabilitační aktivity, které se dělí do tří oblastí – fyzioterapeutická a ergoterapeutická (MUTOH *et al.*, 2016), pedagogická a sociální oblast (BURGON, 2011) a psychická a psychologická (LAC, 2016). Welfare koní se dostává v dnešní době do popředí zájmu po celém světě s rostoucím zaměřením na potřebu vytvoření kodexu v jednotlivých zemích, které by zahrnovali pravidla pro management a zacházení s koňmi během transportu (PADALINO *et al.*, 2016), ustájení (DALLA COSTA *et al.*, 2014) a závodů (WILLIAMS *et* RANDLE, 2017). Využití technologií s automatickým záznamem se může zdát výhodné z hlediska usnadnění práce, ale nezřídka přepis dat, jejich hodnocení a redukce často zabere mnohonásobně delší čas v porovnání s jejich získáním (MARTIN et BATESON, 2007). Bez ohledu na použitou metodu a technologii záznamu je podle RANDLE *et al.* (2017) důležité dodržovat soubor čtyř vědeckých principů – přesnost, spolehlivost, preciznost a vhodnost, aby bylo zajištěno, že data budou měřena dobře (tabulka 3).

Tabulka 3: Principy vědeckého měření a sběru dat (RANDLE *et al.*, 2017)

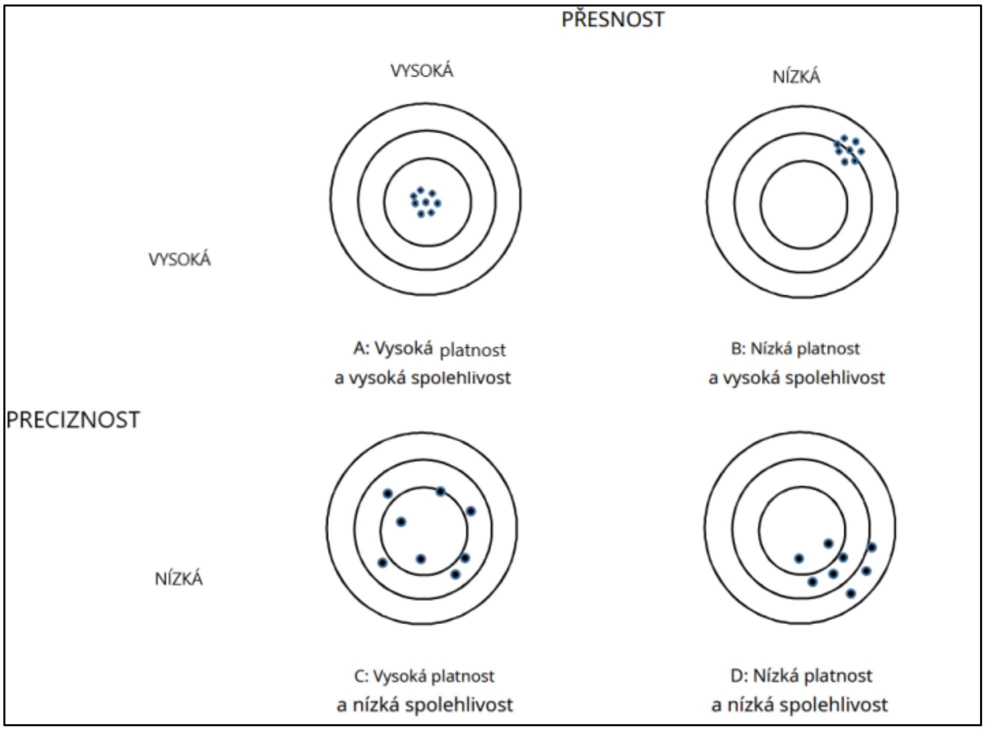
Princip	Definice
Přesnost	Jak dobře měřená hodnota odpovídá skutečné hodnotě.
Systematická chyba	Konstantní chyba, která zůstává stejná pro všechny měření. Vyskytuje se pouze v jednom směru.
Preciznost	Podíl záznamů bez chyby měření.
Náhodná chyba	Náhodná chyba nastane v experimentu kvůli nejistým změnám v prostředí. Vyskytuje se v obou směrech.
Platnost	Nakolik daný diagnostický nástroj měří to, co má.
Spolehlivost	Jak stabilní je měřící zařízení a jak dobře měřená hodnota odráží změny skutečné hodnoty.

Na obrázku 4 je názorně vyobrazen vztah mezi principy vědeckého měření (RANDLE *et al*., 2017). Případ A znázorňuje situaci, kdy jsou náhodná i systematická chyba malé, což vede k platnému měření a výsledkům, které lze považovat za spolehlivé. V případě B je náhodná chyba malá a systematická chyba je velká, což znamená, že výsledek je spolehlivý, ale postrádá platnost, čímž postrádá užitečnost. Varianta C ukazuje velkou náhodnou chybu a malou systematickou chybu, což znamená, že výsledek postrádá spolehlivost i přes vysokou platnost (validitu), a proto je málo užitečný. V případě D jsou náhodná i systematická chyba velké, to znamená, že výsledek postrádá platnost i spolehlivost, a proto není užitečný. HOLMES *et* JEFFCOTT (2010) uvádějí, že sběr a analýza dat z oblasti jezdectví by měly být v souladu se základními principy měření. PIERARD *et al.* (2015) doporučují využití technologií s ověřenými platnými postupy všude tam, kde je to možné.

Tvrzení WARANA *et* RANDLE (2017), že co umíme měřit, umíme i ovládat, zdůrazňuje význam měření, posuzování a hodnocení jezdecké teorie a praxe. U koně i jezdce lze provést celou řadu měření. Lze získávat fyzikální údaje, jako jsou tlak a napětí, lze provádět fyziologická měření, například měření srdeční frekvence a teploty. Mezi možná behaviorální hodnocení lze řadit zaznamenávání stereotypního chování nebo specifické činnosti, které zahrnují žvýkání, polykání nebo mrkání.

Obrázek 4 zobrazuje čtyři možné scénáře založené na principech vědeckého měření se zahrnutím dopadu systematických a náhodných chyb, které mohou vzniknout při použití technologického vybavení pro záznam dat. Pouze, když je zaznamenané měření přesné (tj. systematická chyba je malá) a precizní (tj. náhodná chyba je malá), mohou být získaná data považována za platná (validní) a spolehlivá, a tím zajišťují statistickou analýzu, která může dospět k závěrům skutečně založených na důkazech (RANDLE *et al*., 2015).

.



Obrázek 6: Vztah mezi principy vědeckého měření (RANDLE *et al.*, 2017)

RANDLE *et al.* (2017) se zaměřili na výzkum v oblasti jezdectví. V tabulce 4 je uveden přehled základních principů plánování experimentu ve studiích z oblasti jezdectví, které ve své práci uvádí. Veškerý výzkum by měl dodržovat široce uznávaný soubor principů měření. Vědci by měli stanovit nejvhodnější metodu sběru dat, aby zajistili reprezentativní údaje.

Velký pokrok v posledních desetiletích přineslo používání počítačů a specializovaného statistického softwaru (např. Statistica, SPSS, NCSS, SAS), což odstranilo nutnost provádět manuálně komplikované výpočty. Informační technologie mají – přes nesporně velký užitek – i negativní vliv v tom, že umožňují provádět nejrůznější výpočty, aniž by uživatel přesně chápal, co se počítá (HENDL, 2015).

Tabulka 4: Základní principy návrhu pokusu v jezdectví (RANDLE *et al.*, 2017)

Princip	**Důvod v rámci výzkumu v oblasti jezdectví**
Kontrolní skupina	Možnost porovnat experimentální/testované podmínky s nemanipulovanými
Kontrola faktorů vhodným postupem sběru dat a výběru objektů	Pohlaví koní, věk, předchozí zkušenost, krmení, ustájení, stupeň tréninku
Opakování	Jediné měření je nedostatečné k dosažení vědeckých závěrů
Náhodný výběr	Náhodný výběr testovaných objektů. Vzorky koní nebývají jednotného genotypu nebo fenotypu a může ovlivňovat sledované vlastnosti, proto je náhodný výběr obzvlášť důležitý
Efekt pořadí	Pořadí, ve kterém jsou jedinci testování může ovlivnit výsledky. Konkrétně v jezdectví se může jedná o únavu koní nebo hodnotitelů.
Řízení podmínek pokusu	Zajištění vhodného prostředí k pokusům. Většinou je vhodnější známé prostředí v porovnání s novým, při použití objektů by na ně hodnocení jedinci měli být zvyklí.
Nezávislost měření	Provedená měření by na sobě měla být nezávislá, tedy není opakovaně měřen jeden jedinec.
Nezaujatost hodnotitelů	Bývá problémem u hodnocení koní, které je těžké anonymizovat vzhledem k velkým fenotypovým rozdílům

V etickém rámci je komplikovanou otázkou počet pozorování v plánu pokusu. Vzorky pod 10 jedinců jsou obecně považovány za malé (HOLMES *et* JEFFCOTT, 2010). PIERARD *et al.* (2015) poukazují na to, že hodnotu výzkumu, zejména toho, který zahrnuje malý počet pozorování, lze zvýšit aplikací konzistentní metodiky a výsledky, které mohou být porovnávány napříč studiemi. Jakmile je stanoven počet pozorování, zajištěno etické schválení je zahájeno experimentální testování. Použití technologií ke sběru informací rychle generuje velké objemy dat, které budou mít hodnotu pouze při správném použití (HOLMES *et* JEFFCOT, 2010).

Orientační doporučení pro volbu statistického test vychází z rozdělení náhodných proměnných a jejich středních hodnot ve třech situacích, které se v praxi vyskytují. Jedná se o porovnání výběru s teoretickými předpoklady, porovnání dvou závislých nebo nezávislých výběrů, porovnání více než dvou závislých nebo nezávislých výběrů. V tabulce 5 je uvedeno, jaké metody a statistické testy lze použít při kombinaci různých typů proměnných (HENDL, 2015).

Tabulka 5: Hodnocení vztahu proměnných – rozhodovací tabulka pro výběr metody nebo statistického testu (HENDL, 2015).

Druhá z proměnných	**Jedna z proměnných**					
	spojitá (normální)	**spojitá (nenormální)**	**ordinální pořadová**	**ordinální kategoriální**	**nominální**	**dichotomická**
spojitá (normální)	regrese, korelace					
spojitá (nenormální)	regrese, pořadová korelace podle Spearmana	pořadová korelace podle Spearmana				
ordinální pořadová	pořadová korelace podle Spearmana	pořadová korelace podle Spearmana	pořadová korelace podle Spearmana			
ordinální kategoriální	Kendallův koeficient korelace	Kendallův koeficient korelace	Kendallův koeficient korelace	Kendallův koeficient korelace		
nominální	analýza rozptylu	test Kruskala a Wallise	test Kruskala a Wallise	$\chi 2$ test Fisherův přesný test	$\chi 2$ test Fisherův přesný test	
dichotomická	t-test, z-test	z-test pro velké výběry, Wilcoxonův test	Wilcoxonův test	Přesný Wilcoxonův test, $\chi 2$ test trendu v kontingenční tabulce	$\chi 2$ test Fisherův přesný test	$\chi 2$ test Fisherův přesný test

3 Hypotézy a cíle práce

Výzkumné hypotézy

Pro dosažení cílů byly stanoveny následující výzkumné hypotézy.

- Hmotnost teplokrevných koní v růstu lze odhadnout s využitím vhodných tělesných parametrů.
- S využitím Brodyho funkce lze aktualizovat růstovou křivku teplokrevných hřebců a stanovit pásmo standardu.
- Lze očekávat, že morfometrie spermií ovlivňuje jejich kinematické parametry.
- Kvalitu chlazené dávky ejakulátu teplokrevných hřebců ovlivňuje použité ředidlo.

Cíle práce

Tato práce byla zaměřena na čtyři hlavní cíle.

- Vytvořit vhodný vzorec pro odhad hmotnosti rostoucích teplokrevných koní na základě vybraných tělesných parametrů.
- Sestavit a aktualizovat existující matematický růstový model s využitím Brodyho funkce pro teplokrevné hřebce.
- Vyhodnotit souvislosti morfometrických a kinematických parametrů u spermatu teplokrevných hřebců.
- Porovnat vliv tří vybraných ředidel na kvalitu chlazených dávek teplokrevných hřebců.

4 Experimentální část a výsledky

4.1 Odhad tělesné hmotnosti hřebců z lineárních měření teplokrevných koní

Hmotnost koně je pro chovatele důležitou informací, ať už je to z důvodu přepravy zvířat nebo například dávkování léčiv. Existuje řada vzorců pro odhad hmotnosti u koní, ale všechny jsou vytvořeny pro dospělé jedince.

Cílem této studie bylo vytvořit vzorec pro odhad hmotnosti u rostoucích teplokrevných zvířat založený a lineárních vztazích. Celkem bylo zváženo 524 teplokrevných koní ve věku od 97 do 1290 dnů a u každého bylo provedeno 13 tělesných měření. Na základě největší míry korelace s hmotností byly vybrány pro odhad tělesné hmotnosti čtyři míry: délka těla (DT), obvod hrudníku (OH), výška hrudní kosti (VHK) a přední šířka pánve (PŠP), která se měří mezi zevními hrboly kyčelními. Z těchto čtyř parametrů byl vytvořen vzorec

$$pomocná\ hodnota = \frac{[(2*VHK*OH)*2*DT^2*PŠP]}{10\ 000\ 000}.$$

Byl brán ohled na různou intenzitu růstu u koní v průběhu dospívání, a proto byly vytvořeny 4 věkové skupiny s hraničními hodnotami 365, 650 a 950 dnů. S využitím koeficientů lineární korelace mezi vypočtenými a skutečnými hodnotami hmotnosti byl vytvořen vzorec pro odhad hmotnosti pro každou věkovou skupinu.

$$hmotnost = koefcient\_1 * pomocná\ hodnota + koeficient\_2$$

Hodnoty koeficientu pro koně ve věku do 365 dnů: koeficient_1=4,46; koeficient_2 = 91,12; věk od 365 do 650 dnů: koeficient_1=3,83; koeficient_2 = 135,87; věk od 651 do 950 dnů: koeficient_1=3,60; koeficient_2 = 168,09; věk nad 950 dnů: koeficient_1=3,23; koeficient_2 = 221,55.

V porovnání s předchozími vzorci pro odhad hmotnosti u teplokrevných koní je tento pro teplokrevné koně nejvhodnější. Tento výsledek byl potvrzen statisticky (p-hodnota < 0,001) a hlavním důvodem je zohlednění různé intenzity růstu v průběhu vývoje koní.

Publikační výstupy:

ČOUDKOVÁ, V., SACHELLO, V., ŠTĚRBOVÁ, H., KLEINOVÁ, A., PAPOUŠKOVÁ, Z., MARŠÁLEK, M., KOVANDA, J. Bodyweight Estimation From Linear Measures of Growing Warmblood Horses by a Formula. Journal of Equine Veterinary Science [online]. 2016, 36, 63-68 [cit. 2022-06-12]. ISSN 07370806. Dostupné z: doi:10.1016/j.jevs.2015.09.010

4.2 Růstové křivky vybraných parametrů hřebců českého teplokrevníka

Možnost hodnocení růstu poskytuje chovatelům užitečný nástroj k analyzování vývoje jedinců. Proto je důležité stanovit růstové křivky pro každé plemeno.

Hlavním cílem práce bylo vyhodnotit růstové parametry hřebců českého teplokrevníka (ČT) a stanovit jejich růstové křivky. Celkově bylo zaznamenáno na pěti odchovnách 7488 měření u 117 teplokrevných hřebců pro kohoutkovou výšku měřenou páskou (KVP) a hůlkou (KVH), obvod hrudníku (OH) a obvod holeně (Ohol). Hřebci byli měřeni pravidelně ve věku od 7 do 36 měsíců a pro výpočet byla použita Brodyho rovnice s nelineárními funkcemi s využitím software Statistica.12 (TIBCO®). Pomocí první derivace rovnice byla vypočtena intenzita růstu (přepočtena na denní přírůstek, cm/den). Jako průměrné míry v dospělosti byly získány hodnoty 167,4 ± 0,7 cm, 177,1 ± 0,8 cm, 194,3 ± 0,9 a 21,8 ± 0,1 pro KVH, KVP, OH a Ohol. Nejmenší intenzita růstu byla zaznamenána u KV (0,0026 ± 0,0001cm/den) a naopak nejvyšší u Ohol (0,0032 ± 0,0002 cm/den). Nejvyšší stupeň dospělosti při narození byl odhadnut u KV (70 %) a nejnižší u OH (51 %). Pro KVH byly stupně dospělosti určeny pro věk 6, 12, 18, 30 a 36 měsíců, a to 83, 88, 92, 94, 96 a 97 %. Pro OH byly tyto hodnoty pro stejný věk 76, 84, 90, 94, 96 a 98 %. Odhadnutý věk pro 99% stupeň dospělosti byl pro KVH, KVP. OH a Ohol 42, 44, 46 a 38 měsíců. Z toho vyplývá, že hodnota Ohol se přiblížila asymptotické hodnotě jako první a OH jako poslední ze sledovaných parametrů.

Pro všechny sledované parametry byly sestaveny percentilové a Z-score grafy. Percentilový graf s pásmem standardu mezi 25. a 75. percentilem byl vyhodnocen jako vhodnější pro využití v chovatelské praxi. Tato práce poskytla podrobné informace o růstu hřebců ČT a užitečné ukazatele pro řízení chovu, které jsou využitelné pro teplokrevné hřebce obecně.

Publikační výstupy:

ČOUDKOVÁ, V., VRBOVÁ, A., CIVIŠOVÁ, H., PAPOUŠKOVÁ, Z., MARŠÁLEK, M. The growth curves for some biometric traits in Czech Warmblood stallions. *Livestock Science* [online]. 2022, **255** [cit. 2022-06-12]. ISSN 18711413. Dostupné z: doi:10.1016/j.livsci.2021.104782

4.3 Počítačem řízená analýza morfometrie hlavičky a kinetických parametrů spermatu teplokrevných hřebců

Tato studie byla provedena za účelem stanovení semenných charakteristik teplokrevných hřebců a posouzení vztahu mezi parametry motility spermií a morfometrie hlavičky spermií pomocí počítačem řízené analýzy spermií.

Celkem bylo během připouštěcí sezóny 2016 odebráno 35 ejakulátů od 10 klinicky zdravých a plodných teplokrevných hřebců ve věku 3 až 22 let). Hodnotil se objem ejakulátu (ml), koncentrace spermií (M/ml), pohyblivost spermií kinematickými parametry a morfometrie spermií. Charakteristiky motility, koncentrace spermií a morfometrické parametry spermií byly objektivně stanoveny pomocí Sperm Class Analyzer (SCA, MICROPTIC SL). Celkový průměr ± hodnoty směrodatné odchylky objemu ejakulátu, koncentrace, celkové motility (MOT), progresivní motility, křivočaré rychlosti, přímočaré rychlosti (VAP), přímosti, linearity, amplitudy bočního posunu hlavičky, frekvence křížení drah (BCF), prodloužení a plochy hlavičky byly 50,29 ± 29,88 ml, 210,64 ± 90,98*10^6/ml, 88,68 ± 10,8 %, 36,64 ± 15,36 %, 63,23 ± 15,98 mm/s, 31,02 ± 9,39mm/s, 47,53 ± 14,32mm/s, 65,24 ± 5,72 %, 48,8 ± 8,34 %, 2,68 ± 0,41mm, 7,29 ± 0,84 Hz, 36,2 ± 3,59 %, 15,49 ± 1,54 mm^2, resp.

Mezi hřebci byly signifikantní rozdíly ve všech hodnocených parametrech spermií (p-hodnota < 0,05). Mezi parametry plochy a rychlosti (VSL, VAP a koeficient kolísání WOB) byly průkazné pozitivní korelace. Prodloužení hlavičky spermií pozitivně korelovalo s MOT. Více protáhlé spermie vykazovaly nižší frekvenci průsečíků křivočaré dráhy s průměrnou dráhou.

Publikační výstupy:

KŘÍŽKOVÁ, J., **ČOUDKOVÁ, V.**, MARŠÁLEK, M. Computer-Assisted Sperm Analysis of Head Morphometry and Kinematic Parameters in Warmblood Stallions Spermatozoa. *Journal of Equine Veterinary Science* [online]. 2017, **57**, 8-17 [cit. 2022-06-12]. ISSN 07370806. Dostupné z: doi:10.1016/j.jevs.2017.05.012

4.4 Vliv ředidla na chlazené sperma teplokrevných hřebců

Cílem této práce bylo porovnat ochranný účinek tří mléčných ředidel na chlazené sperma získané od hřebců po delší sexuální pauze (5 až 6 měsíců). Celkem bylo odebráno 21 ejakulátů od 11 hřebců. K ředění byla využita ředidla Kenney, EquiPLus a INRA 96. Hodnoceno bylo sedm parametrů – vitalita spermií (VIT, %), celková motilita (MOT, %), progresivní motilita (PMOT), počet progresivních spermií v mililitru (prog M/ml, milion/ml), průměrná rychlost (VAP, μm/s), křivočará rychlost (VCL, μm/s) a přímočará rychlost spermií (VSL, μm/s). Tyto parametry byly stanoveny metodou CASA 2 hodiny po zpracování spermatu a poté po 24, 48 a 72 hodinách skladování při 5 °C. Byl prokázán významný vliv doby skladování spermatu (p-hodnota < 0,001) na všechny hodnocené parametry. Vliv ředidla byl signifikantní (p- hodnota < 0,05) v případě VIT, MOT, PMOT, VCL, VSL a VAP. Průměrný ochranný účinek ředidla INRA 96 byl významně lepší po celou dobu skladování u všech sledovaných parametrů než u ředidla Kenney (p-hodnota < 0,01) a než u EquiPlus (p-hodnota < 0,05) u hodnot PMOT, VCL a VAP. U všech sledovaných ředidel hodnoty Mot a VIT klesaly lineárně, zatímco PMOT exponenciálně. Nejrychlejší pokles PMOT byl pozorován v průběhu prvních 24 hodin skladování. Výsledky dosažené s ředidlem INRA 96 ukázaly, že kvalita chlazeného spermatu u hřebců odebraných po delší sexuální inaktivitě může být dostatečně udržena nejméně během prvních 24 hodin skladování.

Publikační výstupy:

VOKROUHLÍKOVÁ, J., **ČOUDKOVÁ, V.**, ŠICHTAŘ, J., VRBOVÁ, J., BŮDOVÁ, A., MARŠÁLEK, M., RUTKAYOVÁ, J. Chemically defined caseinate extenders and their effect on the cooled semen collected from stallions with long sexual rest. *Journal of Central European Agriculture* [online]. 2021, **22**(2), 269-279 [cit. 2022-06-12]. ISSN 1332-9049. Dostupné z: doi:10.5513/JCEA01/22.2.2904

5 Přílohy

Kapitola experimentální část a výsledky zahrnuje výsledky a data, která byla publikována v odborných impaktovaných časopisech. Názvy publikací jsou následující.

- Bodyweight Estimation From Linear Measures of Growing Warmblood Horses by a Formula.
- The growth curves for some biometric traits in Czech Warmblood stallions
- Computer-Assisted Sperm Analysis of Head Morphometry and Kinematic Parameters in Warmblood Stallions Spermatozoa
- Chemically defined caseinate extenders and their effect on the cooled semen collected from stallions with long sexual rest

Journal of Equine Veterinary Science 36 (2016) 63–68

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Bodyweight Estimation From Linear Measures of Growing Warmblood Horses by a Formula

Veronika Čoudková [a,*], Vincent Sachello [b], Hana Štěrbová [c], Anna Kleinová [d], Zita Papoušková [e], Miroslav Maršálek [f], Jan Kovanda [g]

[a] Department of Zootechnical Sciences, University of South Bohemia, Veselí nad Lužnicí, Czech Republic
[b] Ecole supérieure des Techniques aéronautiques et de Construction automobile, Sucé sur Erdre, France
[c] Department of Zootechnical Sciences, University of South Bohemia, Zemský hřebčinec Písek, Písek, Czech Republic
[d] Department of Zootechnical Sciences, University of South Bohemia, Lhenice, Czech Republic
[e] Department of Zootechnical Sciences, University of South Bohemia, České Budějovice, Czech Republic
[f] Department of Zootechnical Sciences, University of South Bohemia, České Budějovice, Czech Republic
[g] Department of Vehicles and Ground Transport, Czech University of Life Sciences Prague, Praha 6, Czech Republic

ARTICLE INFO

Article history:
Received 22 June 2015
Received in revised form 15 September 2015
Accepted 18 September 2015
Available online 9 October 2015

Keywords:
Weight estimation
Estimation formula
Warmblood horse
Growth

ABSTRACT

Formulas for the estimation of bodyweight (BW) are generally used when no scale is available. The objective of this study was to develop a suitable formula for the BW estimation of warmblood horses of different age that were based on linear relations. Five hundred twenty-four warmblood horses from 97 to 1,290 days were weighed, and 13 body measurements, BW, and body mass index were taken. Because of the closest linear correlation with weight, four measurements were selected for weight estimation: body length (BL), heart girth (HG), sternum height (SH), and front pelvis width (FPW). The formula value = $[(2 \times SH + HG) \times 2 \times BL \times BL \times FPW]/10{,}000{,}000$ was developed from the four selected parameters. Taking into account the variable growth intensity during horse maturation, four age groups with boundary values of 365, 650, and 950 days were created. Using the coefficients of linear correlation between the value and actual weight, the formula for BW estimation was adjusted for each age group: mass = coefficient_1 × value + coefficient_2 (horse age < 365 days: coefficient_1 = 4.46, coefficient_2 = 91.12; 365 days < horse age < 650 days: coefficient_1 = 3.83, coefficient_2 = 135.87; 651 days < horse age < 950 days: coefficient_1 = 3.60, coefficient_2 = 168.09; horse age > 950 days: coefficient_1 = 3.23, coefficient_2 = 221.55). In comparison with past BW estimation formulas, the new equation is the most suitable for BW estimation in warmblood horses of different age (P value < .001).

1. Introduction

Horse bodyweight (BW) is not always a parameter which horse owners and breeders are able to accurately estimate. However, it is an important parameter describing body development and is a rough equivalent of physiological functions of an organism [1]. A horse's BW can be an important health indicator. Knowing how much a horse weighs and whether it is currently gaining or losing weight can help identify health issues. Low BW in a horse can be caused by health problems (disease or hormone imbalance), old age, parasites, overwork, an unbalanced diet, insufficient feed access (sometimes caused by a low pecking order in the herd), or genetics [2]. Knowing the BW of a horse is important in many facets of horse care, including designing feeding programs and administering

* Corresponding author at: Veronika Čoudková, Mažice 33, 391 81 Veselí nad Lužnicí, Czech Republic.
E-mail address: nika.13@seznam.cz (V. Čoudková).

0737-0806/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2015.09.010

medication. However, horse owners and veterinarians working in the field do not generally have access to a livestock scale for the purpose of obtaining a horse's weight [3]. Drugs and anthelmintic preparations are potentially toxic and should be limited to the dosages dictated by the weight of the animal [4,5]. Sendel [2] found that incorrect BW estimation may result in overdosing or underdosing the animal. Overdosing medicinal products may cause toxicity and colic or other serious complications, including death [2]. Underdosing will not produce the desired effects and, in the case of antibiotics, may lead to antibiotic resistance [2]. Johnson et al [6] found that 88% of horse owners underestimated actual BW by an average of 84.37 kg.

Several researchers have developed BW estimation formulas using body measurements of adult horses [3–5,7–14] and ponies [15–17]. Stanier et al [18] predicted BW of growing Thoroughbreds. Bodyweight of foals at 2 months of age is reaching 25% to 30% of the weight of the adult animal and after the 6 months of age 45%. Height at withers (HWs) of yearlings is corresponding to 91% to 93% of adults, but their weight corresponds to only 62% to 76% of the weight in adulthood. In the second year of life, the growth rate slows down. Two-year-old horses reach 84% to 90% of BW of adult animals [19–21]. Dušek et al [1] described changes in the body constitution of growing horses. Body framework of foals is similar to standing rectangle because HW is greater than body length (BL). Within 12 to 18 months of age, the body framework is changing into the square format. During further growth, BL is increasing faster than HW, so the body frame is similar to a rectangle lying on the longer side.

The objective of this research was to develop a simple formula to estimate the BW of different age groups of warmblood horses based on body measurements.

2. Materials and Methods

Measurement and weighing of horses were done on five farms across the Czech Republic in 2010 to 2012. A total of 524 horses at 97 to 1,290 days of age were measured and weighed on a portable livestock scale KERN UFA 1.5T0.5 (Kern & Sohn GmbH, Balingen, Germany) to the nearest 0.5 kg. In total, 15 variables including 13 body measurements (Fig. 1), body mass index, and BW were determined. Thirteen body measurements including:

- Height at withers measured by the stick
- Height at withers measured by the tape
- Heart girth (HG)
- Carpus circumference (CC)
- Sternum height (SH)
- Height at sacrum
- Height at cross
- Height at root of the tail
- BL
- Front pelvis width (FPW)
- Medium pelvis width
- Breast width of the shoulder joints
- Width of the heart

We first examined the relation between BW and age of horses expressed in days by regression analysis. Next, we evaluated the influence of each of the 13 determined body measurements on horse BW independently of the others. Subsequently, we evaluated the influence of combinations of girth, height, and length measurements on BW. Taking into account the growth physiology of horses, four age categories were created with boundary values of 365, 650,

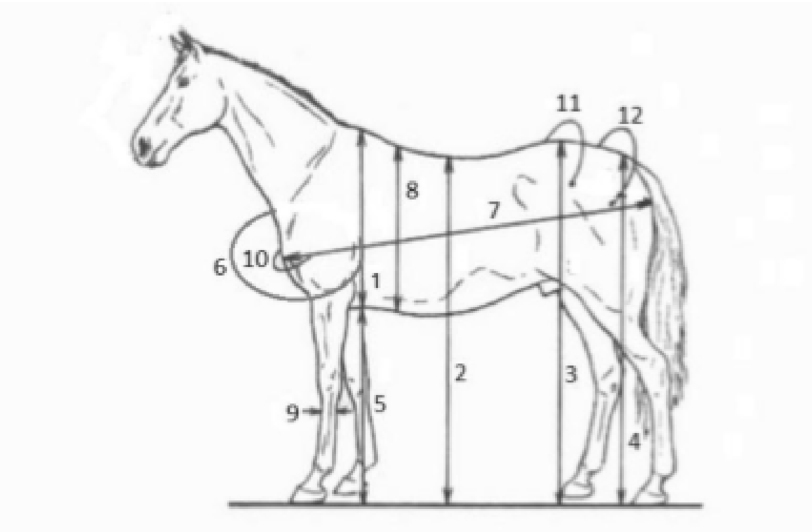


Fig. 1. The representation of body measurements used for the calculation (1 = height at wither measured by the stick/tape; 2 = height at croup; 3 = height at sternum; 4 = height at root of the tale; 5 = sternum height; 6 = width of the heart; 7 = body length; 8 = heart girth; 9 = carpus circumference; 10 = breast width of the shoulder joints; 11 = front pelvis width; and 12 = medium pelvis width) [1].

Table 1
The equations for estimation of BW (kg) from girth, height, and length measurements (cm), which were used in comparison with the new one.

Source	Equation
Shaker et al [8]	BW = height at withers × height at croup × body length × 0.0001
Carroll and Hungtington [5]	$BW = \frac{\text{heart girth}^2 \times \text{body length}}{11,877}$
Hall [10]	$BW = \frac{\text{heart girth}^2 \times \text{body length}}{11,880}$
Marcenac et al [22]	$BW = \left(\frac{\text{heart girth}}{100}\right)^3 \times 80$
Brown et al [23]	$BW = \frac{\text{heart girth}^2 \times \text{body length}}{8,717}$
Milner and Hewit [4]	$BW = \frac{(\text{heart girth} \times 0.39)^{1.97} \times (\text{body length} \times 0.39)^{1.05}}{228.1} \times 0.45$

Abbreviation: BW, bodyweight.

and 950 days from birth. Relationship of the particular parameters (and their combinations) and BW was represented graphically and examined for a linear relationship. Finally, combination of body measurements was selected that showed the highest level of linear relationship with BW.

Consequently, selected parameters were combined to most accurately estimate horse BW for different age groups using a simple formula. Based on this, four body measurements were selected, including:

- Body length—length from the tuber ischium to the point of the shoulder.
- Sternum height—distance from the surface of a platform to the sternum.
- Heart girth—measured around the circumference of the horse, directly behind the shoulder and over the horse's withers.
- Front pelvis width—distance between the external iliac tuberosities.

Fig. 1 illustrates how to take the four selected body measurements.

Actual BW was then compared with existing predicted BW equations [4,5,8,10,22,23] (Table 1) and to the newly developed formula using Spearman's coefficient of correlation. In addition, percent error was calculated for each method using the following formula [18]:

$$Error\ (\%) = \frac{observed\ \ value - predicted\ \ value}{observed\ \ value} \times 100$$

Cross-validation was used as a model evaluation method of suitability of newly developed formula for estimation of BW of growing warmblood horses. The programs MS Office and Statistica 10 were used for the data processing.

3. Results and Discussion

Horses included in the present study represented a broad selection among warmblood horses in the Czech Republic (Table 2). Horse BW ranged from 214 to 712 kg, and age ranged from 976 to 1,290 days. Horses older than 18 months have the body constitution of a rectangular shape lying on the longer side. During next growth, the body constitution of horses does not change [1]. Three-year-old horses reach 92% of BW adult animals [21].

The linear relationship of horse BW to age (in days) shows that age is a poor predictor of BW ($R^2 = 0.59$). This is due to the rapid growth and BW gain observed in the first year [1,19–21].

The growth curve of a horse is not linear and varies with age. Dušek et al [1] determined that the body constitution of a foal changes with increasing age. The initial body framework is similar to a standing rectangle because the withers height is greater than the BL. This gradually changes within 12 to 18 months, taking on a more square shape as BL increases. Between 2.5 and 3 years of age, HW and height at cross tend to become more equal with a slight increase in HW. By age 4 years, most horses tend to become stable and growth is limited. McKiernan [24] reported that the BW of warmblood adult horses ranged from 450 to 650 kg. Between 4 and 5 years of age, also the largest horse breeds are fully mature [19–21].

Table 2
Body measurements, actual BW, and predicted BW (mean ± SD) of growing warmblood horses.

Number of Days From Birth	Number of Horses	FPW (cm)	BL (cm)	SH (cm)	HG (cm)	Actual BW (kg)	Estimated BW[a] (kg)
<365	30	42 ± 4.8	138.2 ± 12.3	84.2 ± 3.9	156.6 ± 14.8	333.6 ± 86.0	333.6 ± 85.0
365–650	248	47.7 ± 2.8	151.3 ± 7.4	86.7 ± 4.2	171.9 ± 8.9	428.3 ± 56.6	428.4 ± 52.1
651–950	201	52.7 ± 2.3	161.6 ± 6.7	88.4 ± 4.3	184.8 ± 7.0	528.8 ± 55.6	528.8 ± 48.6
>950	45	55.6 ± 2.5	165.9 ± 5.2	89.6 ± 4.7	195.8 ± 5.6	593.6 ± 52.1	593.6 ± 40.3

Abbreviations: BL, body length; BW, bodyweight; FPW, front pelvis width; HG, heart girth; SD, standard deviation; SH, sternum height.
[a] BW estimated by new formula: value = [(2 × SH + HG) × 2 × BL × BL × FPW]/10,000,000, mass = coefficient_1 × value + coefficient_2 (horse age < 365 days: coefficient_1 = 4.46, coefficient_2 = 91.12; 365 days < horse age < 650 days: coefficient_1 = 3.83, coefficient_2 = 135.87; 651 days < horse age < 950 days: coefficient_1 = 3.60, coefficient_2 = 168.09; horse age > 950 days: coefficient_1 = 3.23, coefficient_2 = 221.55).

Table 3
R^2 values of linear relationship between body measurements and the actual body weight of growing warmblood horses (P value < .001).

Body Measurement	Number of Days From Birth				
	All Groups	<365	365–650	651–950	>950
HWS	0.83	0.83	0.69	0.63	0.46
HWT	0.80	0.81	0.62	0.56	0.35
HS	0.70	0.77	0.52	0.46	0.42
HC	0.71	0.80	0.62	0.55	0.37
HRT	0.68	0.71	0.57	0.51	0.40
BWS	0.73	0.87	0.63	0.43	0.27
WHG	0.82	0.91	0.73	0.53	0.19
FPW	0.81	0.87	0.69	0.48	0.35
MPW	0.83	0.91	0.75	0.65	0.40
BL	0.83	0.91	0.75	0.65	0.40
SH	0.30	0.42	0.27	0.26	0.25
HG	0.87	0.86	0.76	0.68	0.45
CC	0.70	0.82	0.63	0.56	—
FPW × BL × SH × HG	0.89	0.95	0.84	0.70	0.52

Abbreviations: BL, body length; CC, Carpus circumference; FPW, front pelvis width; HG, heart girth; HWS, height at wither; SH, sternum height; HWT, height at withers measured by the tape; HS, height at sacrum; HC, height at cross; HRT, height at root of the tail; BWS, breast width of the shoulder joints; WHG, width of the heart; MPW, medium pelvis width.

Table 3 shows evaluation of the influence of each of the 13 determined body measurements on horse BW independently of the others and one combination of them. For the estimation of horse BW, the combination of four body measurements was determined to be the most accurate in predicting BW, including SH, HG, BL, and FPW ($R^2 = 0.89$). Heart girth and FWP represented girth measurement, SH height, and BL length measurement. In correlation between HG and BW, Carroll and Hungtington [5] achieved the same result ($R^2 = 0.87$), but in case of BL and HW, they obtain lower R^2 (0.75 and 0.62, respectively). Other authors have also assessed the body measurements of BL and HG as ideal for estimating BW in adult horses [4,5,8,10,22,23] (Table 1). Stanier et al [18] used to monitor growth of Thoroughbreds HG, BL, CC, and left forelimb (distance from the point of the elbow to the midpoint of the proximal sesamoids).

These body measurements were included in a new BW estimation equation for growing warmblood horses:

$$Value = \frac{[(2 \times SH + HG) \times 2 \times BL \times BL \times FPW]}{10,000,000}$$

Formula 1

Subsequently, the basic formula was adjusted by means of coefficients for each of the four age groups to reflected changes in intensity of growth of horses. Fig. 2 represent a linear dependence of the value calculated by the basic Formula 1 (value) and the actual weight of horses, on the basis of which the coefficients for Formula 2 for the particular age groups were determined. The first two groups, which include horses until 650 days of age, achieved correlation coefficient greater than 0.85. In horses, 651 to 950 and >950 days of age correlation were reduced to $R^2 = 0.76$ and $R^2 = 0.60$, respectively (Table 4).

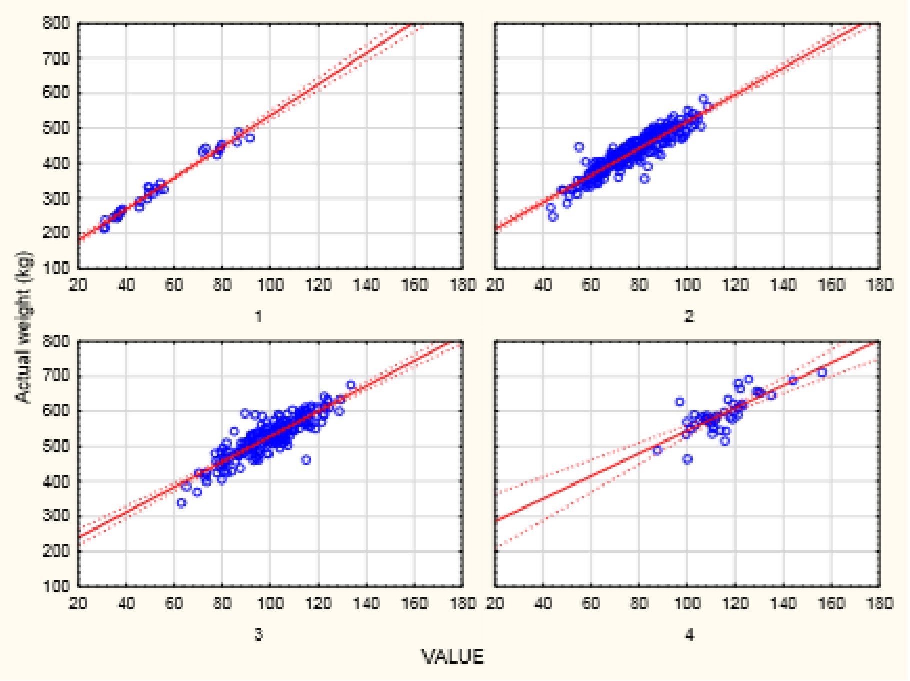


Fig. 2. Linear dependence of the variable value calculated by the basic Formula 1 (value = [(2 × SH + HG) × 2 × BL × BL × FPW]/10,000,000) and the actual weight of horses (kg) for four age groups, on the basis of which the coefficients for Formula 2 for the particular age groups were determined (1: age <356 days, n = 30, actual weight (kg) = 91.12 + 4.46 × value, $P = .0000$, $R^2 = 0.98$; 2: 365 to 650 days, n = 248, actual weight (kg) = 135.87 + 3.83 × value, $P = .0000$, $R^2 = 0.85$; 3: 651 to 950 days, n = 201, actual weight (kg) = 168.09 + 3.60 × value, $P = .0000$, $R^2 = 0.76$; and 4: age > 950 days, n = 45, actual weight (kg) = 221.55 + 3.23 × value, $P = .0000$, $R^2 = 0.60$). BL, body length; FPW, front pelvis width; HG, heart girth; SH, sternum height.

Table 4
Coefficients and R^2 for the calculation of BW of warmblood horses for four age groups by Formula 2; mass = coefficient_1 × value + coefficient_2.

Age of Horse (Days)	Coefficient_1	Coefficient_2	R^2
<365	4.46	91.12	0.98
365–650	3.83	135.87	0.85
651–960	3.60	168.09	0.76
>950	3.23	221.55	0.60

Abbreviation: BW, bodyweight.

Future research should focus on data collection for these age groups and improved BW estimation equations. The reason is that R^2 values of linear relationship between body measurements and the actual BW of horses decrease as the horse age. So, the linearity of growing of warmblood horses decreases with increasing age. Subsequently, R^2 values of linear relationship between variable value, which based on body measurements, decrease as the horse age.

Table 4 shows the coefficients for the calculation of the BW of warmblood horses within age groups. These coefficients are substituted in the Formula 1, and the result equation is listed below.

$$Mass = coefficient\_1 \times value + coefficient\_2 \qquad \text{Formula 2}$$

Results from Formula 2 were compared with six previously published equations (Fig. 3). There were differences between the newly developed equation and previously published BW estimation equations (P value < .000). In all formulas, a high level of correlation between the calculated and actual BW of horses was demonstrated (Spearman's coefficient >0.95). But the interpretation of a difference between the calculated and actual BW for particular formulas showed that actual weight is either underestimated or overestimated. The calculation according to Shaker et al [8] underestimates the BW to the largest extent, by an average of 78 kg. The same result was obtained using the formulas developed by Carroll and Hungtington [5] and Hall [10], which underestimate the weight an average of

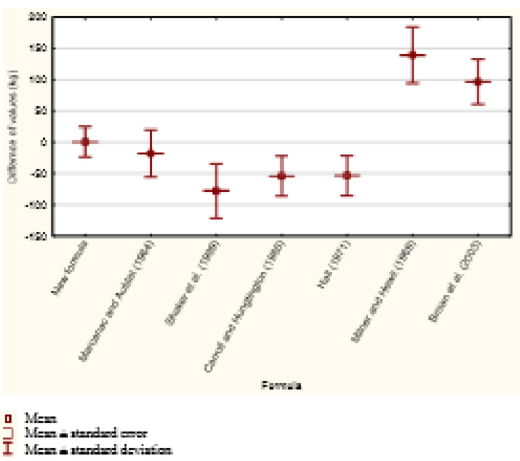


Fig. 3. The box plot comparing differences between calculated weights according to the particular formulas and the actual horse weight (n = 524; P value < .0001).

Table 5
Multiple comparison of differences between BW estimations according to the formulas and actual BW (homogeneous subsets) and mean error of the calculation.

Kruskal–Wallis Test: H (7, n = 4,011) = 3,338.427 P = 0.000

Formula	Mean Error (%)	Difference Mean ± SD	1	2	3	4	5	6
Shaker et al [8]	16.2	−77.9 ± 43.6			[a]			
Carroll and Hungtington [5]	11.9	−54.1 ± 31.8	[a]					
Hall [10]	11.8	−53.4 ± 31.8	[a]					
Marcenac et al [22]	6.7	−17.9 ± 37.7				[a]		
New formula	3.9	0.5 ± 24.3					[a]	
Brown et al [23]	20.5	97.0 ± 34.1						[a]
Milner and Hewit [4]	29.6	139.4 ± 45.1		[a]				

Abbreviations: BW, bodyweight; SD, standard deviation.
[a] P < .001.

54.1 kg and 53.4 kg, respectively. The closest estimation of BW was obtained by using the formula developed by Marcenac et al [22], which resulted in an underestimation in BW of 17.9 kg. Other formulas overestimated BW, including formula developed by Milner and Hewit [4], which overestimated horse BW to the largest extent (+139.4 kg). A comparison of differences between the calculated and actual BW demonstrated a difference (P value < .000) between the formulas (Table 5). The mean error of the calculation according to the formula corresponds to the absolute value of a difference in the estimation of horse BW and actual BW. The highest error of BW estimation was observed in formula developed by Milner and Hewit [4], whereas the newly developed formula estimated the BW of warmblood horses with the lowest error. Among existing BW estimation equations, the lowest error occurred when using equations developed by Marcenac et al [22].

None of the existing equations used for comparison took into the account growing horses. Stanier et al [18] estimated the BW of growing Thoroughbred horses; however, their study was aimed at foals until 540 days of age. And they used to monitoring of growth HG, BL, CC, and left forelimb. Because our 13 body measurements did not include left forelimb, their formula could not be included in the comparison. However, they stated that in the BW estimation of growing Thoroughbred horses, if selected formulas of other authors were used [5,25,26], the mean error of the BW estimation increased with decreasing age of foals.

4. Conclusion

Providing a simple but accurate BW estimation formula for growing warmbloods using body measurements has wide application in the horse breeding industry. Based on the relationship between BW and age, horses were divided into four age categories with boundary values of <365, 365 to 650, 651 to 950, and >950 days from birth. Four parameters including FPW, BL, SH, and HG were selected as the most suitable body parameters to be used for BW estimation. Variable for estimation of horse BW was value = [(2 × SH + HG) × 2 × BL × BL × FPW]/10,000,000.

Linear dependence between actual BW and variable value was constructed for each age category of horses. The resulting coefficients were added to strengthen the original equation: mass = coefficient_1 × value + coefficient_2 (horse age < 365 days: coefficient_1 = 4.46, coefficient_2 = 91.12; 365 days < horse age < 650 days: coefficient_1 = 3.83, coefficient_2 = 135.87; 651 days < horse age < 950 days: coefficient_1 = 3.60, coefficient_2 = 168.09; horse age > 950 days: coefficient_1 = 3.23, coefficient_2 = 221.55).

A comparison of BW estimation to existing formulas shows that warmblood horses BW can be accurately predicted using formulas developed by Marcenac et al [22]. However, the BW estimation formula currently developed resulted in the greatest accuracy when predicting growing warmblood horse BW. Therefore, this equation seems to be the most suitable for the BW estimation of growing warmblood horses.

Acknowledgments

This work would not have been possible without the financial support of Grant Agency of University of South Bohemia GAJU-020/2013/Z.

References

[1] Dušek J, Misař D, Müller Z, Navrátil J, Rajman J, Tlučhoř V, et al. Chov koní. 2nd ed. Praha: Brázda; 2007.
[2] Sendel T. Estimating body weight for horses. Factsheet 2010;10:1–4.
[3] Wagner EL, Tyler PJ. A comparison of weight estimation methods in adult horses. J Equine Vet Sci 2011;31:706–10.
[4] Milner J, Hewit D. Weight of horses: improved estimates based on girth and length. Can Vet J 1969;10:314–6.
[5] Carroll CL, Hungtington PJ. Body condition scoring and weight estimation of horses. Equine Vet J 1988;20:41–5.
[6] Johnson EL, Asquith RL, Kivipelto JK. Accuracy of weight determination of equids by visual estimation. Eleventh Equine Nutrition and Physiology Symposium. Stillwater, Oklahoma, 1989:241.
[7] Hoffmann G, Bentke A, Rose-Meierhöfer S, Ammon C, Mazetti P, Hardarson GH. Estimation of the body weight of Icelandic horses. J Equine Vet Sci 2013;33:893–5.
[8] Shaker MM, Wael AK, Nabeeh MA. A new equation for estimation of horses body weight. Pakistan Vet J 1999;19:216–7.
[9] Gibbs PG, Householder DD. Estimating horse body weight with a simple formula. Texas AM University, College Station: Department of animal science, Equine science program; 2003.
[10] Hall LW. Wright's veterinary anesthesia and analgesia. 7th ed. London: Bailliere Tindall; 1971.
[11] Leighton-Hardman AC. Equine nutrition. London: Pelham Books; 1980.
[12] Martison KL, Coleman RC, Rendahl AK, Fang Z, McCue ME. Estimation of body weight and development of a body weight score for adult equids using morphometric measurements. J Anim Sci 2014;92:2230–8.
[13] Neder GA, Pérez A, Perrone G. Assessment of body weight of the Criollo horse using morphometric measurements: confirmation of published equations for other breeds and development of a new formula. Redvet 2009;10.
[14] Catalano DN, Coleman RJ, Hathaway MR, McCue ME, Rendahl AK, Hansen AM, Martinson KL. Estimation of actual and ideal bodyweight for adult draft and warmblood horse breeds using morphometric measurements. J Equine Vet Sci 2015;35:423–4.
[15] Evans JW, Borton A, Hintz HF, Van Vleck LD. The horse. San Francisco: W.H. Freeman and Company; 1977.
[16] Owen KM, Wagner EL, Dowler LE, Eller WS. Estimation of body weight in ponies. J Anim Sci 2008;86:431.
[17] Bruce AM, Wagner EL, Tyler PJ. Weight estimation in miniature horses and Shetland ponies. J Anim Sci 2010;88:204.
[18] Stanier WB, Kronfeld DS, Hoffman RM, Wilson JA, Harris PA. Weight prediction from linear measurements of growing Thoroughbreds. Equine Vet J 2004;36:149–54.
[19] Jelan ZA, Jeffcott L, Lundenheim N, Osborne M. Growth rates in thoroughbred foals. Pferdeheilkunde 1996;12:291–5.
[20] Pagan JD, Jackson SG, Caddel S. A summary of growth rates of thoroughbreds in Kentucky. Pferdeheilkunde 1996;12:285–9.
[21] Gesellschaft für Ernährungsphysiologie der Haustiere. Empfehlungen zur Energie- und Nährstoffversorgung des Pferdes. Frankfurt am Main: DLG-Verlag; 1994.
[22] Marcenac L-N, Aublet H, D'Autheville P. Encyclopédie du cheval. 1st ed. Paris: Maloine; 1964.
[23] Brown JH, Pilliner S, Davies Z. Horse and stable management. 4th ed. Oxford: Blackwell Publishing; 2003.
[24] McKiernan B. Estimating a horse's weight. Primefact 2007;494:1–3.
[25] Willoughby DP. Growth and nutrition in the horse. London: A.S. Barnes and Co; 1975.
[26] Ensminger ME. Horses and horsemanship. 5th ed. Danville: Interstate Printers and Publishers; 1977.

Livestock Science 255 (2022) 104782

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

The growth curves for some biometric traits in Czech Warmblood stallions

Veronika Čoudková [a,*], Aneta Vrbová [a], Hana Civišová [a], Zita Papoušková [a], Miroslav Maršálek [b]

[a] Department of Animal Husbandry Sciences, Faculty of Agriculture, University of South Bohemia, Studentská 13, 370 05 České Budějovice, Czech Republic
[b] Department of Animal Breeding, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1/1665, 613 00 Brno, Czech Republic

HIGHLIGHTS

- Growth patterns of the Czech Warmblood stallions were studied.
- Brody model was used to describe the growth of four biometric traits.
- Percentile and Z-score growth curves were established.

ARTICLE INFO

Keywords:
Czech Warmblood stallion
Brody function
Body measures
Growth
Z-scores
Percentiles

ABTRACT

The possibility of growth tracking gives breeders the opportunity to analyse animal development and monitor it in daily farm management. Therefore, it is important to establish an ideal growth rate for each breed. The main objective of the present study was to characterise the growth patterns of Czech Warmblood (CW) stallions and establish their growth curves. A total of 7,488 records for withers height (WH) measured by stick (WH_s), WH measured by tape (WH_t), girth (G) and cannon circumference (CC) were obtained from 117 warmblood stallions raised at five rearing facilities for testing young horses (RFT). Data were regularly collected from 7 to 36 months of age. Brody's equation was adjusted using the non-linear (NLIN) procedures of Statistica software, version 12 (TIBCO®). Growth rates (adjusted daily gain [ADG], cm/d) were obtained from the first derivative of the equations. The mean mature sizes were 167.4 ± 0.7 cm, 177.1 ± 0.8 cm, 194.3 ± 0.9 cm and 21.8 ± 0.1 cm, respectively, for WH_s, WH_t, G and CC. The slowest growth rate was observed at WH (0.0026 ± 0.0001) and the fastest at CC (0.0032 ± 0.0002). The highest degree of maturity at birth was predicted at WH (70%) and the lowest at G (51%). The WH_s degree of maturity at 6, 12, 18, 24, 30 and 36 months of age were 83, 88, 92, 94, 96 and 97%, respectively. The G degrees of maturity for the same ages were 76, 84, 90, 94, 96 and 98%. Ages at 99% of mature WH_s, WH_t, G and CC were 42, 44, 46 and 38 months, respectively. It follows that CC approached the asymptotic value as the first one and G as the last one from the monitored biometric traits. Percentile and Z-score curves were established for all evaluated biometric traits. Percentiles with intervals from the 25th to 75th as the standard of CW stallions appeared more appropriate for breeders, as they tend to be intuitive and more easily understood. The present study provided detailed information about the growth of CW stallions, which revealed useful indicators for breeding management.

1. Introduction

The Czech Warmblood (CW) is a sturdy horse with a solid body structure, having many features reminiscent of its carriage drawing ancestors, but with variable movement mechanics. It is quite long-lived, has a good temperament, and is persistent, undemanding and relatively low in maintenance (Edwards, 1994). The goal of breeding the CW is to produce a noble, proper and easy-to-ride horse which, based on its temperament, character, spacious and elastic mechanics of movement and strong health, is suitable for all types of performance in equestrian sports within the International Federation for Equestrian Sports (FEI) disciplines, as well as for leisure activities. The adult horse is of medium body frame with good lines and a solid foundation without obvious genetic defects and diseases. The adult stallion should reach a height at withers of 162–170 cm and a cannon circumference (CC) of 21–22.5 cm. The Hanoverian Warmblood, the Oldenburg Warmblood, the Holstein Warmblood, the Royal Dutch Warmblood (*Koninklijk Warmbloed Paardenstamboek Nederland* [KWPN]) and the Selle Français (SF) are often

* Corresponding author.
E-mail address: karlov00@zf.jcu.cz (V. Čoudková).

https://doi.org/10.1016/j.livsci.2021.104782
Received 4 May 2021; Received in revised form 11 November 2021; Accepted 15 November 2021
Available online 21 November 2021

used to refine the CW (SCHČT, 2013).

Knowledge of the growth curve is important to all animal scientists, regardless of specialisation, who are concerned with the effects of their research and recommendations of lifetime production efficiency (Fitzhugh, 1976). The breeders' aim is to produce foals that exhibit optimal growth and development, but determining how to achieve an appropriate growth rate is a major concern (Pimentel et al., 2017). The majority of studies on horse growth are based on weight, height, chest girth and cannon bone. Of these measurements, height has more practical interest because it is required for the purpose of description and classification of horses, while weight is a measure of an animal's physical condition (Santos et al., 1999). Furthermore, studies have also shown a high incidence of developmental orthopaedic diseases when foals have a high growth rate (Gallio et al., 2014). Fradinho et al. (2016) suggested that growth rate is one of the important parameters that is taken into account when creating the feed ration of horses, and it is therefore of major importance that accurate growth curves are known. Thus, it is essential to establish an ideal growth rate for each breed (Pimentel et al., 2017). Growth evaluation is a part of stallion licensing (*körung*). It is used to select stallions of different breeds suitable for the breeding of certain breeds. The procedure regulates the rules of breeding associations and laws.

The use of mathematical growth models provides an effective way to summarise the information contained in data into a few parameters with biological meaning (Fitzhugh, 1976). Non-linear functions are easy to interpret biologically and can be readily compared across different production systems (Silva et al., 2011). Among the most commonly used growth models are those of Gompertz, Brody, Logistic, Weibull and Richards and von Bertalanffy.

Several reports have been generated on the growth curve of different horse breeds. Great attention is paid to thoroughbreds (Denham and Splan, 2007; Green, 1976; 1969; Hintz et al., 1979; Kocher and Staniar, 2013; Morel et al., 2007; Onoda et al., 2011, 2013; Pagan et al., 1996; Rogers et al., 2004; Staniar et al., 2004; Staniar et al., 2005; Thompson, 1995); other breeds analysed are Caspian horses (Hossein-Zadeh and Ghorbani, 2018), Lusitano horses (Fradinho et al., 2016), Pantaneiro horses (Santos et al., 1999), Criollo horses (Pimentel et al., 2017), Mangalarga Marchador horses (Cabral et al., 2004; Fernandes et al., 2020; Pinto et al., 2005; Rezende, 1998), Arabian horses (Heugebaert et al., 2010; Reed and Dun, 1977) and warmblood horses in Brazil (McManus et al., 2010). Heugebaert et al. (2010) estimated growth curves of weight, and Robert et al. (2013) developed forelimb conformation of Selle Français, Thoroughbred and French Trotter. However, there has not been any report on the growth curve of biometric traits of warmblood horses in Central Europe.

The CW growth curve was compiled in the 1970s by (Dušek, 2011). The aim of this study was itsto update the growth curves of four biometric traits: withers height (WH) measured by stick (WH_s), WH measured by tape (WH_t), girth (G) and CC of CW stallions. These are commonly used in the Czech Republic to evaluate growing stallions. Also, the findings of our study on the use of warmblood breeds in Czech Warmblood breeding provide beneficial information for the analysis of animal development of warmblood horses in general.

2. Materials and Methods

2.1. *Animals and management*

This study included 117 clinically healthy stallions registered in the CW studbook. We conducted a longitudinal study in five rearing facilities for testing young horses (RFTs) located in the Czech Republic (at an altitude of 235 to 675 m above sea level). These facilities were established and are controlled by the state. The stallions had *ad libitum* access to water. Foals were group-fed in large paddocks with compound feeds and grass hay with pasture access. Feed rations were set for winter and summer. A standardised herd health schedule with routine vaccination and deworming programmes was practiced in the RFT.

Stallions were assembled after weaning at the age of six months. The following year, the spring and autumn körungs took place, which included the measurement and inclusion of horses in the growth curve. In addition, the exterior, movement and free jumping were evaluated. At the beginning of the following year, in which the stallions reached the age of three, they were started under saddle. The third körung took place in February, and the stallions were then tested in May. In September, they took a 70-day test, which concluded with performance tests. The stallions that successfully passed all these tests became breed stallions registered in the CW studbook.

2.2. *Data collection*

Body measurements, WH_s, WH_t, G and CC, were taken on a monthly basis from 6 to 12 months, every two months from 12 to 24 months and every three months from 24 to 36 months. WH was measured with a standard measuring stick marked with centimetres (WH_s) and a flexible measuring tape marked with centimetres (WH_t) from the ground to the highest point of the withers (i.e. the spinous process of the fifth thoracic vertebra). G (chest girth measured around the circumference of the horse directly behind the shoulder and over the horse's withers was measured with a flexible measuring tape marked with centimetres (Čoudková et al., 2016). CC (circumference taken at the midsection of the cannon bone) was measured with a flexible measuring tape marked in 0.1 cm increments. All measurements were taken by the same operator.

2.3. *Data analysis*

Brody's non-linear growth model (Brody and Lardy, 1946) was applied to characterise the growth curves for biometric traits. Brody's equation (Eq. [1]) was adjusted using the non-linear (NLIN) procedures of Statistica software, version 12 (TIBCO®). In the model, the variable *y* is described as a function of age, which is represented by the following expression:

$$y_t = A\left(1 - be^{-kt}\right), \tag{1}$$

where y_t is the vector of biometric measurement observations; A, b and k are model parameters to account for scale and shape of the growth curve; and t_i is a vector of time from birth (age in days).

The parameters of this equation can be biologically interpreted. Briefly, A is the asymptotic limit for the observed biometric trait as t approaches infinity and is commonly interpreted as the mean mature size; b is a scaling parameter that defines the degree of maturity at time of birth t = 0 (1 − b); k is a rate constant that establishes the distribution of the sigmoid curve along the time axis and serves both as a measure of growth rate and of change in growth rate (large k values indicate early maturating individuals; small k values indicate late maturing individuals) (Fitzhugh, 1976; Richards, 1959; Staniar et al., 2005).

The proportion of mature size attained at age t (u_t) (Eq. [2]) was calculated according to Fitzhugh (1976):

$$u_t = \frac{y_t}{A} = 1 - be^{-kt}. \tag{2}$$

Another parameter that is derived to determine growth earliness is the age required to reach a certain degree of maturity (t_u) (Eq. [3]). The formula for its calculation can be obtained by expressing t from the equation for the degree of maturity at age t (u_t) as follows:

$$t_u = -\frac{1}{k}\ln\left|\frac{1-u_t}{b}\right|. \tag{3}$$

In this study, the ages required to reach 75% and 99% of mature size, respectively, were calculated ($t_{0,75}$ and $t_{0,99}$).

Accuracy for prediction equations was further evaluated by calculating the percent error (Eq. [4]) (Kocher and Stainar, 2013) and residual standard deviation (RSD) as seen in the following:

$$\text{Error (\%)} = \text{abs}\left(\frac{y_{obs} - y_{pred}}{y_{obs}}\right) * 100. \tag{4}$$

RSD was calculated using the expression RSD $= \sqrt{\text{MSE}}$, with MSE being the mean squared error (Fernandes et al., 2020).

The first derivative of the equation describing the change in a size variable provides the description of the rate of change or growth velocity in that size variable with respect to time (Kocher and Stainar, 2013). For each biometric trait, the first derivative of Brody's equation (Eq. [5]) was calculated with respect to time ($\delta y/\delta t$), expressing the instantaneous rate of gain (average daily gain [ADG], cm/m) at time t (t = days of age) (Fitzhugh, 1976):

$$f'(t) = k[(A - f(t)] = ABke^{-kt}. \tag{5}$$

Usual percentiles with 3rd, 10th, 25th, 50th (median), 75th, 90th and 97th curves and *Z*-scores with cut points in median ± standard deviation (*SD*), median ± 2 SD and median ± 3 SD (Wang and Chen, 2012) were used to establish CW stallion growth curves. Table 1 shows the corresponding values between percentiles and Z-scores for data under normal distribution (mean and *SD* can be used for conversion). Mean and *SD* were established using individual estimates of growth, which were obtained using the Gauss–Newton method in the NLIN procedure in Statistica software, version 12.

3. Results

The parameter estimates of the non-linear growth model of Brody, percent error and RSD for all observed biometric traits are summarised in Table 2. The lowest percent error was observed in WH_s, 2.12%, whereas the highest percent error of 3.39% was for the CC growth model. According to the chosen growth model, the asymptotic mean mature WH_s, WH_t, G and CC were 167.4 ± 0.7, 177.1 ± 0.8, 194.3 ± 0.9 and 21.8 ± 0.1, respectively. The resulting values of scaling parameter b indicated that the highest degree of maturity at t = 0 (u_0 = 70%) was observed in parameters of height (WH_s and WH_t). Predicted WH_s and WH_t at birth (considering t = 1 day of age) were 117.7 cm and 124.3 cm, respectively, which is a credible prediction. In the case of CC, the predicted value at birth was 13.2 cm. The highest value of b in comparison to other biometric traits of this work was recorded in G, and the predicted value at birth was 99.2 cm.

Related to these results are the degrees of maturity (ut) counted at age 7, 12, 18, 24, 30 and 36 months and age at 75% and 99% of maturity (t_u), which are presented in Table 3. In addition, the values of u_t and t_u of other authors considering their growth models are included in the evaluation. The rate at which biometric traits approach asymptotic values is affected by the scaling parameter b and by maturity index k. Parameter b reflects the degree of maturity at birth (u_0), and k serves both as the measure of growth rate and the rate of change of growth rate. In the present study, the highest u_0 was observed at height parameters with the lowest maturity index k (0.0026). In CC, based on the b value, u_0 was lower (61%) compared with WH (70%), while the CC k value was the highest (0.0032); at that point, this biometric trait approached the asymptotic value as the first one, estimated as an age of 38 months. This was followed by growth in height, where the t_{99} estimate was 42 months of age. The estimated age of parameter G asymptotic approach was 44 months. This represented the latest maturation of all the parameters observed, despite the higher growth rate for G than for WH (0.0028), but with the lowest value of u_0 = 51%.

Due to the normal distribution of data, it was possible to use the mean and *SD* (Table 4) to establish *Z*-score and percentile growth curves for the observed biometric traits (Fig. 1). Based on their definition, *Z*-score intervals are larger than percentiles (standard mean range is ± 1 *SD* including 68.26% of the data in *Z*-scores and ± 0.67 *SD* including 50% of the data in percentiles).

In Fig 2., the ADGs of the studied traits were plotted against the day of age from 7 to 36 months. WH_s and WH_t at the age of 7 months reached 83% of the mature value, and ADG was 0.075 cm/day and 0.079 cm/day, respectively. The WH_t ADG curve gradually approached the WH_s curve due to decreasing G ADG values, which affected WH_t ADG. At the age of 1,096 days, both height parameters approached the ADG rate of 0.008 cm/day equally. The G ADG declined from 0.147 cm/day at 213 days to 0.012 cm/day at 1,096 days of age, which is the highest ADG value at this stage. This result confirmed that G is the last trait to approach asymptotic mature size limit. However, the closest approach to the x-axis can be seen in the CC curve, where ADG declines from 0.014 cm/day at 213 days to 0.001 cm/day at 1,096 days of age.

4. Discussion

To better understand the relationship between morphofunctional characteristics and age, the study of animal growth curves has gained prominence for analysing animal development as the method summarises the information from the data into a set of parameters that can be interpreted and applied in daily farm management (Fernandes et al., 2017; Freitas, 2005; Silveira et al., 2011). Non-linear functions with exponential components are most commonly used to describe this type of growth (Valette et al., 2008). According to Tedeschi et al. (2000), the parameters of superior asymptotic value and maturing rate, from non-linear curves (i.e. Gompertz, Brody, Logistic, Weibull and

Table 1
List of usually used percentiles and Z-scores, values converted through mean and standard deviation (SD), modified according to Wang et Chen (2012). Commonly used values of Z-scores and percentiles are highlighted.

Z-scores	Mean, SD	Percentiles
-3	$\bar{x}$ - 3*SD*	0.14th
-2	$\bar{x}$ - 2*SD*	2.28th
-1.88	$\bar{x}$ - 1.88*SD*	**3rd**
-1.29	$\bar{x}$ - 1.29*SD*	**10th**
-1	$\bar{x}$ - *SD*	15.87th
-0.67	$\bar{x}$ - 0.67*SD*	**25th**
0	$\bar{x}$	**50th (median)**
0.67	$\bar{x}$ + 0.67*SD*	**75th**
1	$\bar{x}$ + *SD*	84.13nd
1.29	$\bar{x}$ + 1.29*SD*	**90th**
1.88	$\bar{x}$ + 1.88*SD*	**97th**
2	$\bar{x}$ + 2*SD*	97.72nd
3	$\bar{x}$ + 3*SD*	99.86th

Table 2
Parameter estimates, RSD and standard errors of Brody nonlinear growth model fitted to withers height, girth and cannon circumference as a function of age (days) in Czech warmblood stallions.

Measure[a]	Parameters[b] A (± SE)	b (± SE)	k (± SE)	RSD[c]	Error[d] (%)
WH_s (cm)	167.4 ± 0.7	0.298 ± 0.005	0.0026 ± 0.0001	3.99	2.12
WH_t (cm)	177.1 ± 0.8	0.299 ± 0.005	0.0026 ± 0.0001	4.39	2.15
G (cm)	194.3 ± 0.9	0.491 ± 0.010	0.0028 ± 0.0002	6.3	2.67
CC (cm)	21.8 ± 0.1	0.394 ± 0.020	0.0032 ± 0.0002	0.88	3.39

[a] WH_s – withers height measured by stick, WH_t – withers height measured by tape, G – girth, CC – cannon circumference
[b] A – asymptotic value for WH_s/WH_t/G/CC (interpreted as mean WH_s/WH_t/G/CC at maturity); b – scaling parameter that defines the degree of maturity when age = 0 day (intercept on y axis); k – maturing index (rate that establishes the spread of the curve along time axis)
[c] RSD – residual standard error
[d] Error – mean percent error

Table 3
Degree of maturity (%) and Age at degree of maturity (months) for withers height, girth and cannon circumference at standard ages for the Czech warmblood and other horse breeds.

Measure[a]	Source	Breed	Growth model[b]	Degree of maturity[c] (%)						Age at degree of maturity[d] (months)	
			$A(1-be^{-kt})^M$	u_7	u_{12}	u_{18}	u_{24}	u_{30}	u_{36}	$t_{0.75}$	$t_{0.99}$
WH_s		Czech warmblood	$167.4(1-0.298e^{-0.0020t})$	83	88	92	94	96	97	2	42
	Fradinho et al., 2016	Lusitano horse	$161.5(1-0.944e^{-0.0017t})^{0.17}$	83	89	92	95	96	97	3	54
	Kocher and Stanier, 2013	Thoughbred	$168.9(1-0.935e^{-0.0010t})^{0.18}$	82	87	91	94	96	97	3	59
	Hossein-Zadeh et Ghorbani, 2018	Caspian horse	$100.4(1-0.32e^{-0.08T})$	82	88	92	95	97	98	3	43
WH_t		Czech warmblood	$177.1(1-0.299e^{-0.0020t})$	83	88	93	95	97	98	2	44
G		Czech warmblood	$194.3(1-0.491e^{-0.0028t})$	76	84	90	94	96	98	6	46
	Fradinho et al., 2016	Lusitano horse	$202.9(1-0.985e^{-0.0008t})^{0.21}$	69	76	81	85	87	90	11	125
	Hossein-Zadeh et Ghorbani, 2018	Caspian horse	$100.9(1-0.43e^{-0.08T})$	75	83	89	93	92	97	7	51
CC		Czech warmblood	$21.8(1-0.394e^{-0.0022t})$	80	88	93	96	98	99	5	38
	Fradinho et al., 2016	Lusitano horse	$21.4(1-0.926e^{-0.0010t})^{0.21}$	80	86	90	93	95	96	4	61

[a] WH_s – withers height measured by stick, WH_t – withers height measured by tape, G – girth, CC – cannon circumference

[b] Growth models as functions of age (t – days; T – months) used for u_t nad t_u calculations; when $0 < M < 1$ Richards equation; when $M = 1$ Brody equation; A – asymptotic value for WH_s/WH_t/G/CC; b – scaling parameter that defines the degree of maturity when age = 0 day (intercept on y axis); k – maturing index (rate that establishes the spread of the curve along time axis); M – determines the point of inflection of the curve

[c] Degree of maturity (%) considering the growth model (u_t – degree of maturity at age t [months])

[d] Age required to reach 75% and 99% of mature value considering the growth model (t_u – age [months] at u degree of maturity)

Table 4
Summary of withers height, girth and cannon circumference for age from 7 to 36 months; predicted values calculated by Brody equation; medians, means and standard deviations (SD) of data obtained from individual growth models of biometric traits.

Age (days)	Biometric Measures[a] WH_s (cm) Brody	Median	Mean	SD	WH_t (cm) Brody	Median	Mean	SD	G (cm) Brody	Median	Mean	SD	CC (cm) Brody	Median	Mean	SD
213	138.9	139.8	140.3	3.22	147.2	146.3	146.9	3.25	146.7	145.6	146.2	4.25	17.4	17.5	17.4	0.54
247	141.4	141.7	142.2	3.27	149.2	148.3	149.1	3.43	150.3	149.4	150.0	4.47	17.9	17.9	17.8	0.55
274	143.2	143.6	143.9	3.34	151.1	150.4	151.1	3.64	153.6	153.2	153.5	4.64	18.3	18.2	18.2	0.58
304	145.0	145.4	145.5	3.44	152.9	152.3	152.9	3.81	156.6	156.0	156.7	4.89	18.6	18.6	18.6	0.62
335	146.8	146.8	147.0	3.56	154.5	153.7	154.6	4.00	159.4	158.5	159.6	5.14	18.9	18.9	18.9	0.66
365	148.3	148.1	148.3	3.67	156.0	155.2	156.2	4.17	162.0	161.5	162.2	5.38	19.2	19.3	19.2	0.70
396	149.8	149.3	149.6	3.77	157.5	156.8	157.6	4.33	164.4	164.4	164.6	5.59	19.4	19.6	19.4	0.73
426	151.2	150.4	150.8	3.88	158.8	158.1	158.9	4.46	166.6	166.8	166.9	5.77	19.6	19.8	19.7	0.76
457	152.5	151.5	151.9	3.99	160.0	159.3	160.2	4.57	168.6	169.1	168.9	5.92	19.8	20.0	19.9	0.79
487	153.6	152.4	153.0	4.10	161.2	160.4	161.3	4.65	170.5	171.2	170.7	6.04	20.0	20.2	20.0	0.81
517	154.6	153.4	153.9	4.21	162.2	161.6	162.4	4.71	172.2	173.2	172.4	6.13	20.2	20.3	20.2	0.83
548	155.6	154.2	154.8	4.30	163.2	162.6	163.4	4.75	173.8	175.0	174.0	6.21	20.3	20.5	20.3	0.85
578	156.5	155.0	155.7	4.39	164.2	163.6	164.3	4.78	175.3	176.7	175.4	6.26	20.5	20.7	20.5	0.87
609	157.4	155.7	156.5	4.47	165.1	164.5	165.1	4.79	176.7	178.3	176.7	6.31	20.6	20.8	20.6	0.88
639	158.2	156.4	157.2	4.55	165.9	165.3	165.9	4.79	177.9	179.4	178.0	6.34	20.7	20.8	20.7	0.90
670	158.9	157.1	157.9	4.61	166.6	166.1	166.6	4.80	179.1	180.2	179.1	6.36	20.8	20.9	20.8	0.91
700	159.5	157.8	158.6	4.70	167.3	166.8	167.3	4.81	180.2	180.9	180.1	6.38	20.9	21.0	20.9	0.92
731	160.2	158.5	159.2	4.77	168.0	167.4	168.0	4.82	181.1	181.5	181.1	6.39	21.0	21.1	21.0	0.93
761	160.7	159.1	159.7	4.83	168.6	168.1	168.6	4.83	182.0	182.3	181.9	6.39	21.1	21.1	21.0	0.95
791	161.2	159.6	160.3	4.89	169.2	168.7	169.1	4.83	182.9	183.3	182.7	6.40	21.1	21.2	21.1	0.96
822	161.7	160.2	160.8	4.95	169.7	169.2	169.6	4.85	183.7	184.2	183.5	6.40	21.2	21.2	21.2	0.97
852	162.1	160.9	161.3	4.96	170.2	169.8	170.1	4.86	184.4	185.2	184.2	6.41	21.3	21.2	21.2	0.98
883	162.5	161.4	161.7	4.98	170.7	170.2	170.6	4.88	185.0	185.9	184.8	6.41	21.3	21.3	21.3	0.99
913	162.9	161.8	162.1	4.99	171.1	170.7	171.0	4.88	185.6	186.5	185.4	6.42	21.4	21.3	21.3	1.01
944	163.3	162.4	162.5	5.00	171.5	171.1	171.4	4.89	186.2	187.2	186.0	6.42	21.4	21.3	21.4	1.01
974	163.6	162.9	162.9	5.01	171.9	171.5	171.8	4.90	186.7	187.8	186.5	6.43	21.4	21.3	21.4	1.02
1004	163.9	163.3	163.3	5.02	172.3	171.8	172.1	4.91	187.2	188.3	187.0	6.44	21.5	21.4	21.4	1.02
1035	164.2	163.6	163.6	5.02	172.6	172.3	172.5	4.92	187.6	188.7	187.4	6.45	21.5	21.4	21.5	1.02
1065	164.4	163.9	163.9	5.02	172.9	172.6	172.8	4.92	188.0	189.0	187.8	6.47	21.5	21.4	21.5	1.03
1096	164.6	164.3	164.2	5.02	173.2	172.9	173.1	4.94	188.4	189.0	188.2	6.48	21.6	21.5	21.5	1.03

[a] WH_s – withers height measured by stick, WH_t – withers height measured by tape, G – girth, CC – cannon circumference

Richards), are those that have biological interpretation, being indicators of growth rate. However, according to Ţabatabai et al. (2005), no mathematical model can accurately describe every biological phenomenon.

Many authors have investigated the best fit model for biometric traits (Fradinho et al., 2016; Heugebaert et al., 2010; Hossein-Zadeh and Ghorbani, 2018; López et al., 2000; Marinho et al., 2013; Vallette et al., 2008). Many of them reported the Brody growth model as the most appropriate function for certain traits (Dušek, 2011; Hojjati and Ghavi Hossein-Zadeh, 2018; McManus et al., 2010; Onoda et al., 2013). Akbas et al. (1999) noted that the Brody function is the best fit model because of its simplicity of interpretation and ease of estimation. Brown et al. (1976) and DeNise et al. (1985) stated that the Brody function is better applied to ages over six months, which is the same as with the data presented here where the youngest age is 7 months. Other authors found that while the logistic function underestimates adult weight, Brody overestimates it on average by 30 kg compared with Richards and Von Bertalanffy's function (Perotto et al., 1992). Another reason for the application of Brody's model in this study was our aim to update the CW stallion growth curve so that it could be used as a breed standard.

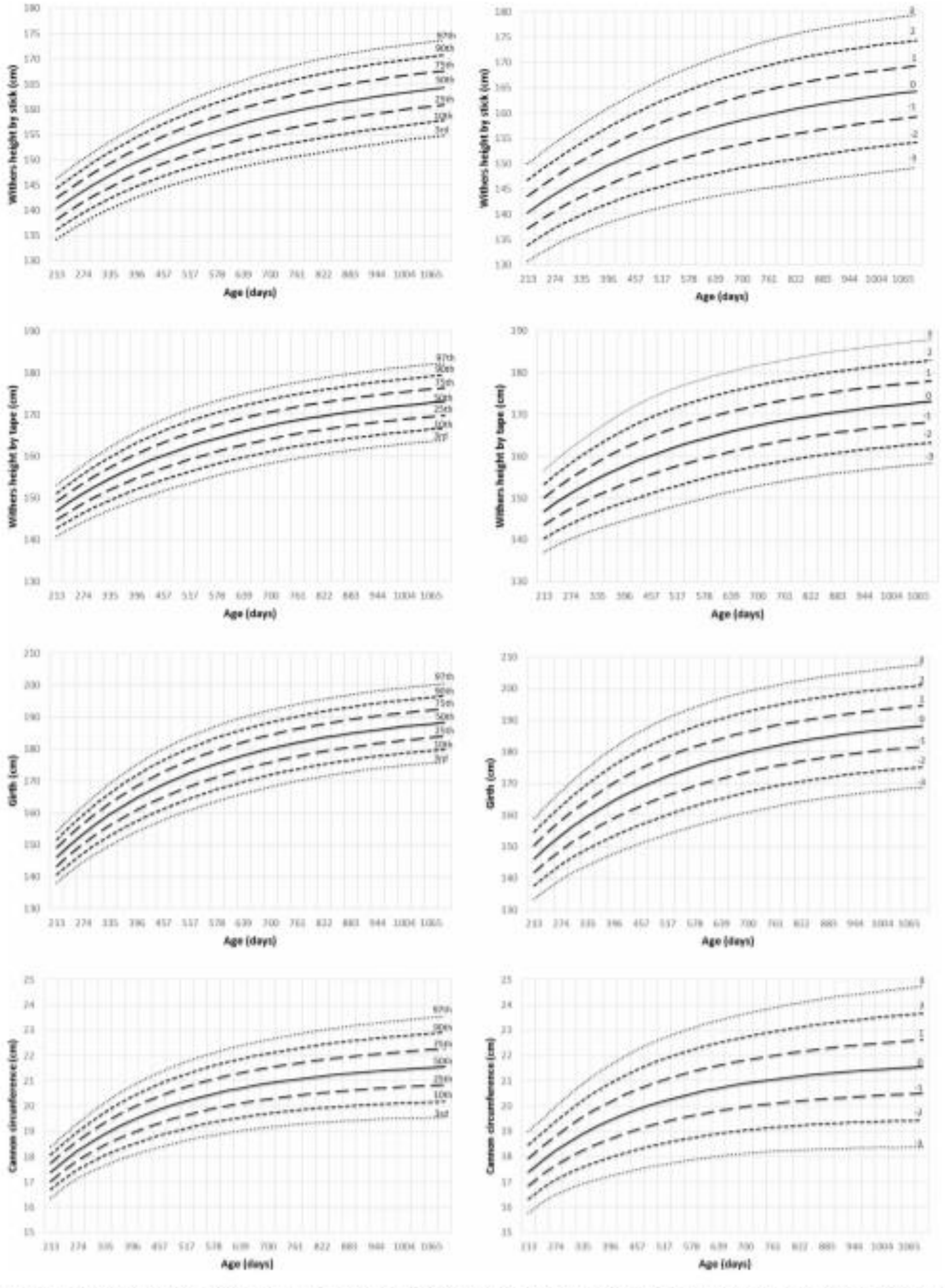


Fig. 1. Growth curves for withers height, girth and cannon circumference obtained by the Brody growth model (left column percentile growth curves; right column - Z-score growth curves). The mean ($z = 0$) halves the normal distribution into two equal and symmetric areas. On both the right and left sides each, the graph can be shown as divided into three parts, according to Z-scores of 1, 2, and 3 SD to the right and Alt+8722 −1, −2, −3 SD to the left, respectively. At each point of these SDs, the corresponding percentile (or cumulative probability) is fixed. As long as the distribution is normal, every SD unit on the x-axis has a specific percentile which is always paired with them. Therefore, on a normal curve, 34.13% of the data lies between 0 and −1 (or +1), 13.59% between −1 and −2 (or between +1 and +2), and 2.14% between −2 and −3 (or between +2 and +3). The remaining 0.14% of the data lies below −3 (or above +3) (Wand et Chen, 2012).

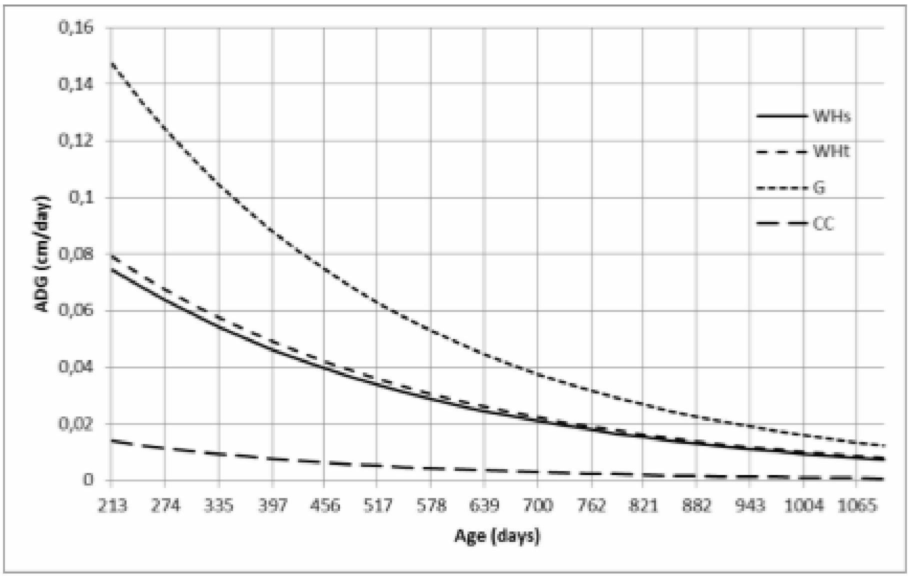


Fig. 2. Average daily gain (cm/day) for observed biometric traits (WH_s – withers height measured by stick, WH_t withers height measured by tape, G-girth, CC – cannon circumference)

Currently, the growth curve by Dušek from the 1970s based on the Brody function is used, as stated in Dušek (2011). McManus et al. (2010) analysed the variables of height and weight and concluded that Brody's model was the most adequate to describe growth of the warmblood breeds reared by the Brazilian Army. Teixeira et al. (2021) estimated the parameters of the Logistical, Gomperts, von Bertalanffy and Brody models for the WH variable of Campolina males with the resulting R^2 of 0.9718 for the Logistical model and 0.9719 for the other models with the similar adult WH (parameter α) of 160 cm.

WH_s and WH_t are two height traits observed during the growth of CW stallions in our study. On average, the estimated WH_t values were 8 cm higher compared with those of WH_s. The value of this parameter was affected by the size of the chest, as a result of which WH_t growth was terminated later ($t_{0.99}$ = 44 months) compared with WH_s ($t_{0.99}$ = 42 months), because there was a later termination of G growth ($t_{0.99}$ = 46 months). The later approaching to the asymptotic value of G growth compared with WH was also noted by other authors (Fradinho et al., 2016; Hossein-Zadeh and Ghorbani, 2018). The growth of foals to height takes place primarily in the first six months of life and also ends first (Kudrna, 1998). In this study, growth was evaluated from the age of seven months, after the period of its maximum intensity. Thompson (1995) examined WH of the Thoroughbred at 20 months. On average, colts had 152.2 cm of WH, which showed an increase in WH by 44.0 cm when compared with the first measurement at 14 days. In our study, CW stallions achieved the highest increases between the 7th and 9th months of life, when the average monthly gain (AMG) for WH_s was 2.14 cm/month, after which there was a slight decrease. From the age of 18 months onward, AMGs fell below 1 cm. This is consistent with the study by Lovšin et al. (2001), who found that the growth dynamics of WH was the steepest in the first 9 months of age. This was confirmed by Jelan et al. (1996) in which the gain in height in Thoroughbred horses was the most rapid from birth to 1 month (8 cm/month). Similar results were obtained by Kocher and Staniar (2013) in Thoroughbred horses, where the gain then declined to 4 cm/month until 6 months of age and then gradually decreased to 1 cm/month until 20 months of age. Dušek et al. (1981) examined WH_t of four different horse breeds at the age of 20 months. Black Kladrub colts, White Kladrub colts, Arab colts and Lipizzan colts measured, on average, 166.9 cm, 163.6 cm, 165 cm and 165.0 cm, respectively. For comparison with these results, an average WH_t of 165.1 cm was estimated for CW stallions at the age of 20 months.

The accuracy of the equations for WH_t and WH_s in our study, characterised as percent errors of 2.15 and 2.12, respectively, was comparable to the result of Kocher and Steinar (2013). RSD for the growth model of WH_s was 3.99 and of WH_t was 4.39. These values are comparable to those found by Fradinho et al. (2016) for the Brody function (RSD = 3.47). We found that the CW stallions reached 75% WH_s in the 2nd month of life, and their height growth approached the asymptotic limit value at the age of 42 months. Fitzhugh and Taylor (1971) stated that the degrees of maturity may be freely compared at the same or different stages, for the same or a different trait, in the same or different animals. Pimentel et al. (2017), Rezende et al. (2007) and Santos et al. (1995) agreed that the body height of foals at birth accounts for about 60% of their final adult height, and at 12 months of age, saddle horses have already attained 85 to 90% of their adult height. This result is in line with our findings that the CW stallions reached 88% of adult height (WH_s and WH_t) at 12 months. The same conclusion was reached by Fradinho et al. (2016). Similar values were also observed by Manso Filho (1998), using data from Mangalarga Marchador foals, and by Souza et al. (1997), using Brasileiro de Hipismo foals, which confirmed that the percentage of final height at the withers was 68% at birth, 86% at 12 months and 91% at 24 months. Staniar et al. (2005) and Willoughby (1975) estimated that 95% of mature WH may have been reached at the age of two years. Our result of a 94% degree of maturity of WH_s at 24 months is in agreement with Cabral et al. (2004), Fradinho et al. (2016) and Martin-Rosset and Juliand (2005) who added that for most of the light breeds, WH already represents 88 to 90% of mature height at one year of age, reflecting the earlier development of the skeletal tissue. Fradinho et al. (2016) further stated that skeletal development follows a similar pattern across breeds.

G decreases gradually from the initial AMG 4.48 cm/month at 7 months of age to AMG 1.93 cm/month at 17 months. From the age of 25 months, the growth of the G slows down even more when the AMG value falls below 1 cm/month respectively. 0.98 cm/month. We estimated that at 7 months of age, the CW stallions reached 76% of their mature G and 94% at 24 months of age. These values indicate that G does not develop at a later age than the other measured parameters.

This is in agreement with Kudrna (1998) who determined that the intensity of height growth is accompanied by growth in depth and width. Among the individual organs and tissues, it is the skin, muscles, blood and stomach that reach the highest growth intensity in post-embryonic development. This was confirmed by Dušek (1981) according to whom, the foal's chest is flatter and widens significantly only in the later growth phase. In our study, G approached asymptotic value at the age of 46 months. The G scaling parameter b for the CW and Lusitano horses was found to be close (0.491 respectively 0.461) (Fradinho et al., 2016).

Depending on the degree of maturity, the growth rate of G in CW (u_{36} = 98%) is comparable to that of Caspian stallions (u_{36} = 97%) (Hossein-Zadeh and Ghorbani, 2018). According to Fradinho et al. (2016), slower chest growth can be seen in Lusitano horses who reach 90% of mature value at 36 months of age. Lusitano breeders believe that these horses reach maturity at a later age.

The potential for growth and production performance of the horse is determined by its genetic background, but the expression of growth-related traits is highly influenced by environmental factors. The individual genetic effect on body size and live weight is significant, and great differences can be found from small ponies to draught horse breeds (Dario et al. 2006; Druml et al., 2008; Fradinho et al., 2020; Molina et al. 1999; Zechner et al. 2001). Across Warmblood populations and linear schemes, published heritabilities indicated the greatest potential for increase of genetic gain for limb conformation by replacing valuating scores for front and hind limb conformation (mostly h^2 = 0.05–0.15) with a set of linear scores (average h^2 = 0.17–0.42) (Stock and Distl, 2006). Moderate to high heritabilities of linear traits make them suitable for selection and genetic improvement (Koenen et al., 1995). The obtained RSD for girth 6.3 is consistent with the RSD of girth for the Brody function in Fradinho et al. (2016).

The CC characterises the strength of the skeleton. The foal is born with long shanks and pasterns whose growth is significantly smaller compared to other biometric traits. However, this does not apply to the CC, which increases in line with other biometric traits (Dušek, 1985). In our study, it was found that CC AMG at its highest value reached 0.41 cm/month and between the 10th and 11th month, it fell below 0.3 cm/month and from the 23rd month onward. We found that AMGs were in the order of hundredths of a cm (0.09). This was confirmed by Martin-Rosset et al. (1983), according to whom, considering the different skeletal segments, the bones in the extremities (e.g. metacarpus) develop earlier in relation to the whole skeleton. Fradinho et al. (2016) stated that the CC parameter did not show the effect of the season, probably because these measures are directly associated with bone development. In CW stallions, we found that CC reached 88% of mature value at the age of 12 months and 96% at 24 months. Similarly, Pimentel et al. (2017) found in their work that from 24 months to 36 months, all growth rates were lower than 10%, except for that of the CC of males, which remained the same as the rate observed from 12 and 24 months of age. CC in females showed no growth after 24 months. Some of the growth studies in the horse pointed out asexual dimorphism in some body measures, which tended to increase with time (Hintz et al., 1979; Saastamoinen, 1990). We found the growth curve parameters for CC in CW stallions (b = 0.343) were similar to those in Fradinho et al. (2016) (b = 0.394). These values indicate that at birth, foals obtain a high degree of maturity of CC, the value of which ranges from 60 to 70%. Large *k*-values indicate early maturating of this parameter in comparison with other observed body measurements. Also, the RSD values (0.88) are comparable to Fradinho et al. (2016), where the RSD for CC was 0.80. The reason for the highest value of percentage error (3.39%) of all monitored biometric traits was most likely due to the difficulty of measuring CC, when it is complicated to accurately measure small increments with a measuring tape marked in 0.1 cm increments. This conclusion is in agreement with those of other authors (Fradinho et al., 2016; Pimentel et al., 2017).

Percentile growth curves are commonly used to monitor the growth of cattle, swine and humans (Corson et al., 2008; Heinrichs and Lammers, 1998; Kuczmarski et al., 2002; Onoda et al., 2013). The advantage of utilising these figures is that they effectively capture the population variation in biometric traits and provide a useful visual reference of what these variables look like in this population. These can be used to monitor individual animal growth and make some comparisons to the population of horses used in studies (Kocher and Staniar, 2013). Percentiles and *Z*-scores are widely used to assess anthropometric measures to help evaluate children's growth and nutritional status. If the data are normally distributed, the data value of the 50th percentile is identical to the mean of the data, and the conversion between percentiles and *Z*-scores values through mean and *SD* is possible (Kocher and Stainar, 2013; Wang and Chen, 2012) A *Z*-score of zero corresponds to the empirical 50th percentile curve (Odona et al., 2013). Using *Z*-scores is an alternative way to obtain empirical percentile growth curves. The *Z*-score is a standardised score that indicates how many *SDs* a data point is apart from the population mean, based on the assumption of normal distributions (Onoda et al., 2013). *Z*-scores are more useful in research, while percentiles are easier for use in clinical settings and by the public (Wang and Chen, 2012). According to Odona et al. (2013), the establishment of percentile growth curves would give us useful indicators for feeding management in order to achieve sound musculoskeletal development or desirable body compositions in growing horses. Percentile growth curves for observed biometric traits in this study seemed to be more accurate in comparison with *Z*-score growth curves, considering values given in the CW breed standard. Another reason is that percentiles are more intuitive and comprehensible for breeders. According to Fernandes et al. (2020), morphological evaluation has great relevance in the selection of animals for breeding, since morphological characteristics are related to the functionality of the equine species. Therefore, selection in the riding horse is primarily based on indicator traits that are accessible at a younger age and have higher heritabilities (Viklund et al., 2010). Due to their correlations with the selection traits (i.e. success in sport riding disciplines), results of standardised performance tests of young horses and evaluations of conformation and gaits are important components of modern Warmblood breeding programmes (Thorén Hellsten et al., 2006; Ducro et al., 2007; Viklund et al., 2010).

Dušek (2011) stated that comparison of the biometric traits of individual foals with a standard is important, especially when comparing all the annual cohorts of foals, which include individuals with large differences in age. In the case of marked deviations from the standard, especially in the minus zones (lower than 25th percentile), it is always necessary to monitor the development of the foal for a longer period and only deselect the foal from the breeding programme in the case of a permanently significant deviation. Wang and Chen (2012) noted that within regular measurements, the growth curve should run in parallel with percentiles, ideally in the range from 25th to 75th percentile. When a biometric trait observed in an individual falls below the 10th or rises above the 90th percentile for a given age and gender, it is advisable to pay more attention to the individual, and if it falls below the 3rd or rises above the 97th percentile, it is necessary to look for the cause of the growth abnormality by anamnestic or laboratory examination and repeat the measurement after a shorter interval.

5. Conclusion

The present study provides a comprehensive overview of the growth patterns of CW stallions. Using the Brody function, we characterised the growth of four biometric traits that are commonly used to monitor growth in CW. The output represents an update of the CW growth curves of WH_a, WH_s, G and CC and the establishment of percentile curves with the standard from the 25th to 75th percentiles. In comparison with the *Z*-score, percentiles are more intuitive and comprehensible for breeders who can thus analyse animal development more simply and apply this monitoring in their daily breeding management. Therefore, the results of this study can provide an effective measurement criterion for planning farm management strategies and early detection of growth abnormalities just by observing the growth curve. Due to the common use of other warmblood stallions in CW breeding, these results also represent beneficial information for the analysis of animal development of warmblood horses in general.

Conflict of interest statement

The authors declare that there are no conflicts of interest to disclose in regard to this work.

Acknowledgements

This work was supported by the Grant Agency of the University of South Bohemia under Grant project No. 028/2019/Z.

References

Akbas, Y., Taskin, T., Erdinc, D., 1999. Comparison of several models to fit the growth curves of Kıvırcık and Dağlıç male lambs. Turkish J. Vet. Anim. Sci. 23, 537–544.

Brody, S., Lardy, H.A., 1946. Bioenergetics and growth. J. Phys. Chem. 2.

Brown, J.E., Fitzhugh, H.A., Cartwright, T.C., 1976. A Comparison of Nonlinear Models for Describing Weight-Age Relationships in Cattle1. J. Anim. Sci. 42, 810–818. https://doi.org/10.2527/jas1976.424810x.

Cabral, G.C., Almeida, F.Q.de, Quirino, C.R., Azevedo, P.C.N.de, Pinto, L.F.B., Santos, E. M., 2004. Avaliação morfométrica de eqüinos da raça Mangalarga Marchador: índices de conformação e proporções corporais. Rev. Bras. Zootec. 33, 1798–1805. https://doi.org/10.1590/S1516-35982004000700018.

Corson, A.M., Laws, J., Laws, A., Litten, J.C., Lean, I.J., Clarke, L., 2008. Percentile growth charts for biomedical studies using a porcine model. Animal 2, 1795–1801. https://doi.org/10.1017/S1751731108002966.

Čoudková, V., Sachello, V., Štěrbová, H., Kleinová, A., Papoušková, Z., Maršálek, M., Kovanda, J., 2016. Bodyweight Estimation From Linear Measures of Growing Warmblood Horses by a Formula. J. Equine Vet. Sci. 36, 63–68. https://doi.org/10.1016/j.jevs.2015.09.010.

Dario, C., Carnicella, D., Dario, M., Bufano, G., 2006. Morphological evaluation and heritability estimates for some biometric traits in the Murgese horse breed. Genet. Mol. Res. 5, 309–314.

DeNise, R., Kersey, S., Brinks, J.S., 1985. Genetic and Environmental Aspects of the Growth Curve Parameters in Beef Cows. J. Anim. Sci. 61, 1431–1440. https://doi.org/10.2527/jas1985.6161431x.

Druml, T., Baumung, R., Sölkner, J., 2008. Morphological analysis and effect of selection for conformation in the Noriker draught horse population. Livest. Sci. 115, 118–128. https://doi.org/10.1016/j.livsci.2007.06.015.

Ducro, B.J., Koenen, E.P.C., van Tartwijk: J.M.F.M. Bovenhuis, H., 2007. Genetic relations of movement and free-jumping traits with dressage and show-jumping performance in competition of Dutch Warmblood horses. Livest. Sci. 107, 227–234. https://doi.org/10.1016/j.livsci.2006.09.018.

Dušek, J., 1981. Hodnocení proporcionality tělesné stavby chovných stád klisen kladrubského vraníka a bělouše. Bull 35, 129–150. VSCHK Slatiňany.

Dušek, J., 1985. Growth of Arabian, Lipizzaner and Old Kladruber foals. Bull 52, 16. VSCHK Slatiňany.

Dušek, J., 2011. Chov koní, 3. ed. Brázda.

Dušek, J., Vondráček, J., Hojek, S., 1981. Growth rates of Arab, Lipitsa and Kladrub horses. Scientia Agriculturae Bohemoslovaca (Czechoslovakia).

Edwards, Elwyn, H., 1994. The Encyclopedia of the Horse, 1. ed. Dorling Kindersley Limited, London.

Fernandes, T.J., Pereira, A.A., Muniz, J.A., 2017. Double sigmoidal models describing the growth of coffee berries. Ciência Rural 47. https://doi.org/10.1590/0103-8478cr20160646.

Fernandes, T.J., Souza, F.A.C.de, Ribeiro, R.A., Cunha, F.O., Meirelles, S.L.C., Moura, R. S.de, Muniz, J.A., 2020. Growth curve for height at withers and body length of Mangalarga Marchador horses. Ciência Rural 50. https://doi.org/10.1590/0103-8478cr20200140.

Fitzhugh, H.A., 1976. Analysis of Growth Curves and Strategies for Altering Their Shape. J. Anim. Sci. 42, 1036–1051. https://doi.org/10.2527/jas1976.4241036x.

Fitzhugh, H.A., Taylor, S.C.S., 1971. Genetic Analysis of Degree of Maturity. J. Anim. Sci. 33, 717–725. https://doi.org/10.2527/jas1971.334717x.

Fradinho, M.J., Bessa, R.J.B., Caldeira, R.M., Ferreira-Dias, G., 2020. Growth and Development of the Lusitano Foal on Extensive Systems. Advances in Animal Health, Medicine and Production. Springer International Publishing, Cham, pp. 178–198. https://doi.org/10.1007/978-3-030-61981-7_9.

Fradinho, M.J., Bessa, R.J.B., Ferreira-Dias, G., Caldeira, R.M., 2016. Growth and development of the Lusitano horse managed on grazing systems. Livest. Sci. 186 https://doi.org/10.1016/j.livsci.2015.06.006.

Freitas, A.R.de, 2005. Curvas de crescimento na produção animal. Rev. Bras. Zootec. 34, 786–795. https://doi.org/10.1590/S1516-35982005000300010.

Gallio, M., Azevedo, M.da S., Brass, K.E., Corte, F.D.D.La, Lopes, L.F.D., 2014. Prevalência de alterações ósseas no tarso de potros Crioulos de até vinte e seis meses de idade. Ciência Rural 44, 1442–1447. https://doi.org/10.1590/0103-8478cr20131171.

Ghavi Hossein-Zadeh, N., Ghorbani, A., 2018. Modeling the growth curves for body weight and some biometric traits in Caspian horses (Equus ferus caballus) using non-linear mixed models. Mamm. Biol. 93 https://doi.org/10.1016/j.mambio.2018.07.004.

Green, D.A., 1976. Growth Rate in Thoroughbred Yearlings and Two Year Olds. Equine Vet. J. 8, 133–134. https://doi.org/10.1111/j.2042-3306.1976.tb03321.x.

Green, D.A., 1969. A Study of Growth Rate in Thoroughbred Foals. Br. Vet. J. 125, 539–546. https://doi.org/10.1016/S0007-1935(17)48714-8.

Heinrichs, A.J., Lammers, B., 1998. Monitoring dairy heifer growth.

Heugebaert, S., Garcia-Launay, F., Trillaud-Geyl, C., Dubroeucq, H.H., Arnaud, G., Bigot, G.G., Valette, J.P., Fleurance, G., Hoch, T, Agabriel, J., Martin-Rosset, W., 2010. Modelling the growth of foals: the first step towards new dietary recommendations. 36e Journée de La Recherche Équine. Le Pin Au Haras, France, Jeudi, pp. 61–68, 4 Mars 2010.

Hintz, H.F., Hintz, R.L., Van Vleck, L.D., 1979. Growth Rate of Thouroughbreds. Effects of Age of Dam, Year and Month of Birth, and Sex of Foal. J. Anim. Sci. 48 https://doi.org/10.2527/jas1979.483480x.

Hojjati, F., Ghavi Hossein-Zadeh, N., 2018. Comparison of non-linear growth models to describe the growth curve of Mehraban sheep. J. Appl. Anim. Res. 46 https://doi.org/10.1080/09712119.2017.1348949.

Jelan, Z.A., Jeffcott, L.B., Lundeheim, N., Osborne, M., 1996. Growth rates in Thoroughbred foals. Pferdeheilkunde 12, 291–295. https://doi.org/10.21836/PEM19960326. Pferdeheilkunde 12, 291–295.

Kocher, A., Staniar, W.B., 2013. The pattern of thoroughbred growth is affected by a foal's birthdate. Livest. Sci. 154, 204–214. https://doi.org/10.1016/j.livsci.2013.03.008.

Koenen, E.P.C., Van Veldhuizen, A.E., Brascamp, E.W., 1995. Genetic parameters of linear scored conformation traits and their relation to dressage and show-jumping in competition Livest. Prod. Sci. 43, 85–94. https://doi.org/10.1016/0301-6226(95)00010-I.

Kuczmarski, R.J., Ogden, C.L., Guo, S.S., Grummer-Strawn, L.M., Flegal, K.M., Mei, Z., Wei, R., Curtin, L.R., Roche, A.F., Johnson, C.L., 2002. Growth Charts for the United States: methods and development. Vital Health Stat 11, 1–190.

Kudrna, V., 1998. Produkce krmiv a výživa skotu. Agrospoj.

López, S., France, J., Gerrits, W.J., Dhanoa, M.S., Humphries, D.J., Dijkstra, J., 2000. A generalized Michaelis-Menten equation for the analysis of growth. J. Anim. Sci. 78 https://doi.org/10.2527/2000.7871816x.

Lovšin, E., Fazarinc, G., Pogačnik, A., Bavdek, S.V, 2001. Growth dynamics of Lipizzan horses and their comparison to other horse breeds. Pflegers Arch. Eur. J. Physiol. 442, r211–r212. https://doi.org/10.1007/s004240100029.

Manso Filho, H.C., 1998. Desenvolvimento corporal de potros da raça Mangalarga-Botucatu, SP Marchador durante a fase de amamentação. Reun. DA SBZ 35, 662–664.

Marinho, K.N., da, S., Freitas, A.R.de, Falcão, A.J.da S, Dias, F.E.F., 2013. Nonlinear models for fitting growth curves of Nellore cows reared in the Amazon Biome. Rev. Bras. Zootec. 42 https://doi.org/10.1590/S1516-35982013000900006.

Martin-Rosset, W., Boccard, R., Jussiaux, M., Robelin, J., Trillaud-Geyl, C., 1983. Croissance relative des différents tissus, organes et régions corporelles entre 12 et 30 mois chez le cheval de boucherie de différentes races lourdes. Ann. Zootech. INRA/EDP Sci. 32, 153–174.

Martin-Rosset, W., Juliand, V., 2005. The growing horse: nutrition and prevention of growth disorders: Nutrition and Prevention of Growth Disorders. Wageningen Academic Publishers.

McManus, C.M., Louvandini, H., Campos, V.A.L., 2010. CURVAS DE CRESCIMENTO NÃO-LINEARES PARA PESO E ALTURA EM QUATRO GRUPOS GENÉTICOS DE CAVALOS. Ciência Anim. Bras. 11 https://doi.org/10.5216/cab.v11i1.5400.

Molina, A., Valera, M., Dos Santos, R., Rodero, A., 1999. Genetic parameters of morphofunctional traits in Andalusian horse. Livest. Prod. Sci. 60, 295–303. https://doi.org/10.1016/S0301-6226(99)00101-3.

Morel, P.C., Bokor, Á., Rogers, C.W., Firth, E.C., 2007. Growth curves from birth to weaning for Thoroughbred foals raised on pasture. N. Z. Vet. J. 55 https://doi.org/10.1080/00480169.2007.36788.

Onoda, T., Yamamoto, R., Sawamura, K., Inoue, Y., Murase, H., Nambo, Y., Tozaki, T., Matsui, A., Miyake, T., Hirai, N., 2013. Empirical growth curve estimation considering multiple seasonal compensatory growths of body weights in Japanese Thoroughbred colts and fillies1. J. Anim. Sci. 91, 5599–5604. https://doi.org/10.2527/jas.2013-6523.

Onoda, T., Yamamoto, R., Sawamura, K., Inoue, Y., Matsu, A., Miyake, T., Hirai, N., 2011. Empirical Growth Curve Estimation Using Sigmoid Sub-functions that Adjust Seasonal Compensatory Growth for Male Body Weight of Thoroughbred Horses. J. Equine Sci. 22 https://doi.org/10.1294/jes.22.37.

Pagan, J.D., Jackson, S.G., Caddel, S., 1996. A summary of growth rates of Thoroughbreds in Kentucky. Pferdeheilkd. Equine Med. 12 https://doi.org/10.21836/PEM19960325.

Perotto, D., Cue, R.I., Lee, A.J., 1992. Comparison of nonlinear functions for describing the growth curve of three genotypes of dairy cattle. Can. J. Anim. Sci. 72, 773–782. https://doi.org/10.1294/jes.22.37.

Pimentel, A.M.H., Rodrigues, W.B., Martins, C.F., Montanez, N.R., Boligon, A.A., Souza, J.R.M.de, 2017. Gender on the growth of Criollo foals from birth to three years of age. Ciência Rural 47. https://doi.org/10.1590/0103-8478cr20150989.

Pinto, L.F.B., Almeida, F.Q.de, Azevedo, P.C.N.de, Quirino, C.R., Cabral, G.C., Santos, E. M., 2005. Análise multivariada das medidas morfométricas de potros da raça Mangalarga Marchador: análise fatorial. Rev. Bras. Zootec. 34, 613–626. https://doi.org/10.1590/S1516-35982005000200031.

Reed, K.R., Dunn, N.., 1977. Growth and development of the Arabian horse. In: Equine Nutrition and Physiology Symposium. St. Louis, Philadelphia. ENP Society, pp. 76–98.

Rezende, A.S.C., 1998. Velocidade de desenvolvimento dos equinos da raça Mangalarga-Marchador. Reun. DA SBZ 35, 273–275.

Rezende, D.M.L.C., Muniz, J.A., Ferreira, D.F., Silva, F.F.e, Aquino, L.H.de, 2007. Ajuste de modelos de platô de resposta para a exigência de zinco em frangos de corte. Ciência e Agrotecnologia 31, 468–478. https://doi.org/10.1590/S1413-70542007000200030.

Richards, F.J., 1959. A Flexible Growth Function for Empirical Use. J. Exp. Bot. 10, 290–301. https://doi.org/10.1093/jxb/10.2.290.

Robert, C., Valette, J.-P., Denoix, J.-M., 2013. Longitudinal development of equine forelimb conformation from birth to weaning in three different horse breeds. Vet. J. 198, e75–e80. https://doi.org/10.1016/j.tvjl.2013.09.037.

Rogers, C., Gee, E., Faram, T., 2004. The effect of two different weaning procedures on the growth of pasture-reared Thoroughbred foals in New Zealand. N. Z. Vet. J. 52 https://doi.org/10.1080/00480169.2004.36458.

Saastamoinen, M., 1990. Factors Affecting Growth and Development of Foals and Young Horses. Acta Agric. Scand. 40, 387–396. https://doi.org/10.1080/00015129009438574.

Santos, S.A., Mazza, M.C.M., Sereno, J.R.B., Abreu, U.G.P. de, Silva, J.A. da, 1995. Avaliação e conservação do cavalo pantaneiro.

Santos, S.A., Souza, G.S., Oliveira, M.R., Sereno, J.R., 1999. Uso de modelos não-lineares para o ajuste de curvas de crescimento de cavalos pantaneiros. Pesqui. Agropecuária Bras. 34, 1133–1138.

SCHČT, 2013. Šlechtitelský program [WWW Document]. URL http://ctavaz.cz/slechtitelsky-program/.

Silva, N.A.M., Lana, A.M.Q., Silva, F., Silveira, F.G., Bergmann, J.A.G., Silva, M.A., Toral, F.L.B., 2011. Seleção e classificação multivariada de modelos de crescimento não lineares para bovinos Nelore. Arq. Bras. Med. Veterinária e Zootec. 63, 364–371. https://doi.org/10.1590/S0102-09352011000200014.

Silveira, F.G.da, Silva, F.F.e, Carneiro, P.L.S., Malhado, C.H.M., Muniz, J.A., 2011. Análise de agrupamento na seleção de modelos de regressão não-lineares para curvas de crescimento de ovinos cruzados. Ciência Rural 41, 692–698. https://doi.org/10.1590/S0103-84782011000400024.

Souza, J.A.T., Gacek, F., Oliveira, J.V., 1997. Desenvolvimento corporal e estabelecimento da puberdade em potras da raça Brasileira de Hipismo. Rev. Bras. Reprodução Anim. 21, 117–120.

Splan, R.K., Staniar, W.B., Denham, S., F, 2007. Growth and conformation changes in warmblood horses from birth to two years of age. In: 20th Equine Science Society Symposium. Hunt Valley, MD, pp. 157–158.

Staniar, W.B., Kronfeld, D.S., Treiber, K.H., Splan, R.K., Harris, P.A., 2004. Growth rate consists of baseline and systematic deviation components in Thoroughbreds. J. Anim. Sci. 82 https://doi.org/10.2527/2004.8241007x.

Staniar, W., Kronfeld, D., Cubitt, T., Byrd, B., Harris, P., 2005. Growth in linear measures of Thoroughbreds: Maturation rates and points of maturity. In: Proceedings of Equine Science Society. Tucson, Arizona.

Stock, K.F., Distl, O., 2006. Genetic correlations between conformation traits and radiographic findings in the limbs of German Warmblood riding horses. Genet. Sel. Evol. 38, 657–671. https://doi.org/10.1186/1297-9686-38-6-657.

Tabatabai, M., Williams, D.K., Zoran, B., 2005. Hyperbolastic growth models: theory and application. Theor. Biol. Med. Model Vol. 2 (2). https://doi.org/10.1186/1742-4682-2-14.

Tedeschi, L.O., Boin, C., Nardon, R.F., Leme, P.R., 2000. Estudo da curva de crescimento de animais da raça Guzerá e seus cruzamentos alimentados a pasto, com e sem suplementação: 1. Análise e seleção das funções não-lineares. Rev. Bras. Zootec. 29, 630–637. https://doi.org/10.1590/S1516-35982000000200040.

Teixeira, G.L., Fernandes, T.J., Muniz, J.A., de Souza, F.A.C., de Moura, R.S., dos Santos Mêlo, R.M.P., 2021. Grotth curves of Campolina horses using nonlinear models. Livest. Sci. 251 https://doi.org/10.1016/j.livsci.2021.104631.

Thompson, K.N., 1995. Skeletal growth rates of weanling and yearling thoroughbred horses. J. Anim. Sci. 73 https://doi.org/10.2527/1995.7392513x.

Thorén Hellsten, E., Viklund, A., Koenen, E.P.C., Ricard, A., Bruns, E., Philipssonа,, P., 2006. Review of genetic parameters estimated at stallion and young horse performance tests and their correlations with later results in dressage and show-jumping competition. Livest. Sci. 103, 1–12. https://doi.org/10.1016/j.livsci.2006.01.004.

Valette, J.P., Robert, C., Denoix, J.M., 2008. Use of linear and non-linear functions to describe the growth of young sport- and race-horses born in Normandy. Animal 2. https://doi.org/10.1017/S1751731107001462.

Viklund, A., Braam, A., Näsholm, A., Strandberg, E., Philipsson, J., 2010. Genetic variation in competition traits at different ages and time periods and correlations with traits at field tests of 4-year-old Swedish Warmblood horses. Animal 4, 682–691. https://doi.org/10.1017/S1751731110000017.

Wang, Y., Chen, H.-J., 2012. Use of Percentiles and Z-Scores in Anthropometry. Handbook of Anthropometry. Springer New York, New York, NY, pp. 29–48. https://doi.org/10.1007/978-1-4419-1788-1_2.

Willoughby, D.P., 1975. Growth and nutrition in the horse. A. S. Barnes.

Zechner, P., Zohman, F., Sölkner, J., Bodo, I., Habe, F., Marti, E., Brem, G., 2001. Morphological description of the Lipizzan horse population. Livest. Prod. Sci. 69, 163–177. https://doi.org/10.1016/S0301-6226(00)00254-2.

Journal of Equine Veterinary Science 57 (2017) 8–17

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Computer-Assisted Sperm Analysis of Head Morphometry and Kinematic Parameters in Warmblood Stallions Spermatozoa

Jana Křížková [a], Veronika Čoudková [a,*], Miroslav Maršálek [b]

[a] Student at University of South Bohemia, České Budějovice, Czechia
[b] Departement of Animal Husbandry Sciences at University of South Bohemia, České Budějovice, Czechia

ARTICLE INFO

Article history:
Received 22 March 2017
Received in revised form 19 May 2017
Accepted 19 May 2017
Available online 26 May 2017

Keywords:
Warmblood stallions
CASA
Sperm evaluation

ABSTRACT

The present study was conducted to determine the seminal characteristics of warmblooded stallions and to assess a relationship between motility parameters of spermatozoa and sperm head dimensions using computer-assisted sperm analysis. In total, 35 ejaculates were collected during the breeding season 2016 from 10 clinically healthy and fertile warmblooded stallions (age 3–22 years). The volume of ejaculate (mL), sperm concentration (M/mL), motility of sperm with kinematic parameters, and sperm morphometry were evaluated. The motility characteristics, sperm concentration, and morphometric parameters of sperm were determined objectively using Sperm Class Analyzer (SCA, MICROPTIC SL). The overall mean ± standard deviation values for volume of ejaculate, concentration, total motility (MOT), progressive motility, curvilinear velocity, straight-line velocity (VSL), average path velocity (VAP), straightness, linearity, amplitude of lateral head displacement, beat-cross frequency, elongation, and area were 50.29 ± 29.88 mL, 210.64 ± 90.98 × 10^6/mL, 88.68 ± 10.8%, 36.64 ± 15.36%, 63.23 ± 15.98 μm/s, 31.02 ± 9.39 μm/s, 47.53 ± 14.32 μm/s, 65.24 ± 5.72%, 48.8 ± 8.34%, 2.68 ± 0.41 μm, 7.29 ± 0.84 Hz, 36.2 ± 3.59%, 15.49 ± 1.54 μm^2, respectively. There were significant differences among stallions in all evaluated parameters of sperm ($P < .05$). There were significant positive correlations between area and velocity parameters (VSL, VAP, and wobble coefficient [WOB]). The elongation positively correlated with MOT. More elongated sperms demonstrated a lower frequency of curvilinear path intersections with the average path.

1. Introduction

Artificial insemination with cooled-shipped semen is the most commonly used method in horse reproduction [1]. However, the efficiency of cooled stallion semen remains limited, as indicated by suboptimal pregnancy rates achieved during artificial insemination with cooled semen (45%–70%) [2,3]. Stallion fertility holds an important place in this area as it is an economically important trait supported by a complex environment and a genetic background [4]. The evaluation of sperm quality is useful in predicting the fertility of stallions and is of a great importance in maximizing the reproductive efficiency in programs of assisted reproduction [5,6]. For the evaluation of semen quality, the most important parameters are the concentration, motility, and morphology of spermatozoa [7,8]. The evaluation of motility is one of the most used parameters for the evaluation of sperm quality [9,10].

Ethical/Animal care and welfare statement: The work described in our article have been carried out in accordance with *EU Directive 2010/63/EU for animal experiments and Ethical guidelines for Journal of Equine Veterinary Science publication.*
Conflict of interest: The authors declare no conflicts of interest.
* Corresponding author at: Veronika Čoudková, Mažice 33, 391 81 Veselí nad Lužnicí, Czech Republic.
E-mail address: nika.13@seznam.cz (V. Čoudková).

0737-0806/$ – see front matter © 2017 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2017.05.012

Morphology is also considered to be a reliable indicator of fertility, and the fertilization capacity of an individual positively correlates with the percentage of sperm cells with normal morphological structure [11,12]. There is a higher frequency of morphometric sperm defects at stallions with low fertility [13]. Fertilizing ability of spermatozoa depends on their shapes and sizes, which affect the course of the acrosomal reaction and sperm penetration of the ovum. Some authors mention a correlation between the morphometric characteristics of spermatozoa and sperm concentration in the ejaculates of stallions [14]. It is also possible that sperm concentration in an ejaculate influences the sperm shape and dimensions, and therefore influencing the motility characteristics and fertilizing ability [15,16]. It has been found that sperm dimensions are highly variable in males belonging to different animal species, breeds within a species, and within individuals of one population [17]. The variation among stallions was generally greater than the variation of ejaculates of one stallion [18]. Some studies have indicated an existence of an association between the sperm head morphometry and stallion fertility. The increase in morphological abnormalities of sperm heads was correlated with a reduction of pregnancy rates [19] and also negatively correlated with the fertility of stallions [20]. Spermatozoa head dimensions of males with decreased fertility differ from the head dimensions of spermatozoa of highly fertile stallions [21,22].

A number of studies indicate that variation in sperm head morphology may be a sensitive biomarker of abnormal chromatin structure and thus of fertility [23,24]. Although sperm head shapes and relative dimensions are considered reliable indicators of a sperm quality, their quantification is the most subjective [25]. However, subjective estimates of a sperm morphology suffer from lack of precision, repeatability, and accuracy [26,27]. The introduction of automated computer-assisted sperm analysis (CASA) systems attempted to overcome the problem of the subjectivity of visual-based assessment methods [25,27–29]. Computer-assisted sperm analysis allows the objective determination of ejaculate parameters (motility parameters, sperm concentration, newest models also have a morphology module) [27]. Computer-assisted sperm analysis has an important and increasing role for assurance of semen quality. Most of today's CASA systems offer the advantage of reduced bias compared with visual (subjective) evaluations. More, they provide outcome measures which could serve as a reliable basis for further semen samples processing used for distribution, and provide an assurance that the semen meets an insemination dose quality requirements [30].

Computer-assisted sperm analysis is the only objective method for motility, progressivity, and hyperactivity sperm sample analysis, and therefore provides an information on fertility potential of the ejaculate. The CASA is based on the capture of successive microscopic images which are then digitalized. The motile spermatozoa observed in these images are subsequently identified in the successive images, thus allowing for the establishment of their trajectories. Finally, the obtained trajectories are mathematically processed, which allows the definition of these trajectories in a numerical form. The results of this processing are reflected in a series of parameters, which precisely define the exact movement for each individual spermatozoon [31]. In the 1990s, the automated sperm morphometry analysis (ASMA) systems were introduced. Although this technology was originally designed for a human sperm, it has been progressively adapted to some animal species [25,28,29,32]. Automated sperm morphometry analysis systems have improved the assessment of sperm morphology with accurate and repeatable measurements of sperm head dimensions of different species including stallions [18,33–35]. Automated sperm morphometry analysis systems provided a series of objective parameters that have facilitated the standardization of morphological semen evaluation [36]. In addition, these automated systems can be used to prove subtle differences in the sperm head dimensions between fertile and subfertile stallions [29]. Currently, the precision of ASMA systems depends on the standardization of analytical variables, on the appropriate sample preparation (washing, fixation, and staining) and on the proper microscopic image analysis [37]. Errors are often the results of differences between ASMA systems, therefore, appropriate settings of CASA play a significant role in the accuracy of the outcomes [38].

The present study was conducted to determine the seminal characteristics of warmblooded stallions and assessment of the relationship between motility, parameters of spermatozoa, and sperm head dimensions including CASA. Sperm Class Analyzer (SCA) was used for objective determination of sperm concentration, motility, and velocity of sperm and morphometric characteristics.

2. Material and Methods

2.1. Stallions

Semen samples were obtained from 10 clinically healthy and fertile warmblooded stallions (age 3–22 years). These stallions were owned by ZH Písek and they have been used in the breeding season 2016 in artificial insemination with fresh semen. The stallions were stabled in the same conditions. All stallions were stabled in boxes at least 5 × 5 m. The feed dose of stallions contained 11 kg of hay, 1 kg of straw, 3 kg of oats, and mineral and vitamin supplementation (PREMIN for breeding stallions, STARFIT granulated feed). The exercise was an integral part of management, 1–4 hours a day, 6 days a week. The exercise was provided in a combination of turn-out in paddock, riding, and mechanical walker.

2.2. Semen Collection and Evaluation

Thirty-five ejaculates were collected in total during the breeding season 2016 (April–July) from stallions (April eight ejaculates, May nine ejaculates, June 10 ejaculates and July eight ejaculates; three, four, three, four, two, four, four, three, four, and four ejaculates from stallion S1, S2, S3, S4, S5, S6, S7, S8, S9, and S10, respectively). The volume of ejaculate (mL), sperm concentration (M/mL), motility of sperm with kinematic parameters, and sperm morphometry were evaluated. The evaluation of fertility was based on the data from the whole breeding season. It is represented as a percentage, and is based on the number of mares diagnosed as pregnant at a particular point in time divided by the total number of mares that have been bred to that stallion up to that point in time. A total of 3,300 spermatozoa was measured from all ejaculates for sperm morphometric analysis. Semen was collected using Colorado artificial vagina. All the stallions were frequently used for semen collection during the breeding season (2–3 times per week). Immediately after a collection, the volume of ejaculate was recorded. Then the semen was diluted with prewarmed (37°C) extender (Ref. 13570/1000, Double Distilled Water, pyrogen-free and sterile, www.minitube.com) at the ratio 1:1–2. After equilibration for 20 minutes at room temperature in the dark, the semen samples were placed in a refrigerator and they were cooled to 5°C.

Then the semen samples were transferred to a laboratory of ZF JU in České Budějovice. The motility characteristic, sperm concentration, and morphometric parameters of sperm were determined objectively using Sperm Class Analyzer (SCA, MICROPTIC SL, Barcelona, Spain). The technology was equipped with an optical microscope (Nikon ECLIPSE E200LED MV Series) with phase-contrast and heated plate, a build in a digital camera (Basler Color) capturing images and transmitting them to a computer (Genuine Intel Core i5).

Motility was evaluated using the Sperm Class Analyzer motility module. This module was based on the analysis of 16 consecutive, digitalized photographic images obtained from a single field. These 16 consecutive photographs were taken in a time lapse of 0.64 seconds, which implied a velocity of image capturing of one photograph each 40 ms. Sperm cells were detected automatically. For the analysis of motility, the negative phase-contrast (Ph−), magnification 100× and green filter were configured. Prewarmed Leja slide (20 microns in depth; Leja Amsterdam, The Netherlands) at the temperature of 37°C was loaded with extended semen (3 μL) and 10 microscopic fields were evaluated. Approximately, 100–200 of sperm cells were analyzed in one microscopic field. Spermatozoa were differentiated according to curvilinear velocity (VCL) of the rapid (>90 μm/s), medium (45–90 μm/s), slow (10–45 μm/s), and static. Spermatozoa with VCL less than 10 μm/s were considered immotile (static). Spermatozoa were considered progressive with at least 75% straightness (STR). Total motility (MOT, %), progressive motility (PMOT, %), VCL (μm/s), straight-line velocity (VSL, μm/s), average path velocity (VAP, μm/s), linearity coefficient (LIN, calculated by dividing VSL to VCL, %), STR coefficient (calculated by dividing VSL to VAP, %), wobble coefficient (WOB, calculated by dividing VAP to VCL, %), amplitude of lateral head displacement (ALH, μm), and beat-cross frequency (BCF, Hz) were measured in semen samples (Fig. 1). The concentration of spermatozoa (M/mL) was objectively determined in the same module, simultaneously with the evaluation of motility. Particle dimensions were 4–75 μm^2. The basic problem of the CASA assessment is that it is not possible to fully focus the sperm in the depth of the chamber, and the blurred sperms then do not have sufficient contrast against the background. The sperm concentration is usually underestimated. For this reason, is recommended the estimation of sperm concentration by other methods. This control assay was performed at the initial calibration of the CASA system by the hemocytometric method.

Morphometric analysis of the sperm heads was assessed using the Sperm Class Analyzer morphology module. Twenty microliters of sperm was placed on a microscopic slide and let air dry. Semen smear was stained with SpermBlue (MICROPTIC SL). All the slides were permanently sealed with Eukitt mounting medium (MICROPTIC SL) and a coverslip. The setting of the microscope for evaluation of sperm head morphometry: blue filter, 100× oil immersion objective, and bright-field. Hundred spermatozoa were analyzed in each semen sample. The spermatozoa were captured randomly in different fields except for those that overlapped each other. The digitized cells were automatically segmented with a range of grey-level values predetermined by the analysis factor (the automatic algorithm to define the contrast between cell and field). Sperm Class Analyzer system detected the boundary of sperm heads and midpieces. Eight morphometric parameters were calculated automatically: four for head size—length (L, μm), width (W, μm), area (A, μm^2), and perimeter (P, μm); and four for head shape calculated from the previous parameters—ellipticity ($ELIP = L/W$), rugosity ($Rugo = 4\pi A/P^2$), elongation ($ELON = [L - W]/[L + W]$) and regularity ($Regu = \pi LW/4A$). The measurements of each individual sperm cell were captured in Excel.

2.3. Statistical Analysis

Data are presented as means ± standard error of the mean for each stallion. Data sets were tested for normal distribution using Kolmogorov–Smirnov test. Positively skewed data were normalized using log transformation. Differences among stallions for each motility parameter and morphometric parameter (ELON, area) were evaluated by analysis of variance followed by Tukey test for multiple comparisons.

The linear relationships between sperm head-related variables including ELON and area and sperm velocity parameters including MOT, PMOT, VCL, VSL, VAP, LIN STR, WOB, and BCF were analyzed using correlation and regression procedures. Statistical significance was designed as $P < .001$ (***), $P < .01$ (**), $P < .05$ (*), and tendencies were declared at $P < .10$ (+).

The programs MS Office and Statistica 10 were used for data processing.

3. Results and Discussion

The semen parameters of ejaculates in the present study were within the physiological values for fertile adult stallions: a mean gel-free volume of 52 mL, 210 million sperm/mL for sperm concentration, 88% for sperm motility, and 73% for normal sperm morphology.

Table 1 shows a summary of the mean values ± standard deviation for semen volume, sperm concentration, and selected morphological and motility sperm parameters of monitored stallions. Morphometry of sperm was studied in many mammalian species. Most studies have concentrated on the sperm head and results show a high variability within species. It is mainly explained by differences in methodology. The stallion spermatozoon head is relatively

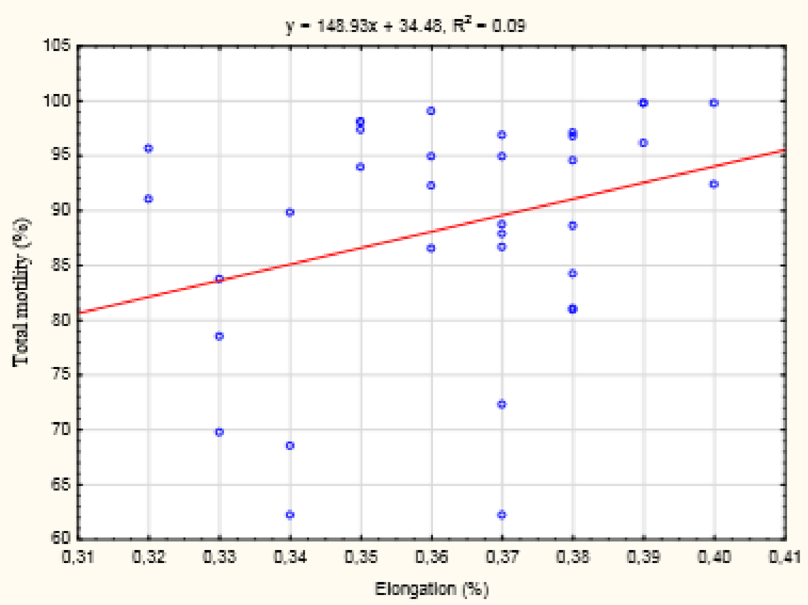


Fig. 1. The linear dependence of sperm elongation (%) and total motility (%) in warmblood stallions.

flat and the longitudinal cut shows oval shape with the anterior being the widest part. The head is relatively flat and a longitudinal section has an approximately elliptical shape with a slightly thicker posterior end. Reported means for the dimension of the stallion sperm head by Věžník et al [9] include 3.2 μm for the maximum the width and 6.3 μm for length. Samper [27] found dimensions 6.6 μm for length and 3.6 μm for the width of sperm heads. Despite the small number of stallions included in most experiments (5–20), all reports indicate a significant stallion effect on sperm head dimension and coefficients of variation ranging from approximately 5%–8% [14,18,19,21,38,39]. In the present study, there were significant differences ($P < .05$) among stallions in all evaluated sperm parameters. The smallest differences were observed in the case of the area. On the contrary, the greatest differences were in PMOT, VAP, ALH, and ELON.

Overview of mean values of stallion sperm head dimensions of different horse breeds measured by other authors is presented in Table 2. Most authors [14,17,19,21,35,39] agree with extent of these dimensions, which were set by Brito [22]. There are only two cases that differ in width (by 0.14 μm [21] and 0.04 μm [19]) and two cases in length (by 0.21 μm [21] and 0.06 μm [19]). One different value for perimeter can be observed (by 0.12 μm [19]). In the present study, the mean value and standard deviation of sperm head length 6.37 ± 0.23 μm are in accordance with the measurements of Brito [22]. Some authors obtained lower lengths [14,17,19,21,35,39]. The mean value for maximal width of sperm head 2.97 ± 0.08 corresponds to most of the authors [14,17,22,35,39]. The mean value of head area 15.49 μm^2 was greater than values observed by other authors [14,17,19,21,35,39], but it corresponds with Brito's measures [22]. Determined sperm head perimeter 16.27 μm was higher than values recorded in stallions by other authors [14,17,19,21,22,35,39]. It relates to greater values obtained for length and width of the sperm head. But width/length ratio value corresponds with all studies included into the comparison. The SCA analyzes the images captured by creating a boundary that matches the external outline of the microscopic image of the spermatozoa and delineating the sperm head. To obtain a correct image, the spermatozoa must contrast with the sample preparation background. Morphometric values obtained with the SCA system can be affected by the staining method. Stain, which provides more intense grey-level values, resulting in enlarged cells, which influence the size morphometric parameters as length, width, area, and perimeter of the head. Also increasing the staining time can enhance the intensity and contrast of the images—this process consequently increased the sperm dimensions [35]. Some substances including dehydration, such as xylene and alcohol, cause germ cells to shrink, and fixatives induce changes in the dimensions of sperm heads [40,41]. Factors affecting sperm dimensions include the osmolality and tonicity of the surrounding medium. It was observed during cryopreservation of semen that sperm placed in hypotonic solution had a tendency to swell, whereas in hyperosmotic solutions they shrunk [42]. In the case of stallion semen, however, there are few studies dealing with this subject. A comparative study by Banazsewska et al [43] showed that the dimensions of stallion sperm stained with SpermBlue were the most similar to the dimensions of sperm in unstained smears.

Determined range of width and length of the sperm head and width/length ratio can be used to estimate the interval for elongation values. It ranges on average between the values 31.58% and 39.86%. These values correspond to

Table 1
Seminal parameters of ejaculates of 10 different Warmblood stallions.

Parameter	Stallions					
	S1	S2	S3	S4	S5	S6
Fertility, %	67.30	69.04	62.50	63.63	75.00	69.00
Volume, mL	83.33 ± 34.00	25.00 ± 50.00	50 ± 0.00	100 ± 14.14	50 ± 0.00	42.5 ± 14.79
Concentration, M/mL	213.49 ± 41.37	193.21 ± 92.57	181.07 ± 26.12	258.58 ± 79.44	206.21 ± 34.06	118.28 ± 16.81
Total motility, %	66.81 ± 3.33^{a}	88.94 ± 9.77^{b}	96.59 ± 0.47^{c}	86.85 ± 1.72^{d}	97.45 ± 0.63^{c}	94 ± 2.72^{e}
PMOT, %	11.29 ± 6.43^{a}	38.29 ± 7.91^{b}	46.84 ± 4.66^{c}	22.71 ± 7.41^{d}	42.35 ± 9.88^{e}	52.85 ± 5.37^{f}
VCL, μm/s	38.61 ± 2.72^{a}	67.81 ± 10.63^{b}	73.19 ± 4.24^{c}	48.91 ± 7.76^{d}	63.79 ± 7.96^{e}	78.9 ± 5.8^{f}
VSL, μm/s	15.97 ± 6.38^{a}	33.46 ± 8.75^{b}	36.35 ± 3.77^{c}	24.51 ± 6.94^{d}	33.82 ± 6.6^{b}	41.98 ± 2.67^{e}
VAP, μm/s	24.92 ± 7.54^{a}	51.19 ± 10.88^{bc}	57.13 ± 5.61^{d}	35.69 ± 7.81^{e}	49.2 ± 8.28^{cf}	67.02 ± 4.45^{g}
STR, %	61.96 ± 8.97^{a}	64.71 ± 3.41^{b}	63.6 ± 0.35^{bc}	67.72 ± 4.46^{d}	68.44 ± 1.89^{d}	62.68 ± 2.27^{ac}
LIN, %	40.46 ± 14.49^{a}	48.58 ± 5.43^{bc}	49.55 ± 2.28^{b}	49.16 ± 6.35^{b}	52.55 ± 3.78^{d}	53.26 ± 1.57^{d}
ALH, μm	2.45 ± 0.32^{a}	2.76 ± 0.29^{b}	2.89 ± 0.03^{c}	2.67 ± 0.12^{d}	2.24 ± 0.08^{e}	2.49 ± 0.24^{a}
BCF, Hz	7.55 ± 1.42^{a}	7.48 ± 1.17^{ab}	6.59 ± 0.09^{c}	6.81 ± 0.42^{c}	7.33 ± 0.07^{abd}	7.04 ± 0.21^{e}
ELON, %	33.73 ± 2.54^{a}	37.06 ± 2.82^{bc}	38.78 ± 3.11^{de}	37.23 ± 2.41^{b}	36.16 ± 3.27^{cf}	35.05 ± 2.93^{g}
Length, μm	6.06 ± 0.15^{a}	6.35 ± 0.34^{b}	6.84 ± 0.38^{c}	6.31 ± 0.29^{b}	6.28 ± 0.42^{b}	6.45 ± 0.45^{d}
Width, μm	$3.00 \pm 2.54^{a,b}$	$2.91 \pm 0.18^{c,d}$	$3.01 \pm 0.16^{b,g}$	2.89 ± 0.16^{c}	$2.95 \pm 0.25^{a,d}$	3.10 ± 0.24^{e}
Perimeter, μm	15.74 ± 0.7^{a}	16.18 ± 0.81^{b}	17.21 ± 0.81^{c}	16.06 ± 0.71^{b}	16.06 ± 3.27^{b}	16.64 ± 1.10^{d}
Area, μm^2	14.95 ± 1.22^{a}	15.23 ± 1.42^{a}	16.91 ± 1.32^{b}	15.00 ± 1.28^{a}	15.25 ± 1.04^{a}	16.48 ± 1.57^{b}

Parameter	Stallions				Overall
	S7	S8	S9	S10	
Fertility, %	58.33	91.66	70.00	64.40	69.09
Volume, mL	65.00 ± 26.00	38.33 ± 16.50	28.75 ± 8.93	25 ± 11.18	50.29 ± 29.88
Concentration, M/mL	158.78 ± 32.23	366.81 ± 61.26	299.59 ± 50.35	140.51 ± 53.66	210.64 ± 90.98
Total motility, %	77.3 ± 11.11^{f}	99.81 ± 0.02^{g}	94.66 ± 4.96^{e}	87.21 ± 6.59^{d}	88.68 ± 10.8
PMOT, %	25.38 ± 6^{g}	48.81 ± 14.49^{c}	39.01 ± 14.36^{bh}	40.75 ± 9.02^{eh}	36.64 ± 15.36
VCL, μm/s	47.91 ± 1.96^{d}	80.84 ± 11.32^{f}	64.91 ± 15.93^{e}	67.13 ± 9.94^{b}	63.23 ± 15.98
VSL, μm/s	28.96 ± 2.1^{f}	31.25 ± 8.49^{g}	32.19 ± 10.01^{bg}	31.58 ± 4.23^{g}	31.02 ± 9.39
VAP, μm/s	39.22 ± 1.75^{h}	52.93 ± 12.36^{b}	47.08 ± 13.58^{f}	50.17 ± 7.39^{c}	47.53 ± 14.32
STR, %	73.76 ± 2.37^{e}	58.49 ± 2.48^{f}	68.18 ± 3.18^{d}	63.28 ± 4.89^{c}	65.24 ± 5.72
LIN, %	60.38 ± 2.19^{e}	37.96 ± 5.23^{f}	49.11 ± 5.03^{b}	47.32 ± 3.99^{c}	48.8 ± 8.34
ALH, μm	2.14 ± 0.3^{f}	3.31 ± 0.05^{g}	2.71 ± 0.27^{bd}	2.99 ± 0.46^{h}	2.68 ± 0.41
BCF, Hz	7.33 ± 0.1^{b}	7.36 ± 0.23^{ab}	7.13 ± 0.23^{de}	8.07 ± 1.18^{f}	7.29 ± 0.84
ELON, %	38.04 ± 3.4^{d}	39.45 ± 2.88^{e}	36.01 ± 2.62^{f}	32.42 ± 3.44^{h}	36.2 ± 3.59
Length, μm	6.60 ± 0.36^{e}	6.48 ± 0.45^{d}	6.29 ± 0.26^{b}	6.01 ± 0.34^{a}	6.37 ± 0.23
Width, μm	$2.96 \pm 0.21^{a,b}$	2.81 ± 0.18^{b}	2.96 ± 0.14^{de}	3.07 ± 0.20^{b}	2.97 ± 0.08
Perimeter, μm	16.75 ± 0.83^{d}	16.25 ± 0.82^{b}	16.10 ± 0.60^{b}	15.74 ± 0.80^{a}	16.27 ± 0.44
Area, μm^2	16.09 ± 1.53^{c}	15.00 ± 1.40^{a}	15.31 ± 1.01^{a}	15.14 ± 1.42^{a}	15.49 ± 1.54

Abbreviations: ALH, amplitude of lateral head; BCF, beat-cross frequency; ELON, elongation; LIN, linearity; PMOT, progressive motility; STR, straightness; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight-line velocity.
Data are presented as mean ± standard deviation.
^{a,b,c,d,e,f,g,h}Values with different superscript letters within rows differ ($P < .05$).

the results we have obtained. Rezagholizadeh et al [38] determined the mean value of the sperm head elongation three times greater than the general observed results, and on the other hand the mean value of the sperm head area three times smaller. Significant differences can be caused by a different measurement method, or by the fact that in this study, stallions of miniature horse breed were used. In most of the studies about semen in pony stallions, following parameters are recorded: motility, PMOT, and percentages of morphologically normal sperm and defects of the acrosome, head, midpiece, and tail [44–48]. But, there is no information about pony sperm head dimensions (width, length, and perimeter). However, across mammals, Gage [49] in his comparative study found no evidence that total sperm size variance was associated with body mass, in contrast with earlier studies. Generally, differences in

Table 2
The comparison of morphometric sperm parameters observed by other authors.

Source	Length, μm	Width, μm	Area, μm^2	Perimeter, μm	Width/Length
Brito [22]	5.33–6.62	2.79–3.26	11.43–16.28	13.76–15.64	0.43–0.52
Gravence et al [21]	5.49	2.65	11.22	13.79	0.49
Gravence et al [39]	5.96	2.95	13.31	15.54	0.49
Waheed et al [17]	5.96	3.06	14.33	14.16	0.51
Davis et al [14]	5.88	2.97	13.22	14.92	0.51
Hidago [35]	5.66	2.85	13.42	15.00	0.50
Casey [19]	5.33	2.75	11.37	13.64	0.52
Present study	6.37	2.97	15.49	16.27	0.47

Table 3
Correlation coefficients of linear relationship between seminal parameters and area and elongation of sperms.

Parameter	Area, μm²	Sig.	ELON, %	Sig.
Total motility, %	0.03	—	0.31	*
PMOT, %	0.22	+	0.14	—
VCL, μm/s	0.22	—	0.15	—
VSL, μm/s	0.31	*	0.13	—
VAP, μm/s	0.32	*	0.10	—
LIN, %	0.24	+	0.07	—
STR, %	0.03	—	0.12	—
WOB, %	0.35	*	0.01	—
ALH, μm	−0.04	—	0.06	—
BCF, Hz	0.00	—	−0.32	*

Abbreviations: ALH, amplitude of lateral head; BCF, beat-cross frequency; ELON, elongation; LIN, linearity; PMOT, progressive motility; STR, straightness; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight-line velocity; WOB, wobble coefficient.
Data are presented as correlation coefficient. Statistical significance $P < .05$ (*) and tendencies were declared at $P < .10$ (+).

dimensions and shape of normal sperm heads are considerable [18,22]. This high variability of sperm morphometric parameters is explained by the different methodology of morphometric analysis. There are suggestions that sperm head dimensions may be associated with fertility. Sperm head length, width, perimeter, and area were greater in subfertile stallions than in fertile stallions. Sperm in subfertile stallions also tended to be more tapered (lower length/width ratio) than in fertile stallions [19,21]. Larger sperm heads found in subfertile stallions may reflect disturbances in spermatogenesis, particularly involving altered chromatin structure. However, it is important to note that subfertile stallions also had lower total sperm number and percentages of motile and normal sperm in the ejaculate [22]. The detection of subtle differences in sperm head morphometry may open new ways to a more accurate and predictive assay for the evaluation of the stallion spermiogramme [50].

Evaluated sperm motility parameters were compared with studies of other authors. Rezagholizadeh et al [38] found values for MOT 87.06%, PMOT 44.54%, VCL 189.33 μm/s, VSL 82.88 μm/s, VAP 113.52 μm/s, STR 72.20%, LIN 45.57%, ALH 6.68 μm, and BCF 34.15 Hz. Their mean values for velocity parameters (VCL, VSP, VAP) differed considerably from our results. We obtained values lower by more than 50%. But calculated parameters, which are relative (STR and LIN), are in accordance with this study. The methodology of morphometric analysis probably caused different mean values of ALH and BCF, which were significantly lower in the present study. However, the choice of frame rate is still conflicting, as it is not only equipment-dependent, but related to species and experimental condition, as well reported by Kraemer et al [51]. In general, more variations are observed for the analysis of different fields than for the repeated analysis of the same fields, so the largest the number of cells analyzed, the more reduced the coefficient variation. Consequently, the precision of the results increases as the number of fields and cell analyses increase. Using digital images of each sperm cells track, CASA machines are able to analyze, by processing algorithms, the motion properties of spermatozoa. To reduce variability and misinterpretation of results, a larger number of cells have to be counted [52]. Differences in mean values of ALH and BCF cause difference in settings CASA system—number of frame per second. A higher number of frame per second (45 at 60 frames/s) in studies [1,38] may have resulted in higher values of ALH, BCF, and sperm velocity. In present study was the acquisition frame rate at 25 per second.

Florez-Rodrigues et al [1] compared in their study different types of diluent in cooled-shipped semen (5°C). Measured parameters in dependence on used diluent were in following ranges: MOT 80.3%–85.4%, PMOT 29.2%–35.6%, VCL 188.7–284.5 μm/s, VSL 68.6–91.8 μm/s, VAP 97.6–151.7 μm/s, STR 59.9%–70.2%, LIN 34.4%–39.4%, ALH 6.7–9.1 μm, and BCF 33.0–36.1 Hz. In both these works, sperm kinematic parameters evaluation was performed using the

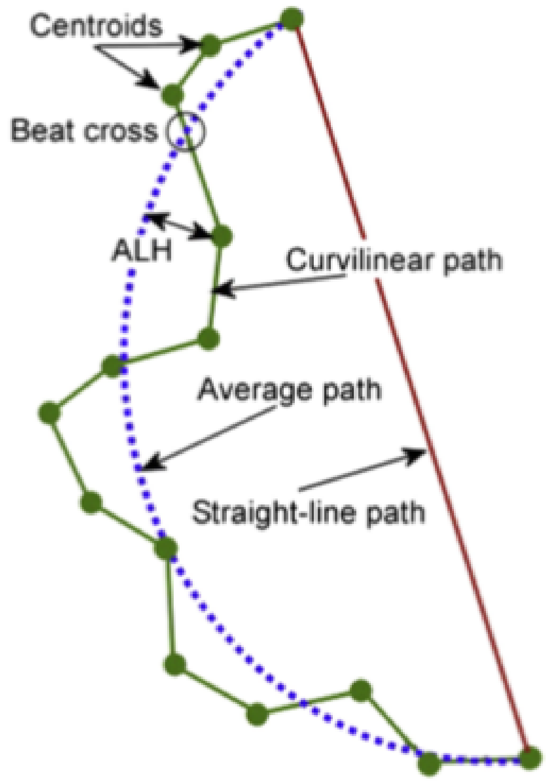


Fig. 2. Illustration showing CASA terminology. Initial image processing provides a centroid for each spermatozoon in the first frame of a scene, and for each cell location of the most probable centroid in successive frames is deduced. Connecting the centroids for a spermatozoon provides its actual trajectory, termed curvilinear path. The time-averaged velocity along this trajectory is termed VCL (μm/s). The average path is computed, and time-averaged velocity along this trajectory is termed VAP (μm/s). A straight-line path from the first to last position of a sperm head is plotted, and velocity along this trajectory is termed VSL (μm/s). For each centroid location, there is a deviation from the average path, and this is termed the ALH (μm). Similarly, there are points where the curvilinear path intersects the average path, and the number of such intersections is termed BCF (number per second) [30]. ALH, amplitude of lateral head displacement; BCF, beat-cross frequency; CASA, computer-assisted sperm analysis; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight-line velocity.

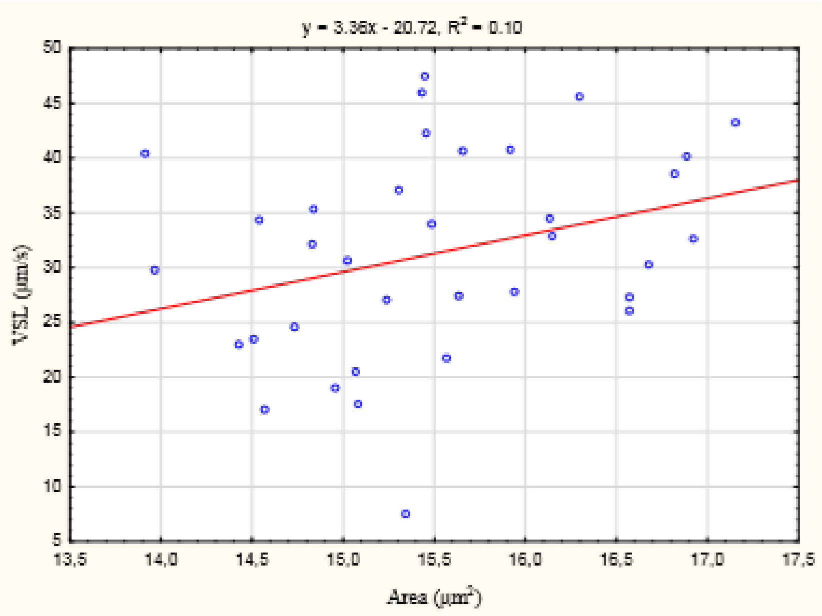


Fig. 3. The linear dependence of sperm area (µm^2) and VSL (µm/s) in warmblood stallions. VSL, straight-line velocity.

HTM, Hamilton-Thorne Research. Using of different CASA system may explain the difference between these results. Obtained sperm characteristics (volume 49.2 mL, concentration 304.8 × 10^6/mL, MOT 88.9%) and kinematic parameters (VAP 40.3 µm/s, LIN 56.13%, STR 64.7%, WOB 64.7%, and BCF 13.56 Hz) by authors Quintero-Moreno et al [31] and De la Cueva et al [53] (VCL 66.4 µm/s, VSL 8.31 µm/s, VAP 40.4 µm/s, LIN 48.6%, STR 72.6%, WOB 61%, and BCF 10.91 Hz), who also used for evaluation SCA system. All these results are in accordance with values which we obtained.

Generally, motility parameters correlated more with the head area than with the elongation. Detailed results are shown in Table 3. Head area was positively correlated with VSL, VAP, and WOB ($P < .05$). A tendency of a positive correlation was observed in the case of PMOT and LIN ($P < .10$).

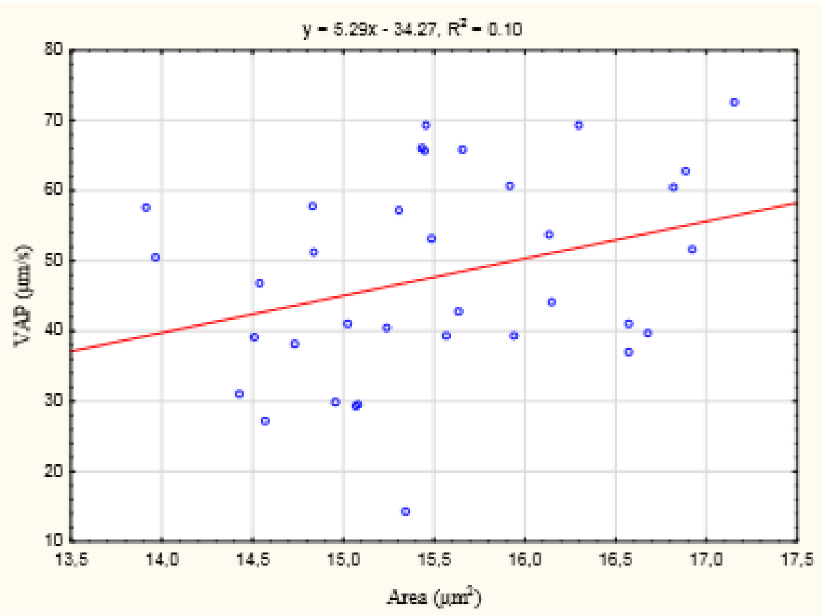


Fig. 4. The linear dependence of sperm area (µm^2) and VAP (µm/s) in warmblood stallions. VAP, average path velocity.

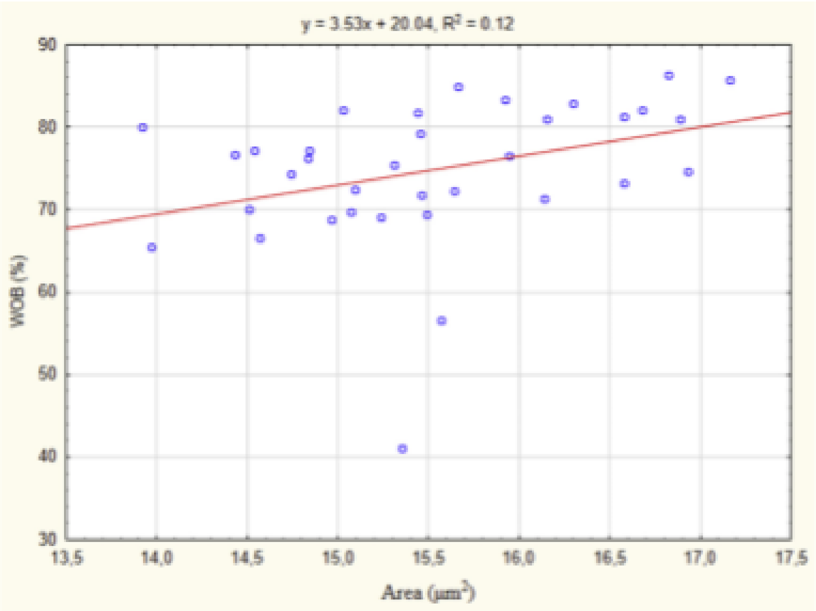


Fig. 5. The linear dependence of sperm area (μm^2) and WOB ($\mu m/s$) in warmblood stallions. WOB, wobble coefficient.

Elongation was positively correlated with the MOT and negatively with BCF. Rezagholizadeh et al [38] in their studies consistently found a positive correlation of head area with mean value of VCL (r = 0.52) and a negative correlation of area and PMOT (r = −0.62), VSL (r = −0.40), STR (r = −0.70), and LIN (r = −0.72). Elongation was in this study positively correlated with PMOT (r = 0.59), VSL (r = 0.32), STR (r = 0.64), and LIN (r = 0.69) and negatively correlated with VCL (r = −0.55). Also, Tourmente et al [54] and Sanchez et al [55] found that increasing the sizes of all cells components leads to the increased sperm size and consequently the sperms are able to produce more energy and move faster. More elongated sperms with a higher ratio of head length to head width would be more hydrodynamically efficient with reduced drag and would pass through the female genital tract with more velocity and STR

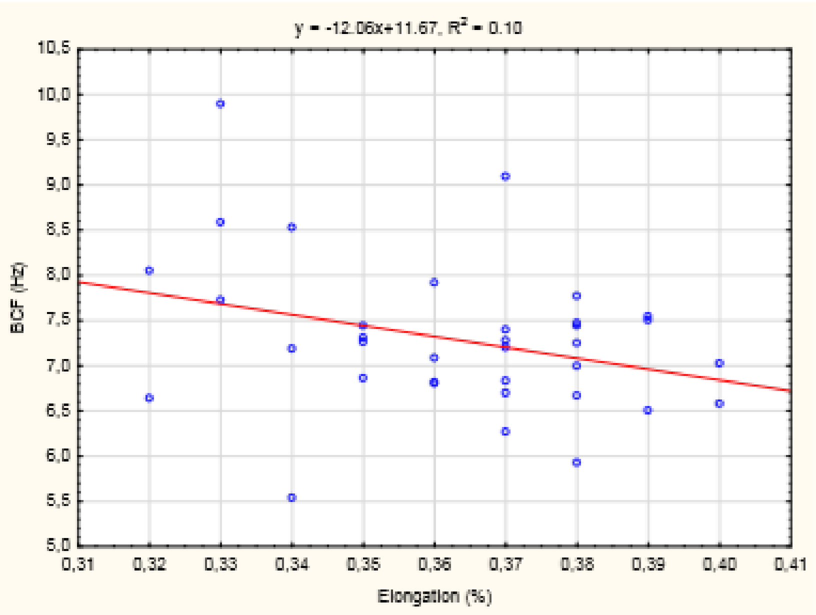


Fig. 6. The linear dependence of sperm elongation (%) and BCF (Hz). BCF, beat-cross frequency.

[56]. Mossman et al [57] proved for zebras, that, several aspects of sperm morphology were positively correlated with sperm velocity, including total sperm length, flagellum length, tail length, and flagellum/head length ratio.

In cases of the significant linear dependence of motility parameters and morphometric sperm parameters have been evaluated using regression analysis. Particular results including regression equation and the value of the coefficient of determination (R^2) are shown in Figs. 2–6. In the cases of the significant dependence of variables, velocity parameters correlated positively with sperm head area, in comparison with the results of Rezagholizadeh et al [38]. In their research, VSL correlates negatively with the area and on the contrary with VCL positively. These results led us to the development of detailed parameters of linear dependence of velocity parameters VSL and VCL. Our results indicate that strong positive correlation ($P < .001$, $r = 0.86$) was found. A positive correlation also shows area and relative parameter WOB (VAP/VCL). This result is related to the positive correlation of velocity parameters and head area, with increasing area VAP increases faster compared with VCL. It indicates that the sperm with the larger head area when moving by average path have a smaller head amplitude. This conclusion is reflected in tendencies of a negative correlation between ALH and head area. In the case of elongation, there were demonstrated a positive correlation with MOT and BCF. Rezagholizadeh et al [38] proved in Caspian miniature stallions positive correlation of elongation with PMOT, VSL, STR, and LIN, and conversely negative correlation with the VCL. In our research, linear dependence of elongation and velocity parameters has not been proven as well as in the dependent parameters. Significant ($P < .05$) negative correlation of elongation and BCF suggests that more elongated sperms demonstrated a lower frequency of curvilinear path intersections with the average path. Also, Pena et al [58] have demonstrated a linear relationship between velocity parameters (VAP, VCL, VSL) and sperm morphometry (length, width, area, ELIP, ELONG) in boar ejaculate.

4. Conclusions

The present study demonstrated mean values for seminal parameters of Warmblood stallions. Significant differences were shown between the observed stallions in all evaluated parameters. Morphometric and velocity parameters are very difficult to objectively discuss and compare with the results of other authors who evaluated stallions of other breeds. More or less different methods for evaluation of semen and sperm measurements were used in various studies. Obtained results and information from the literature show that it has a significant influence on the measured values. Some comparison is possible by relative parameters calculations, for example, STR and LIN.

The present study demonstrated a correlation between area and velocity parameters (VSL, VAP, and WOB). Elongation positively correlated with MOT. More elongated sperms demonstrated a lower frequency of curvilinear path intersections with the average path.

Acknowledgments

This work would not have been possible without the financial support of Grant Agency of University of South Bohemia GAJU-019/2016/Z.

References

[1] Florez-Rodriguez SA, de Arruda RP, Alves MBR, Affonso FJ, Carvalho HF, Lemes KM, Celeghini ECC. Morphofunctional characterization of cooled sperm with different extenders to use in equine-assisted reproduction. J Equine Vet Sci 2014;34:911–7.
[2] Squires EL. Changes in equine reproduction: have they been good or bad for the horse industry? J Equine Vet Sci 2009;29:268–73.
[3] Kiser AM, Brinsko SP, Love CC, Varner DD, Sudderth AK, Blanchard TL. Relationship of sperm quality to fertility after 4 days of cooled storage of equine semen. J Equine Vet Sci 2014;34:602–5.
[4] Sieme H, Distl O. Genomics and fertility in stallions. J Equine Vet Sci 2012;32:467–70.
[5] Colenbrander B, Gadella BM, Stout TAE. The predictive value of semen analysis in the evaluation of stallion fertility. Reprod Domest Anim 2003;38:305–11.
[6] Rodriguez I, Sanz J, Perez C, Felipe M, Dorado J, Hidalgo M. Fertilidad in vivo del esperma congelado-descongelado. I Jornadas de Investigacion en Veterinaria (Cordoba, Spain); 2001. 317–321.
[7] Vantman D, Koukoulis G, Dennison L, Zinaman M, Sherins RJ. Computer-assisted semen analysis: evaluation of method and assessment of the influence of sperm concentration on linear velocity determination. Fertil Steril 1988;49:510–5.
[8] Neuwinger J, Knuth UA, Nieschlag E. Evaluation of the Hamilton-Thorn 2030 motility analyser for routine semen analysis in an infertility clinic. Int J Androl 1990;13:100–9.
[9] Věžník Z, et al. Repetitorium spermatologie a andrologie a metodiky spermatoanalýzy. Výzkumný Ústav Veterinárního Lékařství V Brně. 2004.
[10] Contri A, Gloria A, Robbe D, Valorz C, Wegher L, Carluccio A. Kinematic study on the effect of pH on bull sperm function. Anim Reprod Sci 2013;136:252–9.
[11] Phetudomsinsuk K, Sirinarumitr K, Laikul A, Pinyopummin A. Morphology and head morphometric characters of sperm in Thai native crossbred stallions. Acta Vet Scand 2008;50:41.
[12] Vlasiu T, Groza I, Morar I, Cătană R. The effect of different freezing procedures on sperm head morphometry in stallions. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Vet Med 2008;65:146–51.
[13] Gage MJ, Morrow EH. Experimental evidence for the evolution of numerous, tiny sperm via sperm competition. Curr Biol 2003;13:754–7.
[14] Davis RO, Gravance CG, Casey PJ. Automated morphometric analysis of stallion spermatozoa. Am J Vet Res 1993;54:1808–11.
[15] Banaszewska D, Kondracki S, Wysokinska A. Effect of sperm concentration on ejaculate for morphometric traits of spermatozoas of the pietrain bred boars. J Cent Eur Agric 2009;10:383–95.
[16] Tsakmakidis IA, Lymberopoulos AG, Khalifa TAA. Relationship between sperm quality traits and field-fertility of porcine semen. J Vet Sci 2010;11:151–4.
[17] Waheed MM, Ghoneim IM, Abdou MS. Morphometric characteristics of spermatozoa in the Arabian horse with regard to season, age, sperm concentration, and fertility. J Equine Vet Sci 2015;35:244–9.
[18] Ball BA, Mohammed AO. Morphometry of stallion spermatozoa by computer-assisted image analysis. Theriogenology 1995;44:367–77.
[19] Casey PJ, Gravance CG, Davis RO, Chabot DD, Liu IKM. Morphometric differences in sperm head dimensions of fertile and subfertile stallions. Theriogenology 1997;47:575–82.
[20] Jasko DJ, Lein DH, Foote RH. Determination of the relationship between sperm morphologic classifications and fertility in stallions: 66 cases (1987-1988). J Am Vet Med Assoc 1990;197:389–94.
[21] Gravance CG, Liu IKM, Davis RO, Hughes JP, Casey PJ. Quantification of normal head morphometry of stallion spermatozoa. J Reprod Fertil 1996;108:41–6.
[22] Brito LF. Evaluation of stallion sperm morphology. Clin Tech Equine Pract 2007;6:249–64.
[23] Hingst O, Blottner S, Franz C. Chromatin condensation in cat spermatozoa during epididymal transit as studied by aniline blue and acridine orange staining. Andrologia 1995;27:275–9.

[24] Karabinus DS, Vogler CJ, Saacke RG, Evenson DP. Chromatin structural changes in sperm after scrotal insulation of Holstein bulls. J Androl 1997;18:549–55.
[25] Saravia F, Nunez-Martinez I, Moran JM, Soler C, Muriel A, Rodriguez-Martinez H, Pena FJ. Differences in boar sperm head shape and dimensions recorded by computer-assisted sperm morphometry are not related to chromatin integrity. Theriogenology 2007;2:196–203.
[26] Hidalgo M, Rodriguez I, Dorado J. Influence of staining and sampling procedures on goat sperm morphometry using the Sperm Class Analyzer. Theriogenology 2006;66:996–1003.
[27] Samper JC. Equine breeding management and artificial insemination. St. Louis, Missouri: Saunders Elsevier; 2009.
[28] Esteso MC, Soler AJ, Fernandez-Santos MR, Quintero-Moreno AA, Garde JJ. Functional significance of the sperm head morphometric size and shape for determining freezability in Iberian red deer (*Cervus elaphus hispanicus*) epididymal sperm samples. J Androl 2006;5:662–70.
[29] Hidalgo M, Rodriguez I, Dorado J, Soler C. Morphometric classification of Spanish thoroughbred stallion sperm heads. Anim Reprod Sci 2008;103:374–8.
[30] Amann RP, Waberski D. Computer-assisted sperm analysis (CASA): capabilities and potential developments. Theriogenology 2014;81:5–17.
[31] Quintero-Moreno A, Miró J, Teresa Rigau A, Rodríguez-Gil JE. Identification of sperm subpopulations with specific motility characteristics in stallion ejaculates. Theriogenology 2003;59:1973–90.
[32] Iguer-Ouada M, Verstegen J. Evaluation of the Hamilton Thorn computer based automated system for dog semen analysis. Theriogenology 2001;55:733–49.
[33] Arruda RP, Ball BA, Gravance CG, Garcia AR, Liu IKM. Effects of extenders and cryoprotectants on stallion sperm head morphometry. Theriogenology 2002;58:253–6.
[34] Soler C, De Monserrat JJ, Gutièrrez R, Nunez J, Nunez M, Sancho M, Cooper TG. Use of the SpermClass Analyser® for objective assessment of human sperm morphology. Int J Androl 2003;26:262–70.
[35] Hidalgo M, Rodriguez I, Dorado J, Sanz J, Soler C. Effect of sample size and staining methods on stallion sperm morphometry by the Sperm Class Analyzer. Vet Med Czech 2005;50:24–32.
[36] Yániz JL, Soler C, Santolaria P. Computer assisted sperm morphometry in mammals: a review. Anim Reprod Sci 2015;156:1–12.
[37] Davis RO, Gravance CG. Standardization of specimen preparation, staining, and sampling methods improves automated sperm-head morphometry analysis. Fertil Steril 1993;59:412–7.
[38] Rezagholizadeh A, Gharagozlou F, Niasari-Naslaji A, Akbarinejad V, Ziapour S. Evaluation of sperm characteristics in Caspian stallions using computer-assisted sperm analysis. J Equine Vet Sci 2015;35:505–9.
[39] Gravance CG, Champion Z, Liu IKM, Casey PJ. Sperm head morphometry analysis of ejaculate and dismount stallion semen samples. Anim Reprod Sci 1997;47:149–55.
[40] Gago C, Perez-Sanchez F, Yeung CH, Tablado L, Cooper TG, Soler C. Standardization of sampling and staining methods for the morphometric evaluation of sperm heads in the Cynomolgus monkey (*Macaca fascicularis*) using computer-assisted image analysis. Int J Androl 1998;21:169–76.
[41] Sancho M, Perez-Sanchez P, Tablado L, de Monserrat JJ, Soler C. Computer-assisted morphometric analysis of ram sperm heads: evaluation of different fixative techniques. Theriogenology 1998;50:27–37.
[42] Mc Alister DA. A comparison of motility and head morphology of sperm using different semen processing methods and three different staining techniques. Dissertation presented in partial fulfillment of the requirements for the degree Master of Science in Medical Sciences (MScMedSci-Medical Physiology) at Stellenbosch University. 2010.
[43] Banaszewska D, Czubaszek M, Walczak-Jędrzejowska R, Andraszek K. Morphometric dimensions of the stallion sperm head depending on the staining method used. Bull Vet Inst Pulawy 2015;59:263–70.
[44] Deichsel K, Schrammel N, Aurich J, Aurich C. Effects of a long-day light program on the motility and membrane integrity of cooled-stored and cryopreserved semen in Shetland pony stallions. Anim Reprod Sci 2016;167:68–73.
[45] Van Eldik P, Van Der Waaij EH, Ducro B, Kooper AW, Stout TAE, Colenbrander B. Possible negative effects of inbreeding on semen quality in Shetland pony stallions. Theriogenology 2006;65:1159–70.
[46] Deichsel K, Palm F, Koblischke P, Budik S, Aurich C. Effect of a dietary antioxidant supplementation on semen quality in pony stallions. Theriogenology 2008;69:940–5.
[47] Schrammel N, Deichsel K, Aurich J, Aurich C. A long-day light program accelerates seasonal coat changes but is without effect on semen and metabolic parameters in Shetland pony stallions. Theriogenology 2016;85:946–53.
[48] McDonnell SM, Turner RO. Post-thaw motility and longevity of motility of imipramine-induced ejaculates of pony stallions. Theriogenology 1994;42:475–81.
[49] Gage MJ. Mammalian sperm morphometry. Proc R Soc Lond B Biol Sci 1998;265:97–103.
[50] Severa L, Máchal L, Švábová L, Mamica O. Evaluation of shape variability of stallion sperm heads by means of image analysis and Fourier descriptors. Anim Reprod Sci 2010;119:50–5.
[51] Kraemer M, Fillion C, Martin-Pont B, Auger J. Factors influencing human sperm kinematic measurements by the cell trak computer-assisted sperm analysis systém. Hum Reprod 1998;13:611–9.
[52] Dhurvey M, Gupta VK, Nema SP, Patidar A, Shivhare M, Singh N, Shakya V. Modern semen evaluation techniques in domestic animals: a review. DHR Int J Biomed Life Sci 2012;3:62–83.
[53] De La Cueva FC, Pujol MR, Rigau T, Bonet S, Miró J, Briz M, Rodriguez-Gill JE. Resistance to osmotic stress of horse spermatozoa: the role of ionic pumps and their relationship to cryopreservation success. Theriogenology 1997;48:947–68.
[54] Tourmente M, Gomendio M, Roldan ER. Sperm competition and the evolution of sperm design in mammals. BMC Evol Biol 2011;11:12.
[55] Sanchez MV, Bastir M, Roldan ERS. Geometric morphometrics of rodent sperm head shape. PLoS One 2013;8:e80607.
[56] Malo AF, Gomendio M, Garde J, Lang-Lenton B, Soler AJ, Roldan ERS. Sperm design and sperm function. Biol Lett 2006;2:246–9.
[57] Mossman J, Slate J, Humphries S, Birkhead T. Sperm morphology and velocity are genetically codetermined in the zebra finch. Evolution 2009;63:2730–7.
[58] Peña FJ, Saravia F, García-Herreros M, Núñez martinez I, Tapia JA, Johannisson A, Rodríguez-Martínez H. Identification of sperm morphometric subpopulations in two different portions of the boar ejaculate and its relation to post thaw quality. J Androl 2005;26:716–23.

Chemically defined caseinate extenders and their effect on the cooled semen collected from stallions with long sexual rest

Ředidla s obsahem chemicky definovaných kaseinátů a jejich vliv na chlazené sperma odebrané od hřebců po dlouhé sexuální inaktivitě

Jana VOKROUHLÍKOVÁ[1], Veronika ČOUDKOVÁ[1] (✉), Jiří ŠICHTAŘ[2], Aneta VRBOVÁ[1], Alena BÚDOVÁ[1], Miroslav MARŠÁLEK[3], Jitka RUTKAYOVÁ[1]

[1] Department of Animal Husbandry Sciences, Faculty of Agriculture, University of South Bohemia, Studentská 13, 370 05 České Budějovice, Czech Republic

[2] Department of Veterinary Sciences, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 00 Praha 6-Suchdol, Czech Republic

[3] Czech Hipological Society, Na zlaté stoce 690/3, 370 05 České Budějovice

✉ Corresponding author: nika.13@seznam.cz

Received: May 15, 2020; accepted: April 22, 2021

ABSTRACT

The ability of three milk-based extenders to preserve the motility and viability of cooled shipped semen produced by stallions after a long sexual rest was evaluated. In total, 21 ejaculates from 11 stallions were collected after 5–6 months of sexual inactivity and diluted with Kenney, INRA 96, and EquiPlus. Seven parameters were evaluated – spermatozoa viability (VIT, %), total motility (MOT, %), progressive motility (PMOT, %), number of progressive spermatozoa per ml (prog M/ml, million/ml), average path velocity (VAP, μm/s), curvilinear velocity (VCL, μm/s) and straight-line velocity of spermatozoa (VSP, μm/s). These parameters were determined by CASA 2 hours after semen processing and then in 24, 48 and 72-hour intervals of storage at 5 °C. The effect of storage time was highly significant (P<0,001) for all evaluated parameters. The effect of the extender was significant (P<0.05) for VIT, MOT, PMOT, VCL, VSL and VAP. The ejaculates extended with INRA 96 reached significantly (P<0.01) higher values for all evaluated parameters compared to Kenney extender. The INRA 96 extender significantly increased the values of PMOT, VCL and VAP compared to EquiPlus (P<0.05). In all observed extenders, MOT and VIT decreased linearly whereas PMOT decreased exponentially. The fastest decrease of PMOT was observed within 24 hours of storage. The results obtained from using INRA 96 showed that quality of cooled semen for stallions collected after a long period of sexual inactivity can be sufficiently maintained during at least 24 hours of storage.

Keywords: cooled semen, ejaculate, extender, sperm motility, stallion

ABSTRAKT

Cílem této práce bylo porovnat ochranný účinek tří mléčných ředidel na chlazené sperma získané od hřebců po delší sexuální pauze (5 až 6 měsíců). Celkem bylo odebráno 21 ejakulátů od 11 hřebců. K ředění byla využita ředidla Kenney, EquiPLus a INRA 96. Hodnoceno bylo sedm parametrů – vitalita spermií (VIT, %), celková motilita (MOT, %), progresivní motilita (PMOT), počet progresivních spermií v mililitru (prog M/ml, million/ml), průměrná rychlost (VAP, μm/s), křivočará rychlost (VCL, μm/s) a přímočará rychost spermií (VSL, μm/s). Tyto parametry byly stanoveny metodou CASA 2 hodiny po zpracování spermatu a poté po 24, 48 a 72 hodinách skladování při 5 °C. Byl prokázán významný vliv doby skladování spermatu (P<0,001) na všechny hodnocené parametry. Vliv ředidla byl singnifikantní (P<0,05) v případě VIT, MOT, PMOT, VCL, VSL a VAP. Průměrný ochranný účinek ředidla INRA 96 byl významně lepší po celou dobu skladování u všech sledovaných parametrů než u ředidla Kenney (P<0,01) a než u EquiPlus (P<0,05) u hodnot PMOT, VCL a VAP. U všech sledovaných ředidel hodnoty MOT a VIT klesaly lineárně, zatímco PMOT exponenciálně. Nejrychlejší pokles PMOT byl pozorován v průběhu prvních 24 hodin skladování. Výsledky dosažené s ředidlem INRA 96 ukázaly, že kvalita chlazeného spermatu u hřebců odebraných po delší sexuální inaktivitě může být dostatečně udržena nejméně během prvních 24 hodin skladování.

Klíčová slova: chlazené sperma, ejakulát, ředidlo, motilita spermií, hřebec

INTRODUCTION

The success of equine artificial insemination (AI) depends on maintaining the fertilizing ability of spermatozoa during cold storage. To achieve this goal, several basic and modified extenders have been developed (Ijaz and Ducharme, 1995). Most extenders for equine semen cold storage are based either on skimmed milk or egg yolk (Jasko et al., 1992; Moran et al., 1992; Bergeron and Manjunath, 2006). Milk and milk-based extenders are known to be practical and efficient in protecting equine spermatozoa during cold storage before AI (Michajilov, 1950; Kenney et al., 1975; Palmer, 1984). However, milk is a biological fluid with a complex composition and contains components that could be beneficial or detrimental to sperm survival (Batellier et al., 2001). Some commercial extenders substitute protein-defined milk fraction for milk (Lagares et al., 2012). Fractionating milk has allowed the preparation of purified protein fractions. Among these, native phosphocaseinate (NPPC), composed of total micellar caseins, and β-lactoglobulin were found to be the most effective in supporting the longevity of cold-stored spermatozoa (Batellier, 1997; Batellier et al., 1997). Pellicer-Rubio and Combarnous (1998) state that NPPC is the most efficient component for preserving motility (MOT) and maintaining the fertility of equine spermatozoa stored for 3 days compared to standard skim-milk extenders. Batellier et al. (1997) developed the INRA 96 extender which is a chemically defined medium with NPPC substituting for skimmed milk.

In a healthy stallion, the period of sexual rest is probably the most important aspect of determining the quality of semen (Thompson et al., 2004). It is a common practice for many sport horse stallions to breed a very limited book of mares each breeding season. Therefore, the interval between successive semen collections may be too long to maintain optimal semen quality for a cooled semen shipment program (Samper et al., 2008). The lack of studies performed without stabilizing the extragonadal spermatozoa reserves raises the following questions: what are the semen parameters of the ejaculates of stallions after a long sexual rest? Can the semen parameters in stallions after a long sexual rest be influenced by the choice of an appropriate extender?

The aim of the present work was to evaluate the effect of extenders containing defined milk components with a widely established skimmed milk extender on the motility parameters and viability of stallion spermatozoa obtained after a long sexual rest.

MATERIALS AND METHODS

Stallions

Semen samples were obtained from 11 clinically healthy and fertile stallions (age 3–21 years). Stallions were all involved in a commercial AI program at the National Stud Písek, Czech Republic. The stallions were stabled in the same conditions, i.e. boxes sized at least 5 x 5 m. The stallions' feed dose contained 11 kg hay, 1 kg straw, 3 kg oats, and mineral and vitamin supplements—PREMIN for breeding stallions (VVS Verměřovice, s. r. o., Czech Republic) and STARFIT granulated feed (FIDES AGRO, spol. s. r. o., Czech Republic). Exercise was also an integral part of management, 1–4 hours a day, 6 days a week. It was provided in a combination of turn-out in the paddock, riding, and mechanical walker.

Semen Collection

Twenty-one ejaculates were collected before the beginning of the 2018 breeding season from 11 stallions (n=10 in January, n=11 in February). The sexual rest of the stallions was approximately five to six months.

Semen collection was performed on a phantom using a Colorado model artificial vagina in the presence of a mare. Immediately after collection, the gel fraction was removed, the semen was filtered through sterile gauze and the volume of ejaculate was recorded. Each ejaculate was split into 3 aliquots and one of the 3 extenders, warmed to 37 °C, was added to each aliquot at a 1:1 dilution. After dilution, the semen samples were equilibrated for 20 minutes at room temperature in the dark. After incubation, the semen was cooled to 5 °C at an approximately 0.3 °C/min cooling rate and stored at 5 °C in a refrigerator. The semen samples were then transferred to the laboratory of ZF JU in České Budějovice. Samples were transported for approximately 60 min in a Styrofoam box with cooling elements.

Semen Extenders

Two commercially available extenders (INRA 96 and EquiPlus) and one privately prepared extender (Kenney) were tested.

INRA 96 is patented and available as a ready-to-use solution (Ref. 016441, IMV Technologies, Saint-Ouen-Sur-Iton, France). This chemically defined extender contains Hanks' salts, glucose (67 mM) and lactose (126 mM) supplemented with a purified milk fraction, and native phosphocaseinate (27 g/l).

EquiPlus (Ref. 13570/0202, Minitüb, Tiefenbach, Germany) contains defined caseinates, glucose, sucrose, buffer and antibiotics (lincomycin and spectinomycin). It was used in the powdered form and dissolved in purified water (Double Distilled Water, pyrogen-free and sterile, Ref. 13570/1000, Minitüb, Tiefenbach, Germany) according to the manufacturer's instructions.

The Kenney extender is based on glucose and non-fat milk and contains 2.4 g non-fat dry skimmed milk, 4.9 g glucose monohydrate, 100 ml redistilled water and antibiotics (0.15 g dihydrostreptomycin and 0.15 penicillin). This extender was created in the Písek, Nový Dvůr laboratory especially for National Stud Písek.

Semen Evaluation

Semen samples were evaluated after transporting the cooled semen. Thus, the semen motility was evaluated at the time of insemination rather than directly after collection at the time of processing.

Spermatozoa motility and kinematic parameters were determined objectively using a Sperm Class Analyzer (SCA, MICROPTIC SL, Barcelona, Spain). This apparatus was equipped with a microscope (Nikon ECLIPSE E200 LED MV Series) with phase-contrast,a heated plate (37 C) and a built-in digital camera (Basler Color) to capture images and transmit them to a computer (Genuine Intel Core i5).

Motility was evaluated using the Sperm Class Analyzer motility module. This module was established by analysing 16 consecutive, digitized photographic images obtained from a single field. These 16 consecutive photographs were taken with a 0.64 s time-lapse, which implied an image capturing velocity of one photograph every 40 ms. Sperm cells were detected automatically. Motility was analyzed using negative phase-contrast (Ph-), 100x magnification and a green filter. Semen samples were opened to remove an aliquot for each time and then resealed. For motility analysis, aliquots were prewarmed to 37 °C for 10 minutes. A prewarmed Leja slide (20 microns in depth; Leja Amsterdam, The Netherlands) at 37 °C was loaded with extended semen (3 µL) and 10 microscopic fields were evaluated. The final semen concentrations for analysis were 30–50 x 10^6 spermatozoa cells/ml. Approximately 100–200 spermatozoa cells were analysed in one microscopic field. Parameters evaluated using the Sperm Class Analyzer were total motility (MOT, %), progressive motility (PMOT, %), number of progressive spermatozoa per ml (prog M/ml, %), curvilinear velocity (VCL, µm/s), straight-line velocity (VSL, µm/s) and average path velocity (VAP, µm/s). Spermatozoa were classified according to curvilinear velocity (VCL) as rapid (> 90 µm/s), medium (45–90 µm/s), slow (10–45 µm/s) and static (< 10 µm/s). Spermatozoa were considered progressive with at least 75% straightness (STR).

The viability of spermatozoa was evaluated using the Sperm Class Analyzer vitality module and fluorescent staining FluoVit (MICROPTIC SL). FluoVit is a fluorescent staining solution that permits differentiating the living spermatozoa from the dead spermatozoa using fluorescent dyes (Hoechst and Trihydrochloride Trihydrate, Propidium Iodide). Semen sample (10 µl) was incubated with 1 µl BLUE eppendorf stain (Hoechst 33342, Trihydrochloride Trihydrate 330/380) at 37 °C for 5 minutes and then with 1 µl RED eppendorf stain (Propidium Iodide 536/617) for 5 minutes. After incubation, one drop (4 µl) of stained sample was placed on a glass slide and covered with a glass coverslip. For the analysis of viability, fluorescence, magnification 200x and DAPI filter (EX 330-380, DM 400, BA 420, standard filter for UV) were configured. Heads of membrane-intact (viable) spermatozoa showed bright blue colour, spermatozoa with damaged membranes (dead) were stained red. At least 200 spermatozoa were evaluated per sample and the mean calculated by software provided by SCA. Results are given as percentages of live spermatozoa in the samples.

The chosen parameters were measured for each sample of extended semen immediately after cooling to 5 °C (2 h), and after storage at this temperature for 24, 48 and 72-hour time periods for all extenders.

Statistical Analysis

MS Office and Statistica.12 (StatSoft®, Prague, Czech Republic) were used for data processing. The effect of extender on cold-stored semen characteristics was assessed using analysis of variance with repeated measurements followed by the Tukey test for multiple comparisons. Statistical significance was set at $P<0.001$ (***), $P<0.01$ (**) and $P<0.05$ (*) and tendencies were defined as $0.05<P<0.10$ (+). Relationships among time and dependent variables were estimated using linear regression. Regression analyses were used to evaluate how time was related to the dependent variables (parameters), and to explore the forms of these relationships among all studied extenders. The results, in the form of an equation, are shown together with the coefficient of determination (R^2). The velocity of decrease for the obtained values was evaluated using the first derivative, which can be interpreted as an instantaneous rate of change. Data are presented as means ± standard error of the mean (SEM) unless otherwise indicated.

RESULTS

Table 1 shows the significant effect of extender on VIT, MOT, PMOT, VCL, VSL and VAP ($P<0.05$). As expected, highly significant effect of storage time on all evaluated parameters was manifested ($P<0.001$). The interaction of time and extender was significant for PMOT and VCL ($P<0.05$).

Table 1. The effect of extender and storage time on cold-stored semen motility and vitality assessed by analysis of variance with repeated measurements

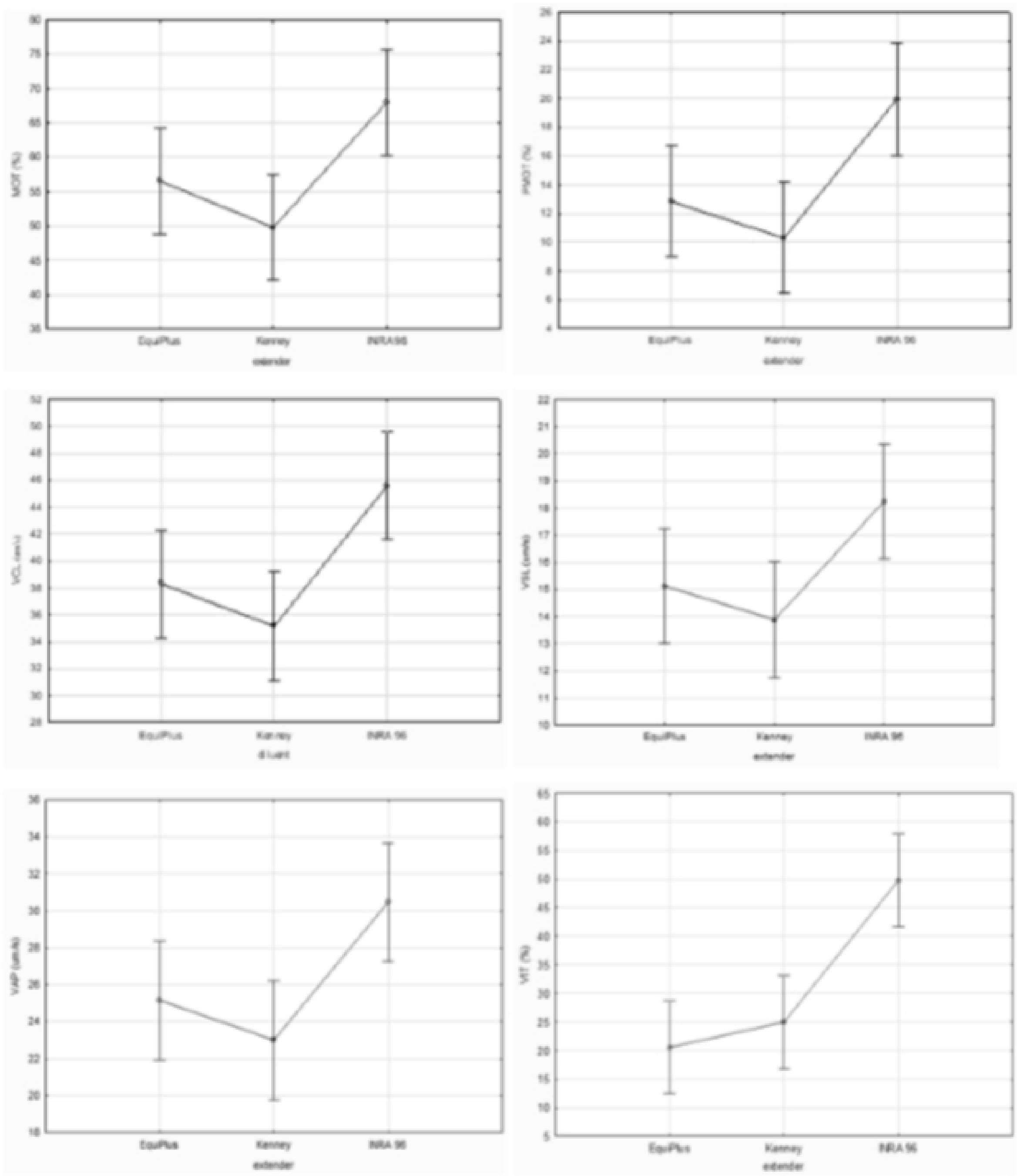
Parameter	extender	time	time*extender
MOT (%)	**	***	-
PMOT (%)	**	***	**
prog M/ml	+	***	-
VCL (µm/s)	**	***	*
VSL (µm/s)	*	***	-
VAP (µm/s)	**	***	-
VIT (%)	***	***	-

Abbreviations: MOT, total motility; PMOT, progressive motility; prog M/mL, million progressive sperm per mL; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight-line velocity; VIT, viability.

Data are presented as statistical significance $P<0.001$ (***), $P<0.01$ (**), $P<0.05$ (*), tendencies were declared at $P<0.10$ (+), $P>0.10$ (-).

The results of multiple comparisons using the Tukey HSD test (Figure 1) demonstrate that the overall protective effect of INRA 96 extender was significantly better than for semen diluted with Kenney ($P<0.01$). The VIT, PMOT, VCL and VAP were higher for ejaculates extended with INRA 96 ($P<0.05$) than those extended with EquiPlus. No difference was found between Kenney and EquiPlus extender.

To illustrate the effect of three different semen extenders in detail, the cooled semen parameters (motility characteristics) were evaluated at 2, 24, 48 and 72-hours (Table 2). Our results show that spermatozoa motility parameters differed significantly among extenders.



Abbreviations: MOT, total motility; PMOT, progressive motility; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight-line velocity; VIT, viability

Figure 1. Graphs of multiple comparisons of INRA 96, EquiPlus and Kenney using the Tukey HSD test within evaluated parameters

Table 2. Effect of extender on stallion semen motility parameters (mean ± SEM) for ejaculates (n=21) stored at 5 °C up to 72 hours

Parameter	2 hours			24 hours		
	EquiPlus	Kenney	INRA 96	EquiPlus	Kenney	INRA 96
MOT (%)	86.86 ± 3.39^{1}	82.92 ± 4.12^{1}	94.25 ± 1.48^{1}	65.79 ± 5.14^{a,b,2}	59.45 ± 5.64^{a,2}	79.09 ± 4.19^{b,2}
PMOT (%)	32.40 ± 3.78^{a,1}	27.49 ± 3.95^{a,1}	47.53 ± 3.68^{b,1}	12.27 ± 2.74^{2}	9.94 ± 2.97^{2}	19.93 ± 3.26^{2}
prog M/mL	76.13 ± 12.29^{a,b,1}	65.83 ± 11.15^{a,1}	103.68 ± 16.06^{b,1}	30.89 ± 7.54^{2}	22.79 ± 6.15^{2}	46.50 ± 9.64^{2}
VCL (µm/s)	60.72 ± 3.61^{a,1}	55.05 ± 3.87^{a,1}	74.28 ± 3.62^{b,1}	39.45 ± 2.69^{2}	35.98 ± 2.85^{2}	46.47 ± 3.17^{2}
VSL (µm/s)	28.99 ± 2.20^{a,b,1}	26.74 ± 2.24^{a,1}	35.01 ± 2.13^{b,1}	15.05 ± 1.55^{2}	13.85 ± 1.66^{2}	18.38 ± 1.73^{2}
VAP (µm/s)	45.45 ± 2.99^{a,1}	41.34 ± 3.16^{a,1}	56.33 ± 2.89^{b,1}	25.51 ± 2.27^{2}	23.35 ± 2.49^{2}	30.85 ± 2.64^{2}
Parameter	**48 hours**			**72 hours**		
	EquiPlus	Kenney	INRA 96	EquiPlus	Kenney	INRA 96
MOT (%)	48.64 ± 5.83^{a,b,3}	34.78 ± 4.42^{a,3}	59.64 ± 5.02^{b,3}	28.42 ± 5.01^{4}	22.04 ± 3.70^{4}	38.90 ± 5.55^{4}
PMOT (%)	4.80 ± 1.50^{a,b,2,3}	2.72 ± 0.72^{a,2}	8.39 ± 2.04^{b,3}	1.87 ± 0.60^{a,b,3}	1.05 ± 0.29^{a,2}	3.92 ± 0.88^{b,3}
prog M/mL	11.28 ± 3.46^{2}	5.73 ± 1.34^{2}	20.98 ± 6.46^{2,3}	4.38 ± 1.47^{a,b,2}	2.69 ± 0.75^{a,2}	9.07 ± 2.73^{b,3}
VCL (µm/s)	28.92 ± 2.03^{3}	26.44 ± 1.24^{3}	33.03 ± 2.54^{3}	24.18 ± 1.43^{3}	23.16 ± 1.12^{3}	28.55 ± 1.66^{3}
VSL (µm/s)	9.58 ± 0.82^{3}	8.61 ± 0.57^{2,3}	11.15 ± 1.05^{3}	6.91 ± 0.59^{3}	6.37 ± 0.38^{3}	8.49 ± 0.62^{3}
VAP (µm/s)	16.63 ± 1.48^{3}	15.16 ± 0.90^{3}	19.15 ± 1.90^{3}	13.02 ± 1.07^{3}	12.19 ± 0.61^{3}	15.50 ± 1.06^{3}

Abbreviations: MOT, total motility; PMOT, progressive motility; prog M/mL, million progressive sperm per mL; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight-line velocity

^{a,b} Different superscripts in a row within individual time periods indicate significant differences among extenders (*P*<0.05)

^{1,2,3,4} Different superscripts within a row indicate significant differences among time periods within individual extender (*P*<0.05)

Generally, the values of all spermatozoa motility parameters were the highest in the INRA 96 extender over the monitored time. At the 2-hour interval, PMOT, VCL and VAP were significantly higher in INRA 96 compared to the other extenders. The prog M/mL and VSL were significantly higher in INRA 96 compared to Kenney. At 24-hours, MOT was significantly higher in INRA 96 compared to Kenney. PMOT tended toward a significant difference (P=0.06) between these two extenders. Twenty-four hours later (48-hours), only MOT was significantly higher in INRA 96 compared to Kenney and PMOT and prog M/mL tended toward a significant-difference (P=0.05 and 0.07, respectively). At the end of the experimental period (72-hours), MOT did not differ between INRA 96 and Kenney (P=0.07), while PMOT and prog M/mL significantly differed between these two extenders.

Figure 2 illustrates that spermatozoa viability differed significantly among extenders over time. In all four periods of storage, spermatozoa viability was significantly higher in INRA 96 than in Kenney and EquiPlus. At 2 hours, viability in INRA 96 was significantly higher than in Kenney (by 27.82%) and EquiPlus (by 22.06%). After 24, 48 and 72 hours of storage at 5 °C, the percentage of membrane-intact spermatozoa was significantly lower in semen diluted with Kenney (by 30.9, 29.3 and 28.9%, respectively) and EquiPlus (by 27.3, 24.8 and 25.0%, respectively) than in semen diluted with INRA 96.

Finally, the differences in the decrease of VIT, MOT and PMOT can be seen in Table 3. For all evaluated parameters, values of R^2 ranged from 0.15 to 0.64. In the cases of MOT and VIT, a linear regression was observed.

Table 3. The dependence of semen motility and viability on time evaluated by regression (velocity of decrease in % per hour, prog M/mL per hour and µm/s per hour)

Parameter	Extender	R^2	First Derivative	Velocity of Decreasing in				Average velocity of Decreasing in			Absolute values of decreasing in		
				2 h	24 h	48 h	72 h	2-24 h	24-48 h	48-72 h	0-24 h	24-48 h	48-72 h
VIT	Kenney	0.27	y = -0.27	-0.27	-0.27	-0.27	-0.28	-0.27	-0.27	-0.27	-6.41	-6.41	-6.41
	EquiPlus	0.21	y = -0.23	-0.23	-0.23	-0.23	-0.23	-0.23	-0.23	-0.23	-5.56	-5.56	-5.56
	INRA96	0.15	y = -0.23	-0.23	-0.23	-0.23	-0.23	-0.23	-0.23	-0.23	-5.41	-5.41	-5.41
MOT	Kenney	0.56	y = - 0.88	-0.88	-0.88	-0.88	-0.88	-0.88	-0.88	-0.88	-21.01	-21.01	-21.01
	EquiPlus	0.48	y = - 0.82	-0.82	-0.82	-0.82	-0.82	-0.82	-0.82	-0.82	-19.66	-19.66	-19.66
	INRA96	0.53	y = - 0.79	-0.79	-0.79	-0.79	-0.79	-0.79	-0.79	-0.79	-19.05	-19.05	-19.05
PMOT	Kenney	0.64	$y = -1{,}35e^{-0.056x}$	-1.35	-0.35	-0.09	-0.74	-0.74	-0.19	-0.05	-17.78	-4.64	-1.21
	EquiPlus	0.52	$y = -1{,}66e^{-0.054x}$	-1.66	-0.45	-0.12	-0.93	-0.93	-0.25	-0.07	-22.33	-6.11	-1.67
	INRA96	0.60	$y = -2{,}15e^{-0.046x}$	-2.15	-0.71	-0.24	-1.30	-1.00	-0.43	-0.14	-31.20	-10.34	-3.43

Abbreviations: MOT, total motility; PMOT, progressive motility; VIT, viability

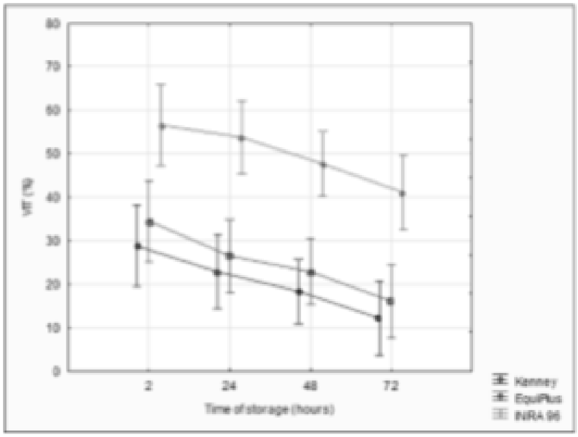


Figure 2. Percentage of vital spermatozoa in semen diluted in three different extenders during storage at 5 °C for 72 hours

In addition, an exponential regression was observed for PMOT, where the velocity of decrease was the fastest in the first 24 hours of storage. In the case of VIT and MOT, the slowest decrease was recorded for the INRA 96 extender (0.23% per hour and 0.79% per hour, respectively). The fastest decrease was observed in the Kenney extender (VIT 0.27% per hour and MOT 0.88% per hour). The opposite trend was observed in PMOT. The fastest decrease was found in the INRA 96 extender. The mean decrease in the first 24 hours in the INRA 96 extender was 1.30% per hour, 0.93% per hour in EquiPlus and 0.74% per hour in Kenney.

DISCUSSION

This study is the first study describing the quality of cooled semen extended with three milk-based extenders in breeding stallions after long sexual rest.

We have demonstrated that the semen characteristics of cold-stored semen were affected by the extender used and the storage time elapsed. Our findings related to ejaculate characteristics of stallions with along sexual rest are consistent with previous studies demonstrating the effect of extender (Webb et al., 2009; LeFrapper et al., 2010) and storage time on stallion semen parameters (Aurich et al., 2007; Janett et al., 2012) when collecting experimental semen samples preceded by repeated semen collection.

In the present study, the differences observed in semen motility among the extenders used agree with the findings of other authors for stallions without sexual rest (Pagl et al., 2006; LeFrapper et al., 2010; Janett et al., 2012; Neuhauser et al., 2018;). The good results for semen motility obtained when INRA 96 extender was used correspond with the special composition of this extender (Pillet et al., 2008). It is advantageous to use native phosphocaseinate as a protective agent in INRA 96 extender (da Silva et al., 2012). Moreover, other substances contained in this extender help to protect spermatozoa obtained after a long sexual rest against the harmful effects of prolonged storage at 5 °C during the 72-hour interval.

The different effects of extenders on spermatozoa motility parameters during prolonged preservation (Table 2) in our study correspond with previously published data (Pagl et al., 2006; Aurich et al., 2007; LeFrapper et al., 2010). The main objective of semen processing in preparation of a cooled insemination dose is to find the best extender that will maintain satisfactory quality during shipping until insemination (Heckenbichler et al., 2011). According to the motility parameters evaluated in our study, the best choice, from the extenders tested, would be INRA 96 when shipping takes about 24 hours in the case of irregularly collected stallions. Our results are in agreement with other studies, although performed with semen from regularly collected stallions, where semen extended with INRA 96 maintained appropriate quality for 24 hours after dilution (LeFrapper et al., 2010; Ortega-Ferrusola et al., 2011; Janett et al., 2012; Bolaños et al., 2014). Nevertheless, in subsequent periods (after 48 and 72 hours of storage at 5 °C), the EquiPlus and INRA 96 equalized. These results are inconsistent with LeFrapper et al. (2010) but the potential weakness of their study is that CASA thresholds are lacking and only two stallions were included. This may be an insufficient number for observation due to individual stallion variability, which was observed in previous studies (Webb et al., 2009; Janett et al., 2012). An insufficient number of observations could have caused a fluctuating effect on the observed parameters of stallion spermatozoa. In this study semen samples were obtained from 11 clinically healthy and fertile stallions and the effect of extender on cold-stored semen characteristics was assessed using analysis of variance with repeated measurements. The benefit of Repeated Measures Design is that of reduced variance and bias of the model's error by controlling for factors that cause variability among subjects. The error term contains only variability within subjects and not the variability among subjects. By controlling for differences among subjects, this type of design can show much more statistical power.

As regards the detailed motility parameter results, it seems that compared to caseinate extenders, Kenney is the less favourable choice. This result is in agreement with previous studies where semen extended with Kenney reached lower quality in vitro compared to EquiPro (Pagl et al., 2006) or INRA 82, which is a previous version of INRA 96 (Ijaz and Ducharme, 1995). These results were supported by an in vivo study (Batellier et al., 2001). The Kenney extender was developed in 1975 (Kenney et al., 1975) and it represents a basic and cheap option for breeders or equine reproductive centres; however, the composition is very simple. Even when the milk in Kenney extender was substituted by 0.6% sodium caseinate, the total and progressive motility, kinematic parameters and percentage of spermatozoa with intact plasma membrane did not differ between Kenney and Kenney + 0.6% caseinate after 24-hour storage (Martins et al., 2016).

Only spermatozoa both motile and with intact plasma membrane can fertilise an oocyte in vivo (Yanagimachi, 1994) and thus, it is of interest to assess changes in these parameters over time during storage in different semen extenders (Love, 2012).

The sperm motility is highly correlated with sperm viability in stallions. Thus, despite individual variation among stallions, 70 - 80% of the sperm in a fresh ejaculate from a normal stallion are expectedly viable (Sieme, 2009). Theoretically, every motile cell is viable but not all viable cells are motile (Nagy et al., 1999). While the integrity of the plasma membrane covering the principal pieces of the sperm can be assessed by analysis of sperm motility, the integrity of the plasma membrane covering the acrosome is focused on the integrity of the outer acrosomal membrane using microscopy, fluorometers and flow cytometry. Fluorescent stains bind to and stain the DNA of sperm possessing defects of post-acrosomal plasma membrane of the sperm head. Non-viable cells can be determined using membrane-impermeable nucleic acid stains, which penetrate through a damaged plasma membrane and result in fluorescent signals of nuclei of dead spermatozoa. Propidium iodide cannot cross intact cell membranes, it gains access to nuclear DNA only when cell membranes are damaged and non-viable. Bound to nucleic DNA, PI fluoresces red in response to excitation (Sieme, 2009). The fluorochromes used here label the DNA in the sperm head and therefore indicate the sperm head membrane status only. Sperm membrane damage usually starts over the flagellar subdomain, therefore there are sperm cells which have intact head membrane (so they are classified as viable) but are immotile due to the damaged tail membrane (Nagy et al., 1999). The reason for the lower values of sperm viability found in this study is not clear (the procedure of preparation of the preparation for viability assessment in the SCA module was followed exactly and was the same for all evaluated samples, as well as the analysis of samples). Based on lower percentage of viable sperm compared to the percentage of motile sperm, poor calibration of SCA vitality module for viability analysis may be a possible explanation, leading to underestimation of percentage of sperm viability in the sample. Dhurvey et al., (2012) confirm that the disadvantages of CASA are related to the extreme need of validation, quality control and standardization of the measures realized. It is vital to use appropriate optics and the best illumination possible to enhance the contrast of the spermatozoa heads, which in turn facilitate the manual selection of thresholds. Nevertheless, these results are significant in the relative comparison of the values in terms of the effect of time and extenders used.

In our study, spermatozoa viability differed significantly among extenders over time. Our results related to plasma membrane status are different compared to previously published data where no significant effect of milk caseinates was found in regularly collected stallions (Pagl et al., 2006; Janett et al., 2012). The situation changed when semen was centrifuged before cooling (Pagl et al., 2006). After 24 and 72 hours of storage, the extender containing defined milk protein preserved the plasma membrane status at a significantly higher level (Pagl et al., 2006). The positive effect of INRA 96 on spermatozoa plasma membrane during storage could be a consequence of NPPC action where this part of the extender decreases efflux of cholesterol and thus preserves the phospholipid:cholesterol ratio important for maintenance of proper fluidity of spermatozoa membrane (Bergeron and Manjunath, 2006). This positive effect is quite important when semen needs to be stored for a long time period in 5 °C in order to retain its fertilizing capacity. This positive effect of NPPC is also supported by high concentration of sugars present in INRA 96 which create physical protection and energy source for the spermatozoa cells during preservation (Pillet et al., 2008). Therefore, semen diluted with INRA 96 extender has the best predispositions for maintaining its viability and for subsequent use for insemination from among the tested extenders in our study.

Prolonged cold storage of ejaculated sperm usually causes decreased motility and kinematic parameters (Rigby et al., 2001; Pagl et al., 2006; Bolaños et al., 2014; Love et al., 2015;). Also, Price et al. (2008) and Webb et al. (2009) suggested that the motility and velocity of equine semen are often impaired progressively during storage; this has been confirmed in extenders such as INRA 96 and equivalent (Webb et al., 2009). These findings agree with our results in which a decrease in the values of all evaluated parameters can be seen. Halo et al.'s (2018) results for stallion spermatozoa motility and progressive motility showed a decreasing trend in each time interval. However, after six hours their results showed a rapid percentage drop-off in semen parameters. In our study, INRA 96 kept the highest PMOT in comparison with the other two extenders during the whole storage period. Batellier et al. (2001) stated that INRA 96 is an efficient extender for preserving fertility potential of spermatozoa for at least 24 h. It also seems to protect spermatozoa cells during long-term storage with more efficiency than traditional milk extenders.

CONCLUSIONS

In conclusion, a defined milk protein extender results in comparable or better semen parameters during cold storage than an extender based on skimmed milk when the semen is from stallions after a long sexual rest. The extenders with defined caseinate components (INRA 96 and EquiPlus) showed a slower decline of PMOT at 48 and 72 hours of storage in comparison with skimmed milk-based extender. Moreover, when diluted with INRA 96, the extender containing NPPC, the quality is prolonged in comparison to other extenders (EquiPlus, Kenney) for at least to 24 hours post- collection and dilution. The best results obtained with extender INRA 96 showed that the semen collected after a long period of sexual abstinence could be used for producing insemination doses of cooled semen during the first 24 hours of storage.

ACKNOWLEDGEMENTS

This work was supported by the Grant Agency of the University of South Bohemia under Grant [project No.028/2019/Z].

REFERENCES

Aurich, C., Seeber, P., Müller-Schlösser, F. (2007) Comparison of different extenders with defined protein composition for storage of stallion spermatozoaat 5 °C. Reproduction of Domestic Animals, 42, 445-448. DOI: https://doi.org/10.1111/j.1439-0531.2006.00828.x

Batellier, F. (1997) Identification, purification et m´ecanismesd'action d'´el´ementscontenus dans le lait agissantsur les spermatozoıdes ´equins. Thesis, Franc¸ois Rabelais University, Tours, France.

Batellier, F., Magistrini, M., Fauquant, J., Palmer, E. (1997) Effect of milk fractions on survival of equine spermatozoa. Theriogenology, 4, 391-410. DOI: https://doi.org/10.1016/s0093-691x(97)00250-1

Batellier, F., Vidament, M., Fauquant, J., Duchamp, G., Arnaud, G., Yvon, J.M., Magistrini, M. (2001) Advances in cooled semen technology. Animal Reproduction Science, 68,181-190. DOI: https://doi.org/10.1016/s0378-4320(01)00155-5

Bergeron, A., Manjunath, P. (2006) New insights towards understanding the mechanisms of sperm protection by egg yolk and milk. Molecular Reproductive Development, 73, 1338-1344. DOI: https://doi.org/10.1002/mrd.20565

Bolaños, J.G., Morán, A.M., da Silva, C.B., Dávila, M.P., Muñoz, P.M., Aparicio, I.M., Pena, F.J. (2014) During cooled storage the extender influences processed autophagy marker light chain 3 (LC3B) of stallion spermatozoa. Animal Reproduction Science, 145, 40-46. DOI: https://doi.org/10.1016/j.anireprosci.2014.01.001

da Silva, M.A.C., Seidel, G.E., Squires, E.L., Graham, J.K., Carnevale, E.M. (2012) Effects of components of semen extenders on the binding of stallion spermatozoa to bovine or equine zonae pellucidae. Reproduction, 143, 577-585.
DOI: https://doi.org/10.1530/REP-11-0099

Dhurvey, M., Gupta, V. K., Nema, S. P., Patidar, A., Modern semen evaluation techniques in domestic animals: a review Shivhare, M., Singh, N., & Shakya, V. (2012). DHR-IJBLS, 3(1), 62-83.

Halo, M., Tirpák, F., Kováčik, A., Lípová, P., Gren, A., Massányi, P. (2018) Biochemical parameters of seminal plasma affect motility trans of stallion spermatozoa. Journal of Microbiology, Biotechnology and Food Sciences, 7, 472-474.
DOI: https://doi.org/10.15414/jmbfs.2018.7.5.516-518

Heckenbichler, S., Deichsel, K., Peters, P., Aurich, C. (2011) Quality and fertility of cooled-shipped stallion semen at the time of insemination. Theriogenology, 75, 849-856.
DOI: https://doi.org/10.1016/j.theriogenology.2010.10.027

Ijaz, A., Ducharme, R. (1995) Effect of various extenders and taurine on survival of stallion sperm cooled to 5 °C. Theriogenology, 44, 1039-1050. DOI: https://doi.org/10.1016/0093-691X(95)00290-O

Janett, F., Sacher, K., Hassig, M., Thun, R. (2012) Quality of raw and of cold-stored semen in icelandic stallions. Journal of Equine Veterinary Science, 32, 590-595.
DOI: https://doi.org/10.1016/j.jevs.2012.02.004

Jasko, D.J., Hathaway, J.A., Schaltenbrand, V.L., Simper, W.D., Squires, E.L. (1992) Effect of seminal plasma and egg yolk on motion characteristics of cooled stallion spermatozoa. Theriogenology, 37, 1241-52. DOI: https://doi.org/10.1016/0093-691X(92)90180-Y

Kenney, R.M., Bergman, R.V., Cooper, W.L., Morse, G.W. (1975) Minimal contamination techniques for breeding mares: techniques and preliminary findings. In: Proceedings of American Association of Equine Practitioners, 327-336.

Lagares, M.A., Martins, H.S., Carvalho, I.A., Junior, C.A., Souza, M.R., Penna, C.F.A.M., Cruz,B.C., Stahlberg, R., Henry, M.R.J.M. (2012) Caseinate protects stallion sperm during semen cooling and freezing. Cryo Letters, 33, 213-218.

LeFrapper, L., Walston, L., Whisnant, C.S. (2010) Comparison of various extenders for storage of cooled stallion spermatozoa for 72 hours. Journal of Equine Veterinary Science, 30, 200-204.
DOI: https://doi.org/10.1016/j.jevs.2010.02.007

Love, C.C. (2012) Measurement of concentration and viability in stallion sperm. Journal of Equine Veterinary Science, 32, 464-466.
DOI: https://doi.org/10.1016/j.jevs.2012.06.015

Love, C.C., Noble, J.K., Standridge, S.A., Bearden, C.T., Blanchard, T.L., Varner, D.D., Cavinder, C.A. (2015) The relationship between sperm quality in cool-shipped semen and embryo recovery rate in horses. Theriogenology, 84, 1587-1593.
DOI: https://doi.org/10.1016/j.theriogenology.2015.08.008

Martins, H.S., Souza, M.R., Penna, C.F.A.M., Da Silva, G.C., Côrtes, S.F., Stahlberg, R., Lagares, M.A. (2016) Milk, caseinate and lactoferrin addition to equine semen cooling extenders. Andrologia, 48, 950-956. DOI: https://doi.org/10.1111/and.12523

Michajilov, N.N. (1950) Sperm dilution in the milk. Czech. Vet. Mag., January 10, abstracted in Journal of the American Veterinary Medicine Association, 117, 337.

Moran, D.M., Jasko, D.J., Squires, E.L., Ammann, R.P. (1992) Determination of temperature and cool rate induced cold shock in stallion spermatozoa. Theriogenology, 38, 999-1012.
DOI: https://doi.org/10.1016/0093-691X(92)90114-7

Nagy, S., Hazas, G., Papp, A. B., Ivancsics, J., Szasz, F., Szasz Jr, F., ... & Foote, R. H. (1999). Evaluation of sperm tail membrane integrity by light microscopy. Theriogenology, 52(7), 1153-1159.
DOI: https://doi.org/10.1016/S0093-691X(99)00207-1

Neuhauser, S., Gösele, P., Handler, J. (2018) The effect of four different commercial semen extenders on the motility of stallion epididymal sperm. Journal of Equine Veterinary Science, 62, 8-12.
DOI: https://doi.org/10.1016/j.jevs.2017.10.015

Ortega-Ferrusola, C., Johannisson, A., Vega, F.J.P., Tapia, J.A., Rodriguez-Martinez, H., Dalin, A.M., Morrell, J.M. (2011) Effect of Different Extenders and Seminal Plasma on the Susceptibility of Equine Spermatozoa to Lipid Peroxidation After Single-Layer Centrifugation, Through Androcoll-E. Journal of Equine Veterinary Science,31, 411-416. DOI: https://doi.org/10.1016/j.jevs.2011.01.010

Pagl, R., Aurich, J.E., Müller-Schlösser, F., Kankofer, M., Aurich, C. (2006) Comparison of an extender containing defined milk protein fractions with a skimmed milk-based extender for storage of equine semen at 5 °C. Theriogenology, 66, 1115-1122.
DOI: https://doi.org/10.1016/j.theriogenology.2006.03.006

Palmer, E. (1984) Factors affecting stallion semen survival and fertility. In: Proceedings of the 10th International Congress on Animal Reproduction, Urbana – Champaign II 3:377.

Pellicer-Rubio, M.T., Combarnous, Y. (1998) Deterioration of goat spermatozoa in skimmed milk-based extenders as a result of oleic acid released by the bulbourethral lipase BUSgp60. Journal of Reproduction and Fertility, 112, 95-105.
DOI: https://doi.org/10.1530/jrf.0.1120095

Pillet, E., Batellier, F., Duchamp, G., Furstoss, V., Le Vern, Y., Kerboeuf, D., Magistrini, M. (2008) High fertility rates with stallion sperm cryopreserved in INRA96® based extender are not predicted by in vitro parameters. Animal Reproduction Science, 3, 339-340.
DOI: https://doi.org/10.1016/j.anireprosci.2008.05.116

Price, S., Aurich, J., Davies-Morel, M., Aurich, C. (2008) Effects of oxygen exposure and gentamicin on stallion semen stored at 5 and 15 degrees C. Reproduction of Domestic Animals, 43, 261-6.
DOI: https://doi.org/10.1111/j.1439-0531.2007.00888.x

Rigby, S.L., Brinsko, S.P., Cochran, M., Blanchard, T.L., Love, C.C., Varner, D.D. (2001) Advances in cooled semen technologies: seminal plasma and semen extenders. Animal Reproduction Science, 68, 171–181.
DOI: https://doi.org/10.1016/S0378-4320(01)0015-3

Samper, J.C. (2008) Equine Breeding Management and Artificial Insemination E-Book. Elsevier Health Sciences. ISBN. 978-1-4160-5234-0

Thompson, J.A., Love, C.C., Stich, K.L., Brinsko, S.P., Blanchard, T.L., Varner, D.D. (2004) A Bayesian approach to prediction of stallion daily sperm output. Theriogenology, 62, 1607-1617.
DOI: https://doi.org/10.1016/j.theriogenology.2004.03.015

Webb, G.W., Dean, M.M., Humes, R.A., Heywood, J.S. (2009) A comparison of the ability of three commercially available diluents to maintain the motility of cold stored stallion semen. Journal of Equine Veterinary Science,29, 229-32.
DOI: https://doi.org/10.1016/j.jevs.2009.03.003

Yanagimachi, R. (1994) Mammalian fertilization. In: Knobil E, Neill JD, editors. The physiology of reproduction, 2nd ed., vol. 1. New York: Raven Press; 189-317.

6 Závěr

Na základě výsledků popsaných v této disertační práci lze závěry shrnout do následujících bodů.

- Hmotnost teplokrevných koní lze s využitím lineárních vztahů tělesného měření odhadnout, a to vzorcem upraveným pro 4 věkové skupiny. Tím je zohledněna různá intenzita růstu v průběhu dospívání teplokrevných koní.

- S využitím Brodyho funkce byl sestaven růstový model pro teplokrevné hřebce ve věku od 7 do 36 měsíců. Tato funkce aktualizuje současnou křivku s pásmy růstu, která se využívá při třídění teplokrevných hřebců. Jako nejvhodnější pro využití v chovatelské praxi se ukázalo sestavení percentilových pásem v porovnání se Z-score grafem. Pásmo standardu bylo určeno v intervalu mezi 25. a 75. percentilem.

- Prokázala se souvislost mezi morfometrickými a kinematickými parametry u spermatu teplokrevných hřebců. Mezi parametry plochy a rychlosti (VSL, VAP a koeficient kolísání WOB) byly průkazné pozitivní korelace. Prodloužení hlavičky spermií pozitivně korelovalo s MOT. Více protáhlé spermie vykazovaly nižší frekvenci průsečíků křivočaré dráhy s průměrnou dráhou.

- Byl prokázán významný vliv doby skladování spermatu (p-hodnota $< 0,001$) na všechny hodnocené parametry. Vliv ředidla byl signifikantní (p- hodnota $< 0,05$) v případě VIT, MOT, PMOT, VCL, VSL a VAP. Průměrný ochranný účinek ředidla INRA 96 byl u všech sledovaných parametrů významně lepší než účinek ředidla Kenney (p-hodnota $< 0,01$) a EquiPlus (p-hodnota $< 0,05$) u hodnot PMOT, VCL a VAP, a to po celou dobu skladování.

7 Seznam literatury

ABBEY, A., RANDLE, H. 2016. Equitation pedagogic practice: use of a ridden horse ethogram to effect change. *Journal of Veterinary Behavior*.: Clin. Appl. Res. 15, 80.

AMANN, R. P., WABERSKI, D. 2014. Computer-assisted sperm analysis (CASA): Capabilities and potential developments. *Theriogenology* 81(1), 5-17.

BACK, W., CLAYTON, H. M. 2013. Equine Locomotion, second edition. Saunders Ltd., U.S.

BARNARD, S., MATTHEWS, L., MESSORI, S., PODALIRI-CUPLIANI, M., FERRI, N., 2016. Laterality as an indicator of emotional stress in ewes and lambs during a separation test. *Animal Cognition*, 19, 207–214.

BATT, L., BATT, M., MCGREEVY, P. 2007. Two tests for motor laterality in dogs. *Journal of Veterinari Behavioral*. 2(2), 47-51.

BRODER, E. D., ANGELONI, L. M. 2014. Predator-inducedphenotypic plasticity of laterality. *Animal Behaviour*, 98, 125–130.

BROWN, J. H., PILLINER, S., DAVIES, Z. 2003. Horse and stable management. 4th ed. Oxford: Blackwell Publishing.

BUDÍKOVÁ, M., KRÁLOVÁ, M., MAROŠ, B. 2010. Průvodce základními statistickými metodami. *Praha: Grada*. Expert (Grada). ISBN 978-80-247-3243-5.

BURGON, H. L., 2011. 'Queen of the World': experiencs of 'at-risk' young people participating in equine-assisted learning/therapy. *Journal of Social Work Practice*. 25, 165–183.

CARROLL, C. L., HUNGTINGTON, P. J. 1988. Body condition scoring and weight estimation of horses. *Equine of Veterinary Journal*. 20, 41–45.

CASKOVÁ, V. 2021. Biomechanika pohybu koně a jeho přenos na jezdce

CATALANO, D. N., COLEMAN, R. J., HATHAWAY, M. R., McCUE, M. E., RENDAHL, A. K., HANSEN, A. M., MARTINSON, K. L. 2015. Estimation of actual and ideal bodyweight for adult draft and warmblood horse breeds using morphometric measurements. *Journal of Equine Veterinary Science*. 35, 423–4.

COLENBRANDER, B., GADELLA, B. M., STOUT, T. A. E. 2003. The predictive value of semen analysis in the evaluation of stallion fertility. *Reproduction of Domestic Animals.* 38, 305–11.

CONTRI, A., GLORIA, A., ROBBE, D., VALORZ, C., WEGHER, L., CARLUCCIO, A. 2013. Kinematic study on the effect of pH on bull sperm function. *Animal Reproduction Science*. 136, 252–9.

CONTRI, A., VALORZ, C., FAUSTINI, M., WEGHER, L., CARLUCCIO, A. 2010. Effect of semen preparation on casa motility results in cryopreserved bull spermatozoa. *Theriogenology*. 74(3), 424-435.

COSTA, M. D., MENDES, L. J, MARUCH, S., RAMIREZ, P. A., MENESES, A. C. A., MARTINS NETO, T., RUAS, J. R. M., CHAMONE, J. M. A. 2016. Effect of genetic composition in conformation traits in horse. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia*. 68, 1629–37.

DALLA COSTA, E., MINERO, M., LEBELT, D., STUCKE, D., CANALI, E., LEACH, M. C. 2014. Development of the horse grimace scale (HGS) as a pain assessment tool in horses undergoing routine castration. PLoS One 9, e92281.

DOBEŠ, J. 1977. Jezdectví a dostihový sport. Vyd. 1. Praha: Státní zemědělské nakladatelství, 338 s.

DUŠEK, J. 2007. Chov koní. Vyd. 2., přeprac. *Praha: Brázda*. ISBN 80-209-0352-6.

DUŠEK, J. 1998. Hodnocení tělesné stavby koní z hlediska její souměrnosti. *Jezdectví*, 46, 11, 22-23.

FITZHUGH, H. A., 1976. Analysis of Growth Curves and Strategies for Altering Their Shape. Journal of Animal Science. 42, 1036–1051.

FRADINHO, M. J., BESSA, R. J. B., FERREIRA-DIAS, G., CALDEIRA, R. M., 2016. Growth and development of the Lusitano horse managed on grazing systems. *Livestock Science*. 186

FREITAG, G. P., DE LIMA, L. G. F., JACOMINI, J. A., KOZICKI, L. E., RIBEIRO, L. B. 2021. An Accurate Image Analysis Method for Estimating Body Measurements in Horses. *Journal of Equine Veterinary Science.* 101

GANDI, E. A., BONDI, A., HOGG, R., PIGOTT, T. M. C., 2014. A preliminary investigation of the use of inertial sensing technology for the measurement of hip rotation asymmetry in horse riders. *Sports Technology*. 7, 79–88.

GEHRKE, E. K., BALDWIN, A., SCHILTZ, P. M., 2011. Heart Rate Variability in horses engaged in equine—assisted activities. *Journal of Equine Veterinary Science*. 31, 78–84.

GIBBS, P. G., HOUSEHOLDER, D. D. 2003. Estimating horse body weight with a simple formula. Texas AM University, College Station: Department of animal science, *Equine science program*.

HAJIČ *et al*. 1995. Obecná zootechnika, JU ZF České Budějovice, 165 s.

HALL, L. 1971. W. Wright’s veterinary anesthesia and analgesia. 7th ed. London: Bailliere Tindall.

HENDL, J. 2015. Přehled statistických metod: analýza a metaanalýza dat. Páté, rozšířené vydání. *Praha: Portál*. ISBN 978-80-262-0981-2.

HIDALGO, M., RODRIGUEZ, I., DORADO, J., SANZ, J., SOLER, C. 2005. Effect of sample size and staining methods on stallion sperm morphometry by the Sperm Class Analyzer. *Veterinary Medicine Czech republic*. 50(1), 24-32.

HIDALGO, M., RODRIGUEZ, I., DORADO, J., SOLER, C. 2008. Morphometric classification of Spanish thoroughbred stallion sperm heads. *Animal Reproduction Science*. 103(3-4), 374–378.

HINCHCLIFF, K. W., KANEPS, A. J., GEOR, R. J., 2008. Equine Exercise Physiology: The Science of Exercise in the Athletic Horse. *Saunders Elsevier*, London.

HINTZ, H. F., HINTZ, R. L., VAN VLECK, L. D., 1979. Growth Rate of Thouroughbreds. Effects of Age of Dam, Year and Month of Birth, and Sex of Foal. *Journal of Animal Science*. 48.

HOFFMANN, G., BENTKE, A., ROSE-MEIERHÖFER, S., AMMON, C., MAZETTI P., HARDARSON, G. H. 2013. Estimation of the body weight of Icelandic horses. *Journal of Equine Veterinary Science*. 33, 893–5.

HOLMES, M., JEFFCOTT, L. 2010. Equitation science, rider effects, saddle and back problems in the horses: can technology provide the answer? (Editorial). *Veterinary Journal*. 184, 5–6.

HUG, F., TURPIN, N. A., GUÉVEL, A., DOREL, S., 2010. Is interindividual variability of EMG patterns in trained cyclists related to different muscle synergies? *Journal of Applied Physiology*. 108, 1727–1736.

JAKUBEC, V., GOLDA, J., ŘÍHA, J. 1998. Šlechtění masných plemen skotu, *Rapotín*, 32-36s.

KAUFFMANN, S., CLINE, CH. 2020. *Kopyta: kompletní průvodce*. Přeložil Dominika ŠVEHLOVÁ. Jihlava: Arcaro, ISBN 978-80-907983-1-1.

LAC, V., 2016. Horsing around: gestalt equine pyschotherapy as humanstic play therapy. *Journal of Human Psychology*. 56, 194–209.

LOCHNER F. K., MILNE D. W., MILLS E. J. 1980. In vivo and in vitro measurement of tendon strain in the horse. *American Journal of Veterinary Research*.41, 1929-37.

ŁUSZCZYŃSKI, J., MICHALAK, J., PIESZKA, M. 2019. Assessment of methods for determining body weight based on biometric dimensions in Hucul horses. *Roczniki Naukowe Polskiego Towarzystwa Zootechnicznego*. 15(4), 9-20.

MARCENAC, L. N., AUBLET, H., D'AUTHEVILLE, P. 1964. Encyclopédie du cheval. 1st ed. *Paris: Maloine*.

MARŠÁLEK, M. 2008. Chov koní – popis posuzování, šlechtění. *Jihočeská univerzita Zemědělská fakulta*, České Budějovice, 109 s.

MARTIN, P., BATESON, P. 2007. Measuring Behaviour: An Introductory Guide, second ed. Cambridge University Press, Cambridge.

MARTISON, K. L., COLEMAN, R. C., RENDAHL, A. K., FANG, Z., MCCUE, M. E. 2014. Estimation of body weight and development of a body weight score for adult equids using morphometric measurements. *Journal of Animal Science*. 92, 2230–2238.

McDONNELL, S. M. 2002. The Equid Ethogram: A Practical Field Guide to Horse Behavior. *Eclipse Press*, Kentucky. 384 s.

McLEAN, A. N., McGREEVY, P. D. 2010. Ethical equitation: capping the price horses pay for human glory. *Journal of Veterinary Behavior*. Clin. Appl. Res. 5, 203–209.

MELOUN, M., MILITKÝ, J. 1993. Statistické zpracování experimentálních dat.

MILNER, J., HEWIT, D. 1969. Weight of horses: improved estimates based on girth and length. *Canadian Veterinary Journal*. 10, 314–316.

MOYER, W., ANDERSON, J. P. 1975. Lameness caused by improper shoeing. *Journal of American Veterinary Medicin Association*. 166, 47-52.

MUTOH, T., MUTOH, T., TAKADA, M., DOURMUA, M., IHARA, M., TAKI, Y., TSUBONE, H., IHARA, M. 2016. Application of a tri-axial accelerometry-based portable motion recorder for the quantitative assessment of hippotherapy in children and adolescents with cerebral palsy. *Journal of Physiology and Theriogenology Science*. 10, 2970–2974.

MUTOH, T., MUTOH, T., TAKADA, M., DOUMURA, M., IHARA, M., TAKI, Y., TSUBONE, H., IHARA, M. 2016. Application of a tri-axial accelerometry-based portable motion recorder for the quantitative assessment of hippotherapy in children and adolescents with cerebral palsy. *Journal of Physical Therapy Science*. 2016. 28(10), 2970-2974

NATH, S. R., MOORE, F. D. 1992. 3rd. Growth analysis by the first, second, and third derivatives of the Richards function. *Growth Dev Aging*. 56(4), 237-47.

NEDER, G. A., PÉREZ, A., PERRONE, G. 2009. Assessment of body weight of the Criollo horse using morphometric measurements: confirmation of published equations for other breeds and development of a new formula. Redvet. 10.

NEUWINGER, J., KNUTH, U. A., NIESCHLAG, E. 1990. Evaluation of the HamiltonThorn 2030 motility analyser for routine semen analysis in an infertility clinic. Internationl Journal of Andrology.13, 100–9.

NORTH, S., HALL, C., ROSHIER, A. L., MANCINI, C., 2015. HABIT: horse automated behaviour identification tool: a position paper. In: ACI@BHCI (Animal Compueter Interaction Workshop), British HCE, 13th–17th July 2015, University of Lincoln, Lincoln.

NOVOTNÁ, A., SVITÁKOVÁ, A., PŘIBYL, L., VOSTRÝ, L. 2018. *Spolehlivost předpovědi plemenných hodnot pro skokovou výkonnost u teplokrevných plemen koní*. Praha: Výzkumný ústav živočišné výroby. ISBN isbn:978-80-7403-201-1.

PADALINO, B., RADIAL, S. L., HALL, E., KNIGHT, P., CELI, P., JEFFCOTT, L., MUSCATELLO, G. 2016. Survey of horse transportation in Australia: issues and practices. *Australian Veterinary Journal*. 94, 349–357.

PARÉS I CASANOVA, P.-M. 2011. A Nonlinear Model for Estimating Hoof Surface Area in Unshod Meat-type Horses. *Journal of Equine Veterinary Science*. 31(7), 379-382.

PIERARD, M., HALL, C., KÖNIG VON BORSTEL, U., AVERIS, A., HAWSON, L., MCLEAN, A., NEVISON, C., VISSER, K., MCGREEVY, P. 2015. Evolving protocols for research in equitation science. *Journal of Veterinary Behavior*. Clin. Appl. Res. 10, 255–266.

PIMENTEL, A. M. H., RODRIGUES, W. B., MARTINS, C. F., MONTANEZ, N. R., BOLIGON, A. A., SOUZA, J. R. M. 2017. Gender on the growth of Criollo foals from birth to three years of age. *Ciˆencia Rural* 47.

POOL, R. R., MEAGHER, D. M. 1990. Pathologic findings and pathogenesis of racetrack injuries. *Veterinary Clinics of North America Equine Practice*. 6, 1-30.

PŘIBYL, J., PŘIBYLOVÁ, J., VOSTRÝ, L. 2008. Šlechtění v globalizujícím světě. In: Sborník referátů ze semináře: Aktuální problémy chovu a šlechtění koní v ČR. 21.-22.11. *MZLU v Brně,* 12-15 s.

PŘIBYL, J. 1997. Šlechtění skotu a jeho vliv na jednotlivé chovy. Institut výchovy a vzdělávání Mze ČR, 35 s.

QUARANTA, A., SINISCALCHI, M., FRATE, A., VALLORTIGARA, G. 2004. Paw preference in dogs: relations between lateralised behaviour and immunity. *Behavioural Brain Research*. 153. 521–525.

QUINTERO-MORENO, A., MIRÓ, J., RIGAU, A. T., RODRIGUEZ-GIL, J. E. 2003. Identification of sperm subpopulations with specific motility characteristics in stallion ejaculates. *Theriogenology* 59(9), 1973-1990.

RANDLE, H., BUTTON, L. 2008. Fulfilling the educational aims of the international society for equitation science (ISES). In: Murphy, J., Hennessey, K., Wall, P., Hanley, P. (Eds.), Proceedings of the Fourth International Society for Equitation Science Conference. 1st–4th August 2008, RDS, Dublin, 103 s.

RANDLE, H., CHALLACOMBE, J., ABBEY, A. 2015. The influence of handler experience on the behaviour of horses in an educational environment: heart Rate and Visual Analogue Scores. In: Heleski, C.R., Merkies, K. (Eds.), Proceedings of the Eleventh International Society for Equitation Science Conference. 5th–8th August 2015, University of British Columbia, Vancouver, B.C., Canada, 52 s.

RANDLE, H., STEENBERGEN, M., ROBERTS, K., HEMMINGS, A. 2017. The use of the technology in equitation science: A panacea or abductive science?. *Applied Animal Behaviour Science*. 190, 57-73.

RIEMERSMA, D. J., VAN DEN BOGERT, A. J., JANSEN, M. O., et al. 1996. Influence of shoeing on ground reaction forces and tendon strains in the forelimbs of ponies. *Equine Veterinary Journal*. 28, 26-32.

RICHARDS, F. J., 1959. A Flexible Growth Function for Empirical Use. *Journal of Experimantal Botany*. 10, 290–301.

RODRIGUEZ, I., SANZ, J., PEREZ, C., FELIPE, M., DORADO, J., HIDALGO, M. 2001. Fertilidad in vivo del esperma congelado-descongelado. I Jornadas de Investigacion en Veterinaria (Cordoba, Spain). 317–321.

SENDEL T. 2010. Estimating body weight for horses. *Factsheet*. 10, 1–4.

SHAKER, M. M., WAEL, A. K., NABEEH, M. A. 1999. A new equation for estimation of horses body weight. *Pakistan Veterinary Journal*. 19, 216–7.

SCHCT.CZ. Svaz chovatelů českého teplokrevníka: Šlechtitelský řád ČT. http://www.schct.cz/, [cit. 2022-05-10].

SCHNEIDER, L. A., DELFABBRO, P. H., BURNS, N .R. 2013. Temperament and lateralization in the domestic dog (Canis familiaris). *Journal of Veterinary Behavior*. 8, 124-134.

SILVA, N. A. M., LANA, A. M. Q., SILVA, F., SILVEIRA, F. G., BERGMANN, J. A. G., SILVA, M. A., TORAL, F. L. B. 2011. Seleç˜ao e classificaç˜ao multivariada de modelos de crescimento n˜ao lineares para bovinos Nelore. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia* 63, 364–371.

SOBOTKOVÁ, E., JISKROVÁ, I., SOMERLÍKOVÁ, K. 2006. Analýza exteriéru starokladrubského koně chovaného v národním hřebčíně Kladruby nad Labem. *Acta Univesitatis Agriculturae et Silviculturae Mendelianae Brunensis*. 54(5), 117-128.

SOLER, C., DE MONSERRAT, J. J., GUTIÉRREZ, R., NUNEZ, J., NUNEZ, M., SANCHO, M., COOPER, T. G. 2003. Use of the Sperm-Class Analyser® ford objective assessment of human sperm morphology. *International Journal of Andrology*. 26(5), 262-270.

SOROKO, M., HOWELL, K., 2016. Infrared Thermography: Current Applications in Equine Medicine. *Journal of Equine Veterinary Science*. 27, 532-541.

SOVÁK, M. *Výchova leváků v rodině* [online]. 7. vyd. Praha: SPN, 1985 [cit. 2022-04-10]. Knižnice speciální pedagogiky. Dostupné z: http://krameriusndk.nkp.cz/search/handle/uuid:fac24840-c0a9-11e4-9541-005056827e51

SOVRANO, V. A., ANDREW, R. J., 2006. Eye use duringviewing a reflection: Behaviourallateralization in zebrafishlarvae. *Behavioural Brain Research*, 167, 226–231.

STANIAR, W., KRONFELD, D., CUBITT, T., BYRD, B., HARRIS, P., 2005. Growth in linear measures of Thoroughbreds: Maturation rates and points of maturity. *Proceedings of Equine Science Society*. Tucson, Arizona.

SVITÁKOVÁ, A., NOVOTNÁ, A., PŘIBYL, J. 2018. *Spolehlivost předpovědi plemenných hodnot pro znaky zevnějšku hodnocených lineárním popisem u teplokrevných plemen koní*. Praha: Výzkumný ústav živočišné výroby. ISBN isbn978-80-7403-200-4.

TAVERNIER, A. 1992. Is the performance at 4 years in jumping informative for later results? 43rd Annual Meeting of the European Association for Animat Production, 13-17.8.1992, Madrid, Spain.

URBAN, T. 2011. Koncept heritability. Virtuální svět genetiky 1 [online]. Brno [cit. 2022-04- 26]. Dostupné z: http://user.mendelu.cz/urban/vsg1/populace/pop_heritab.html

VALETTE, J. P., ROBERT, C., DENOIX, J. M., 2008. Use of linear and non-linear functions to describe the growth of young sport- and race-horses born in Normandy. *Animal* 2.

VANTMAN, D., KOUKOULIS, G., DENNISON, L., ZINAMAN, M., SHERINS, R. J. 1988. Computer-assisted semen analysis: evaluation of method and assessment of the influence of sperm concentration on linear velocity determination. *Fertil Steril*. 49, 510–5.

VESELÁ, Z. 2003. Analýza růstu u genové rezervy "starokladrubský kůň", diplomová práce, Česká zemědělská univerzita v Praze, Fakulta agronomická Katedra genetiky a obecné zootechniky, 51 s.

VĚŽNÍK Z, et al. 2004. Repetitorium spermatologie a andrologie a metodiky spermatoanalýzy. Výzkumný Ústav Veterinárního Lékařství V Brně.

VINČÁLEK, J. Podkovářství. 2015. Vyd. 2. *Zlín: Tigris*. ISBN 978-80-7490-052-5.

VON BORELL, E., LANGBEIN, J., DESPRÉS, G., HANSEN, S., LETERRIER, C., MARCHANT-FORDE, J., MARCHANT-FORDE, R., MINERO, M., MOHR, E., PRUNIER, A., VALANCE, D., VEISSIER, I., 2007. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals—a review. *Physioloy Behavior*. 92, 293–316.

VOSTRÝ L. *a kol*. 2011. Analysis of Czech cold-blooded horses: genetic parameters, breeding value and the influence of inbreeding depression on linear description of conformation and type characters. 2011. *Czech Journal of Animal Science*. 56, 217-230.

WAGNER, E. L., TYLER, P. J. 2011. A comparison of weight estimation methods in adult horses. *Journal of Equine Veterinary Science*. 31, 706–10.

WARAN, N., RANDLE, H., 2017. What we can measure, we can manage: The importance of developing robust welfare indicators for use in Equitation. *Journal of Veterinary Behavioral*. Clin. Appl. and Res. 10, 81-89.

WELLS, D. L. 2003. Lateralised behaviour in the domestic dog, Canis familiaris. *Behavioural Processes*. 61. 27–35.

WILLIAMS, J. M., RANDLE, H., 2017. Is the expression of stereotypic behavior a performance limiting factor in animals? *Journal of Veterinary Behavioral*. Clin. App. and Res. Special issue Captive animal responses: contextualising stereotypic, abnormal and compulsive behaviours.