Breeding soundness evaluation and reproductive management in Baldras sport horses

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Abstract

Morphometric characterization, breeding soundness evaluation and reproductive management of 33 sport horses (14 mares and 19 stallions) belonging to Palace Administration was carried out for 6 months from January to June/2014. Animals were also subjected to condition scoring, and detailed evaluation through ultrasonography, semen analysis and fertility test using AI. The overall age, BCS and body weight were 15±5.7 years, 5.2±1.3 and 327.7±33.3 Kg, respectively. Stallions were significantly older than the mares while mares were heavier with relatively poorer BCS. Body weight was significantly correlated with girth both in mares ($p<0.05$; $r=0.97$) and stallions ($p<0.05$; $r=0.68$). Comparison of body morphometry among mares, studs and neutered stallions showed a significant difference ($p<0.05$) in loin length, front and hind leg height, and head length. BCS and scrotal circumference of the breeding stallions (n=12) were highly correlated ($P<0.05$; $r=0.60$). The length of estrous cycle and estrus in the breeding mares were 16.1±3.5 days and 7-12 days, respectively. There was a significant difference ($P<0.05$) in the total number of follicles among the mares. The mean (±SD) diameters of the first-largest and pre-ovulatory follicles were 24.3±9.23 mm and 29.4±9.19 mm, respectively. There was a significant difference ($P<0.05$) in the diameter of the preovulatory follicles among the breeding mares. Fresh semen was generally characterized by poor motility (<50%), small live percent (54.3%), high gel volume (>40%) and very poor freeze ability (<10% in total motility of frozen thawed semen) however, fertility of fresh semen was 50%. Poor performances are signs of inbreeding that also render the studs unfit for breeding. A proper reproductive management is required to mitigate further loss of performance and influence of inbreeding depression.

Keywords: breeding soundness, morphometry, semen analysis, sporting horses, ultrasonography

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Introduction

In equestrian activities horses are trained and ridden for practical working purposes such as in police work or for controlling herd animals on a ranch, competitive sports including dressage, endurance riding, reining, show jumping, tent pegging, vaulting, polo, horse racing, driving, and rodeo. Horses are also driven in harness racing, at horse shows and in other types of exhibition, historical reenactment or ceremony, often pulling carriages. In some parts of the world, they are still used for practical purposes such as farming. In Ethiopia the donkey, horse and mule populations are estimated at 6.2, 2.0 and 0.38 million, respectively (CSA, 2010) however, equines have a prominent position only in the agricultural and transport sectors of the rural and several urban setups. The horses administered under the horse sport and recreation directorate of the palace administration were primarily kept for few types of horse sporting; show jumping and polo. In this regard they represent the only horse population in Ethiopia primarily bred for sporting. However, the viability of this particular horse population is at stake unless some measures are taken. Anecdotal information suggests the presence of uncontrolled crossing with the indigenous breeds. Through times, smaller horses and those with different kinds of malformation had been born.

The other major problem observed in this population is aging. Most of the stocks are old, some of which were on sport activities for longer time and others were used for renting purposes. Currently a good proportion of the mares remains sub-fertile and rarely produces live foals. An instance of breeding during the recent past (2013), only 36% (4 out 11) mares became pregnant after 3-7 services. Further, there are only few breeding stallions that could be used as breeding stud in the future. Aging, poor conception, reproductive disorders and high prevalence of sub-fertility have also been prevalent A number of factors play a role in poor reproductive performance of horses in general and sub-fertility in the mares in particular such as aging, stress, inbreeding, reproductive disorders, nutritional problems, and hormonal imbalance.

In most of the farm animal species, sire selection is made on the basis of their fertility (Loomis and Graham, 2008). This aspect is not usually considered for stallions because top sires are always chosen for their excellence in sportive performances or for their genetic background. For this reason, fertility and freezability of stallion sperm presents a high variability, ever since the development of efficient freezing protocols (Loomis and Graham, 2008).

Assisted reproductive techniques such as artificial insemination (AI) have been used to improve genetic diversity and performance in equines. Employment of breeding soundness evaluation is critical in the selection of the best animal. Semen evaluation in equine includes gross evaluation of raw semen for color and concentration (sperm/ml), volume, motility and morphological abnormalities (Macpherson, 2001). Measurement of scrotal circumference, testicular size, daily sperm production per gram (DSP/g) of testis etc, should be determined as part of breeding soundness examination. In modern equine production, typically mares are also examined for breeding soundness before purchase or breeding or when a mare is sub fertile. Such evaluation includes examination of the external genitalia, per rectum ultrasonography of the internal genitalia and ovaries, vaginoscopy, endometrial swab for aerobic culture, and biopsy of the endometrium for histological evaluation (Squires *et al.*, 1988; Macpherson, 2001).

Although the horse sporting activity is still undergoing with the Palace Administration horses, there has been no attempt to curb the prevailing problems and improve the performance. So the objectives of this study are: to perform a morphometric characterization of the horse population; carryout a breeding soundness evaluation of the mares and stallions and evaluate quality of semen for AI, cryobanking and fertility.

Materials and Methods

This study was conducted within Addis Ababa (Balderas) located at about 12km from the Palace Administration at 9^0N latitude and 38^0E longitude 2400 meters above sea level. Addis Ababa has an annual rainfall of 1800 ml which falls during the long rainy season extending from June to September and short rain fail extending from March to May. The mean annual maximum and minimum temperature ranges are 23 ^{0}C and 10.7 ^{0}C respectively (AACA, 2004).

The study involved a total 33 horses (14 mares, 12 non castrated stallions and 7 castrated stallions) aged between 3 and 23 years (as per their record) and all belonging to the Horse Sport and Recreation Service Directorate of the Palace Administration. The horse breeds of Palace Administration were imported between 1921 and 1957 from England, Germany, Middle East and few Slovakian counters as a gift to Emperor Hailesilasie. Other local indigenous horses were also given to the King from different provincial governors around 1925 and some of these horses were cross-bred with the exotic horses. Further, this particular horse population has been interbred for quite a long period of time.

Information from retrospective record shows a relatively better phenotypic and genotypic performance of the previous animals (Balderas horses, 1927-1987; unpublished document) compared to the current population.
All the horses were housed in a modern stall, fed on hay and concentrate supplement. Water was given *ad libtum*. They were regularly dewormed, and received vaccination against rabies and African horse sickness. The horses were also screened annually for brucellosis and tuberculosis. Except for the country of origin, the pedigree record of these animals has been discontinued over 30 years ago and hence it was not possible to assign them to a specific breed of horse.

Morphometric characterization

Body morphometry of both the mares and stallions was recorded using ordinary tape and equine weight-tape. Head-tail length, height at the wither, loin length, front and hind leg height, tail length, neck length and circumference, and head length were measured as described in Gomez *et al*, (2009) and Takaendengana, *et al.*, (2011). Body weight was computed using equine weight- tape.

Breeding soundness evaluation (BSE) of mares and stallions

BSE was performed only for breeding mares and stallions (14 mares and 7 breeding stallions). BCS was determined on 1 - 9 scale (1= poor; 9=extremely fat) according to Henneke et al, (1984). Testicular length and width, and scrotal circumference were measured in stallions using a flexible tape and Vernier caliper. Further BSE were determined through semen analysis for the stallions and ultrasonography for the mares.

Ultrasonographic evaluation of reproductive organs of mares

Transrectal examination was preformed to evaluate uterine and ovarian cycle (Squires *et al.*, 1988). Serial scanning of the mare's uterus, and ovarian structures were made for two consecutive cycles as described in Ginther, (1995) using Aloka B-mode, real time ultrasound scanner with a 5 MHz linear array transducer (Aloka 500, S. Korea). Uterine cross sectional diameter, ovarian follicles and corpus luteum were measured using the internal electronic caliper of the ultrasound system. The presence of endometrial fold was assessed during scanning to determine eminent ovulation. Ovarian activity was determined from distribution of the total number of follicles, and diameter of the three largest follicles in each ovary (Ginther, 1995). Data were recorded on a predesigned format and sonograms were taken by videographic printer. Mares suspected to have uterine pathologies after ultrasonography were subjected to endometrial swabbing for further studies using a standard bacteriology.

Semen analysis and cryobanking

A total of 7 breeding stallions were selected based on their history and future plan as a breeder for the farm for this evaluation. Semen was collected using Colorado model equine artificial vagina (Agatech, USA). The internal temperature of the EAV was adjusted to 47- 49^{0}C and a non-spermicidal jelly (KY jelly, France) was used for lubrication. Each stallion was teased against an estrous mare for few minutes until full erection was attained and the penis was washed off using warm water. Two to three false mounting were allowed before the penis was directed to the AV. Libido was scored during teasing on a 1 - 4 scale (1=poor; 4=excellent). Immediately after collection, the semen was transported to the laboratory for gross and microscopic evaluation according to method described in Macpherson, (2001). The pHs, color, and volume (ml) were also recorded immediately. An aliquot of semen was removed to evaluate total motility (%), progressive motility (%), concentration and percent live/dead. Sperm concentration was measured using hemocytometer and was expressed as number of cells per ml using the formula given by Bearden *et al.*, (2004). Percent live was determined from 200 sperm cells counted after a smear of a mixture of 5µl aliquot of semen, equal volume of 1% Eosin and 2.6% of sodium citrate. The smear was dried very fast to avoid false positive results

$$\text{No of sperm/ml} = \text{no of sperm in } 0.1 \text{ mm}^3 \times 10 \times \text{dilution rate} \times 100$$

The remaining semen was then centrifuged at 3000 rpm for 10 minutes to harvest the sperm pellet after which it was re-suspended using INRA-96 equine semen extender (IMV technologies, France). It was then transported in ice box (at +6 to +9^{0}C) to CVMA Animal Reproduction Laboratory. The semen was then equilibrated for 2.5 – 3 hours, filled into a 0.5 ml straw, smoked 4 cm above a liquid nitrogen vapor in a Styrofoam box for 9 minutes after which the straws were plunged immediately into the liquid nitrogen for permanent cryopreservation. After 24 hrs, cryopreserved semen was thawed at 37^{0}C in a water bath for 1 minute and evaluated for motility (total and progressive) to determine freezability of the semen.

Artificial insemination and fertility test

Artificial insemination ware conducted on eight selected mares (based on their record of relatively good breeding history) using extended semen (INRA-96, IMV, France) both as fresh and chilled (+4°C) to determine the fertility. The mares were first induced into estrus by an intramuscular injection of 1 ml closprostenol-sodium (Estrumate, Vet Phrama, Germany). Fresh semen was centrifuged at 3000 rpm for 10 minutes and re-suspended volume by volume in INRA-96 equine semen extender. The concentration was later adjusted to 200 million live sperm per insemination. Each mare was scanned ultrasonically to confirm the presence of a preovulatory follicle before insemination. Fresh semen was deposited in an intrauterine location twice (before and after ovulation) to improve the chance of fertilization. The chilled semen was thawed to +37°C for 1 minute and inseminated the same as the fresh semen. Pregnancy was tested ultrasonically three weeks post AI.

Data management and analysis

All the data collected were entered into a Microsoft excel sheet. The data was later on analyzed using SPSS for windows (Version 15, USA). Descriptive statistics (means, SD and proportions) were used to describe morphomteric variables, follicular data and semen parameters. Pearson's correlation was used to determine relationship between morphometric variables. Paired sample T-test, independent sample T-test, one way ANOVA and GLM were applied to evaluate the effect of fixed factors such as age, breeding status, BCS, body weight, and scrotal circumference on follicular activity in mares and semen parameters in stallion. The significance level was assessed at $p<0.05$.

Results

Morphometric characterization and breeding soundness evaluation

The overall age, BCS and body weight for all horses were 14.5 ±5.6 years, 5.4±1.4 and 344.1 ± 50.3 Kg, respectively. Stallions were significantly older than the mares with good condition while mares were heavier with relatively poorer BCS. Details of the body morphometry are given in Table 1. Most (71%) of the mares are >12 yrs and while 80% of stallions are >10 yrs showing that this is an aging horse population. Average age is 16.8 yrs for breeding stallions and 14.1 yrs for breeding mares which is too old for breeding equines. Bad vices are widespread among the mares and stallions. None of the horses were conditioned, and hence behaviorally manageable.

Table 1: Summary of mean (±SD) of body morphometry in mares and stallions.

Measurement	N	Mares Mean ± SD (Range)	N	Stallions Mean ± SD (Range)
Age [years]	14	14.1±5.8 (3 - 22)	19	15.1±5.7 (5-23)
Body weight [Kg]	14	341.1±29.8 (228-458)	19	312.9±39.0 (229-390)
BCS	14	4.8±1.4 (3 - 8)	19	5.3±1.6 (3-8)
Head to tail [cm]	14	194.2 ±9.0 (176-207)	19	189.7±10.2 (166-206)
Girth [cm]	14	165.4±5.6 (155-185)	19	162.7±5.7 (155-173)
Height at the wither [cm]	14	146.4±4.7 (139-154)	19	147.0±6.7 (143-162)
Loin length [cm]	14	76.0±5.6 (68-86)	19	81.3±6.8 (71-95)
Tail length [cm]	14	127.4±6.5 (115-137)	19	128.1±7.5 (117-139)
Front leg height [cm]	14	142.2±5.6 (134-152)	19	139.5±6.0 (132-151)
Hind leg height [cm]	14	135.1±4.8 (130-144)	19	134.6±4.9 (125-143)
Neck length [cm]	14	75.0±6.3 (62-85)	19	70.4±9.7 (58-99)
Neck circumference [cm]	14	85.5±6.4 (70-93)	19	89.6±6.1 (81-104)
Head length [cm]	14	59.0±3.7 (52-64)	19	55.3±4.3 (51-70)

Comparison of size with existing record shows a 9-11% reduction in size for the mares and 4-9% reduction for stallions. Various conformational problems were also identified such as long neck, ewe-neck, club foot; coon foot, high rump, and sway-back. Body weight was significantly correlated with girth both in mares ($p<0.05$; $r=0.97$) and stallions ($p<0.05$; $r=0.68$). The proportion of the loin length and neck length in relation to the head tail length showed a significant difference ($p<0.05$) showing that loin length is independent of neck length. Stallions with longer neck could have either longer loin or shorter loin relative the head-tail length. Contrary to this, mares showed linear relation between loin and neck length.

Comparison of differences in body morphometry was conducted among the mares, stallions and castrated males. There was a significant difference ($p<0.05$) in loin length, front leg height, hind leg height and head length. Castrated horses had the longest loin length (83.3 ±5.7 cm) followed by the stallions and mares (77.4 ±4.2 cm and 76 ±5.5 cm), respectively and the smallest front leg height and head length compared to the mares and stallions.

Testicular parameters were closely similar between the left and right testis (Table 2). The ratio of body weight to scrotal circumference was relatively smaller ranging from 8.3:1 to 12:1. There was a significant correlation ($P<0.05$; $r=0.60$) between BCS and scrotal circumference (SC) of the breeding stallions.

Table 2: Testicular parameters of stallions

Testicular parameter	N	Mean	Std. D
Scrotal circumference	7	38.05	4.13
Left testicular length	7	17.33	2.73
Right testicular length	7	17.25	2.89
Left testicular width	7	6.00	0.85
Right testicular width	7	5.75	1.06

Ultrasonography

The mean (±SD) length of estrus cycle and estrus in the breeding mares were 16.1±3.5 days and 7-12 days, respectively. Summary of other reproductive parameters is given in Table 3. The mean (±SD) diameter of the CL was 25.3±4.9 mm. Distribution of the total number of follicles and the diameter of the preovulatory follicles among the mares was highly variable ($P<0.05$). The mean diameter for the preovulatory follicle was 29.4±9.19 mm.

Table 3: Results of ultrasonic evaluation of the reproductive organs of mares (n=14)

Ultrasonic measurement	No scanning	Mean (±SD)	Minimum	Maximum
Uterine diameter [mm]	81	35.1±5.8	19.2	49.2
Total number of follicles	81	16.2±6.1	6.0	32.0
Total number of follicles in LOV	81	8.3±3.2	4.0	16.0
First large in LOV [mm]	81	19.6±6.9	4.6	38.1
Second large in LOV [mm]	81	14.9±4.7	4.2	29.0
Third large in LOV [mm]	81	12.0±4.1	3.0	23.0
Total number of follicles in ROV	81	8.0±3.5	1.0	19.0
First large in ROV [mm]	81	22.0±9.4	8.6	49.7
Second large in ROV [mm]	81	16.1±5.9	5.9	43.7
Third large in ROV [mm]	81	12.6±3.9	4.0	20.4

TNF= Total number of follicles; LOV= Left ovary; ROV= Right ovary;

Inactive ovaries, and abnormal uterine fluid (Figure 1) were detected in at least 6 mares, but endometrial samples were negative for any bacterial complication.

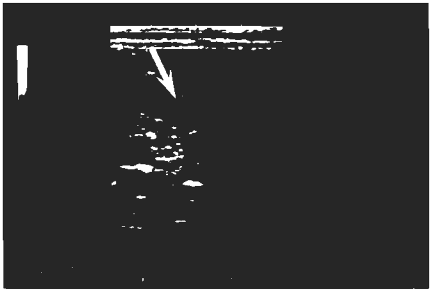

Figure 1. Sonogram of the right uterine horn of a mare indicating presence of fluid (Arrow)

Semen analysis and AI

The color of the raw semen was mainly creamy with a range from light to deep creamy. The average pH was 7 for all stallions. The volume of gel-free semen ranged from 8 to 118 ml while the concentration was in the order of 51 million/ml for the smallest to 361 million/ml for the highest. Other semen parameters are summarized in Table 4. The stallions lacked consistency in their semen parameters and there was no correlation between libido, semen volume and concentration. Since all the stallions were untrained, time to ejaculation was much longer (>1hr).

Table 4: Libido and semen parameters of stallions during collection of semen using EAV

Semen parameter	Stallions ID						
	001	002	003	004	005	006	007
Libido score (out of 4)	4	3.5	4	4	3.5	2.5	3
Gel volume	35	30	40	25	120	35	35
Gel-free volume	65	83	56	102	8	65	118
Total motility [%]	90	70	60	40	10	0	80
Progressive motility [%]	85	60	65	65	20	0	85
Chilled semen motility	75	60	50	25	8	0	65
Post-thaw motility [%]	50	22	28	1	5	0	40
Concentration [mill/ml]	197	361	290	310	280	310	51
Live percent [%]	90	70	70	45	20	0	80
Freezability [%]	50	22	28	1	5	0	40
No straws cryobanked	140	222	175	0	0	0	195

Result of the pregnancy diagnosis carried out at Day 21 post insemination showed a pregnancy rate of 50% (4/8), however, only one maintained pregnancy to the second term.

Discussion

In this particular investigation where the horses have been randomly interbred, it has been obvious that most of the morphometric data have changed over the years. Ancestors of the same horse population used to have heights as big as 172cm while currently the largest measurement was 162cm. Most of the morphometric findings in the present study are lower than what are known for equestrian horse. Further, body morphometry and conformation are also known to influence the performance of sport horses (Langlois, 1979; Holmström *et al.*, 1990).

Documentation of existing genetic resources, including the description of the phenotypic characteristics, and performance are important management activities (Ruane, 1999; Duchev and Groeneveld, 2006). One reason for smaller morphometric findings might be the random breeding over several years, which has lead to inbreeding depression (Cervantes *et al.*, 2011). Population size has also a direct relationship with the rate of inbreeding, fitness and the amount of genetic variation lost due to random genetic drift with small population being more prone to such problem as observed in this horse population.

Signs of phenotypic retrogression in both the mares and stallions are generally rampant. This will affect the sport performance by leading to loss of balance during sporting (Langlois, 1978). BCS is within the range for equestrian (4-6) and breeding horses (5–7) (Ellis, 2000; Šveistienė, 2010, 2010; Takaendengana, *et al.*, 2011). However the body weight and height are below the optimal 460-535Kg and 153 to 163.2cm, respectively for most equestrian and breeding horses (Ellis, 2000; Warren, 2002). The current finding about correlations among some of the morphomteric data such as BCS, height, body weight and girth agrees with previous findings (Hennke *et al.*, 1983). Conformational problems found in the present study such as vulvar deformities, lordosis, and weak foot tendons have also been reported previously in relation to inbreeding (Stull, 1997; McIlwraith, et al, 2003). Various reports also have similarity with the current finding on differences in body morphometry between the mares and stallion (Takaendengana, *et al.*, 2011; Gómez, *et al.*, 2009; 2012). Bad vices observed in almost all horses are indications of apparent grooming problem.

The lack of training is apparent both in the mares and stallions; this has a direct substantial negative effect on their value both as a sport horse and breeding animals (Houpt and McDonnel, 1993; Stout, 2005). The temperament of the stallion is very important, for ease of management and as a heritable trait. A stallion of a quiet and kind disposition is a great asset and will be much easier and safer to handle (Davies Morel, 2008).

The breeding soundness evaluation has demonstrated that both the mares and stallions have poor performance (Fayrer-Hosken, and Caudle, 1989; McKinnon and Voss 1993; Blanchared *et al.*, 2001; Davies-Morel, 2008). Libido was very poor in some stallions. At least three horses were unable to cover a mare showing a clear weakness in their hind quarter which could be due to aging and inexperience.

The ultrasonic findings were closely similar with previous reports (Ginther 1995; Alemayehu Lemma, 2004; Alemayehu Lemma *et al.*, 2006). Follicular number and diameter distribution were within the ranges. The diameter of the preovulatory follicle was also within the range of previous studies on mares elsewhere. Mares also showed typical estrus behavior during heat. However, some mares had inactive ovaries which might be either due to inbreeding depression or old age.

Results of semen analysis were very poor for most stallions supposed to be breeding sires for the farm. Total motility of fresh equine semen is reported to be in the order of 80-100% while acceptable post-thaw motility is[3] 35% (Loomis and Graham, 2008). The present result of semen analysis for most of the studs was characterized generally by poor prefreeze motility and very poor post-thaw motility, small live percent and very high proportion of gel relative to semen volume. Report by Vidament, 2005 and Loomis and Graham, 2008 confirms that stallions with higher initial pre-freeze motility are likely to have higher post-thaw motility (≥40%) showing a good freezability. If the stallion's condition is very poor, spermatogenesis may also suffer (Davies-Morell, 2008; Petrunkina, *et al.*, 2007). High gel volume and very poor freezability could also be associated with signs of inbreeding depression (Watson, 2000; Varner, 2007). Although fertility seemed relatively good with AI, early embryonic loss might be due to the effect of inbreeding.

Conclusion

In conclusion, the current population is characterized by a relatively smaller body morphometry due to the protracted random breeding and due to the uncontrolled genes mixing from European and Arabian horse breeds. Mare ovarian activities are within the ranges of other findings however, the breeding soundness evaluation of the stallions shows a very poor performance for most horses. Generally, the particular sporting horse population can be considered unfit for breeding on its own. Moreover, the difficulty in handling and bad vices for most mares and stallions confirms that most of the horses have not been well groomed from the early foal-hood. Signs of inbreeding depression characterized by phenotypic retrogression, conformational problem, and poor semen parameters. Although early embryonic loss was high, fertility results with assisted reproductive techniques (ultrasonography and timed AI) can be considered fairly good. The sport horse owner is advised to make clear distinction between breeding and sporting horses and introduce strict informed selection procedures. Further, an introduction of new gene (possibilities through AI with imported semen) is mandatory to avert some of the effects of inbreeding.

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References

AACA, 2004. Addis Ababa city administration proclamation establishment for Sub-City and Kebele in Addis Ababa, Ethiopia.

Bearden, H.J, Fukuay, J.W. and Willard, S. T., 2004. Applied animal reproduction. 6th Edition, Mississippi State University, USA, 427.

Blanchard, T.L, Brinsko, S.P., Varner D.D., Rigby, S.L. and Hurtgen, J.P., 2001. Evaluation of testicular size and function in 1-3 year old stallions. Proceedings of the annual convention of the AAEP, 47:232-35.

Blanchard, T.L., Brinsko, S.P. and Rigby, S.L., 2002. Effects of deslorelin or hCG administration on reproductive performance in first postpartum estrus mares. *Theriogenol.*, 58,165-16.

Cervantes, I., Goyache, F., Molina, A., Valera, M., and Gutérrez, J.P., 2011. Estimation of effective population size from the rate of coancestry in pedigreed populations. *J. Anim. Breed. Genet.*, 128, 56–63.

CSA,2010. Agricultural sample survey Volume II. Reports on livestock and livestock characteristics. (Private peasant holdings), Statistical Bulletin. Central Statistics Authority of Ethiopia (CSA), PP 16-19.

Davies Morel, M.C.G., 2008. Equine Reproductive Physiology, Breeding and Stud Management 3rd Edition. Cambridge University Press, Cambridge, UK, 378

Duchev, Z., and Groeneveld, E., 2006. Improving the monitoring of animal genetic resources on National and International level. Arch. Anim. Breed., 49, 532–544.

Ellis, P., 2000. Condition scoring and weight estimation of horses; State of Victoria, Department of Natural Resources and Environment. Agricultural notes, 1-4

Fayrer-Hosken, R.A. and Caudle, A.B., 1989. Stallion fertility evaluation; part III. Equine Practice, 11, 30-34.

Ginther, O.J., 1995. Ultrasonic Imaging and Animal Reproduction: Fundamentals Book 1. 82: 147-155.

Gómez, M.D., Valera, M., Molina, A., Gutiérrez, J. P. and Goyache, F., 2009. Assessment of inbreeding depression for body measurements in Spanish Purebred (Andalusian) horses. *Lives. Sci.*, 122, 149–155.

Gómez. M.D, Azor, P.J, Alonso, M.E, Jordana, J and Valera, M., 2012. Morphological and genetic characterization of Spanish heavy horse breeds: Implications for their conservation. *Lives. Sci.,* 144, 57–66.

Henneke, D.R., Potter, G.D., Kreider, J.L. and Yeates, B.F., 1983. Relationship between Condition Score, Physical Measurements and Body Fat Percentage in Mares. *Equi. Vet. Jour.,* 15, 371 – 372.

Henneke, D.R., Potter G.D, and Kreider T.L., 1984. Body condition during pregnancy and lactation and reproductive efficiency of mares. *Theriogenol.*, 21, 897.

Holmström, M., Magunsson, L.E and Philipsson, J., 1990. Variation in conformation of Swedish Warmblood horses on conformational characteristics if elite sport horses. *Equi. Vet. Jour.,* 22, 186-193.

Houpt, K.A. and McDonnel, S.M., 1993. Equine stereotypies. Conti. Edu. Art. #5 Louisiana State University, 15:1265-1271.

Langlois, B., 1979. French results on the analysis of relationship between morphology and gallop, trot and jumping abilities in horses. 30th Annual Meeting of the EAAP, Harrogate, UK.

Langlois, B., Froideveaux, J., Lamarche, L., Legault, C., Legault, P., Tassencourt, L., Théret, M., 1978. Analysis of relationships between morphology and gallop, trot and jumping abilities in horses. *Ann. Genet Sci. Anim.*, 10, 443-474.

Lemma, A., 2004. Case studies on reproductive activity of equines in relation to environmental factors in central Ethiopia. PhD Thesis, Humboldt University of Berlin. Books on demand GmbH, Nordersteds, Germany, pp 157.

Lemma, A., Schwartz, H.J., and Bekana, M., 2006. Application of ultrasonography in the study of the reproductive system of tropical Jennies (species *Equus asinus*). *Trop. Anim. Hlth Prod.*, 38, 267-274.

Loomis, P.R., and Graham, J.K., 2008. Commercial semen freezing: individual male variation in cryosurvival and the response of stallion sperm to customized freezing protocols. *Anim. Reprod. Sci.,* 105, 119-128.

Macpherson, M.L., 2001. How to evaluate semen in the field. Proceedings of the Annual Convention of the AAEP. Vol. 47/AAEP Proceedings; pp 412-16

McKinnon, A.O. and Voss, J.L. 1993. Equine Reproduction. Lea and Febiger, Philadelphia, London, 465-471.

McIlwraith, C.W., Anderson, T.M., and Sanschi, E.M., 2003. Conformation and Musculoskeletal Problems in the Racehorse. *Clin. Tech. Equi. Prac.*, 2, 339-347.

Petrunkina, A.M., Waberski, D., Günzel-Apel, A.R., and Töpfer-Petersen, E., 2007. Determinants of sperm quality and fertility in domestic species. *Reprod.,* 134, 3-17.

Ruane, J., 1999. Selecting breeds for conservation. In: Oldenbroek, K. (Ed.), Gene banks and the Management of Farm Animal Genetic Resources. Idodl Press, the Netherlands, 59–73.

Squires, E., McKinnon, A., and Shideler, R., 1988. Use of ultrasonography in reproductive management of mares. *Theriogenol.*, 29, 55-70.

Stout T.A.E., 2005. Modulating reproductive activity in stallions: A review, Anim. Reprod. Sci., 89, 93–103

Stull, C.L., 1997. Physiology, Balance, and Management of Horses During Transportation. Proceeding of Horse Breeders and Owners Conference, Red Deer, Alberta, Canada, January 10-12.

Šveistienė, R., 2010. Changes in the Lithuanian heavy draught horse population after the introduction foreign stallions. 61st Annual Meeting of the European Association for Animal Products . Heraklion, Crete Island, Greece, 23rd -27th August 2010

Takaendengana, B.J, Noorc, R.R, and Adiania, S., 2011. Morphometric Characterization of Minahasa Horse for Breeding and Conservation Purposes. *Med. Petern.,* 34, 99-104.

Varner, D., 2007. From a Sperm Eye View-Revisiting Our Perception of This Intriguing Cell; Proceeding of the 53rd Annual Convention of the American Association of Equine Practitioner, December 1-5, 2007, Orlando, Florida.

Vidament, M., 2005. French field results (1985–2005) on factors affecting fertility of frozen stallion semen. *Anim. Reprod. Sci.*, 89, 115–136.

Warren, L.K., 2002. Body Condition Scoring Your Horse. Agri-facts, Agdex 20, 1-3.

Watson, P.F., 2000. The causes of reduced fertility with cryopreserved semen. *Anim. Reprod. Sci.*, 60-66.