

Theses of doctoral (PhD) dissertation

**Conformation and morphological
characterisation in the Hungarian native
donkey, and changes in plasma melatonin
concentration during pregnancy in
indigenous donkey and sheep breeds**

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Table of Contents

Background and Objectives of the PhD Dissertation	4
Background	4
Research Objectives	6
New Scientific Results of the Dissertation	8
Results of Conformational and Morphological Studies in the Hungarian Parlagi Donkey	8
Results of the Investigation of Plasma Melatonin Concentration During Pregnancy in Indigenous Donkey Mares	10
Results of the Investigation of Plasma Melatonin Concentration During Pregnancy in Indigenous Ewes	14
Summary of new scientific results	18
Own Publications Forming the Basis of the Dissertation ...	20
References	22

Background and Objectives of the PhD Dissertation

Background

Statistical data on global donkey populations are largely based on estimates, as both global and regional registries of the species are typically incomplete [1]. This situation has particularly adverse consequences for the survival of breeds with small population sizes, since inadequately implemented or entirely absent breed reconstruction programmes may lead to accelerated genetic erosion, crossbreeding of populations, and, in some cases, the complete extinction of certain breeds [2, 3].

In Hungary, the Hungarian Parlagi donkey is the only officially recognised donkey breed, which, according to Decree 4/2007 (I.18.) of the Ministry of Agriculture and the Ministry of Environment and Water [4], is listed among the protected indigenous and endangered agricultural animal breeds. However, the registration of the population and the documentation of breeding records still face considerable practical difficulties, which necessitated the revision of previous breeding conditions and the development of a modern breed reconstruction programme incorporating specific measures [5]. To date, no comprehensive investigation has been carried out on the Hungarian Parlagi donkey that would integrate results from multiple scientific disciplines and provide a complex description of the breed's conformational, morphological and physiological characteristics and

their interrelationships. Nevertheless, conformation and morphological studies are of fundamental importance for breed conservation, as they enable the identification of individuals eligible for the main studbook, support the refinement of the breed standard, and provide guidance for the development of an appropriate breeding strategy.

One of Hungary's most important indigenous sheep breeds, the Tsigai (Cigája), plays a key role in sustainable, landscape-based livestock farming systems [6]. It tolerates poorer quality pastures well, is agile, and possesses excellent maternal traits, which makes it well-suited for grazing-based management. Its production is economically valuable in terms of milk, meat and wool alike [7]. The breed exhibits a moderate degree of seasonal reproductive activity, with oestrus activity peaking in autumn, a pattern that aligns well with the melatonin rhythm regulated by natural photoperiodicity [8].

The hormone melatonin plays a central role in the regulation of daily and annual physiological cycles. Its secretion in the pineal gland is closely linked to light exposure, particularly the duration of illumination, and therefore serves as a highly sensitive indicator of photoperiodic changes [9]. Melatonin fulfils several essential functions in relation to reproduction [10], immune system activity [11], and metabolism [12, 13]. Its exogenous application [14], or the manipulation of its secretion by controlled lighting programmes [15], offers opportunities in livestock management and breeding to artificially influence hormonal cycles. In

this way, it may contribute to improving reproductive performance [16], modifying seasonal reproductive activity, enabling out-of-season breeding [17], or stabilising the physiological condition of animals [18].

These considerations justify the investigation of melatonin concentration in indigenous farm animal breeds, particularly to deepen our understanding of reproductive biological processes. With respect to the perinatal period, very limited or no data are available concerning serum melatonin levels around parturition in domestic animals. Based on findings from human studies [19, 20], we hypothesised that plasma melatonin concentration increases in late pregnancy before parturition and further assumed that the hormonal cyclicity described in horses is also present in donkeys.

Research Objectives

The dissertation had two principal aims. First, to conduct a comprehensive investigation of the conformational and morphological traits of the Hungarian Parlagi donkey, thereby contributing to the preservation of genetic resources and providing new scientific findings to support the conservation and reconstruction of the breed. Second, the research sought to characterise the endocrine features of indigenous donkey and sheep breeds, thereby promoting a deeper understanding of reproductive biology and physiological processes. The specific research objectives addressed within the framework of the dissertation are detailed below:

Conformational and Morphological Studies

- Our objective was to perform a comparative analysis of the body measurements, body indices, and coat colour varieties of registered Hungarian Parlagi donkey individuals.
- We aimed to process conformation and herd structure data in order to refine the breed standard and identify individuals suitable for inclusion in the main studbook.
- The linear conformation assessment system was adapted to the Hungarian Parlagi donkey, enabling the classification of structural weaknesses and faults, thereby providing guidance for the breeders' association.

Investigation of the Role of Melatonin

- Another focus of our research was the determination of plasma melatonin concentrations in pregnant and non-pregnant donkey mares and in ewes, to reveal hormonal characteristics associated with reproductive status.
- We further aimed to assess whether, through statistical evaluation, changes in melatonin levels could be interpreted not only in relation to daily rhythms but also at the level of annual cycles, with particular attention to alterations occurring during the progression of pregnancy.
- In addition, we set out to determine the nocturnal melatonin concentrations of ewes and their newborn lambs in the period immediately following parturition

New Scientific Results of the Dissertation

The dissertation summarises the results of our investigations conducted on the indigenous Hungarian Parlagi donkey (*Equus asinus*) and the Tsigai sheep (*Ovis aries*). In comparing the coat colour varieties of the Hungarian Parlagi donkey mare population, we were the first to apply the linear conformation assessment system in donkeys, and we demonstrated its practical applicability.

In analysing the plasma melatonin concentrations of the two indigenous livestock species, we were the first to determine both the circannual and circadian rhythms of plasma melatonin levels in donkey mares. Furthermore, we were also the first to describe the changes in melatonin concentration in ewes throughout the entire gestation period and during the early postpartum phase, with particular emphasis on the effect of season.

Results of Conformational and Morphological Studies in the Hungarian Parlagi Donkey

During our morphological investigations, we evaluated the body measurements and conformational traits of a total of 65 Hungarian Parlagi donkey mares during 2020–2021, based on data collected from nine domestic farms. The surveys included the determination of body measurements, body indices, live weight and body condition, in addition to performing linear conformation assessments. Based on the statistical analyses and conformation evaluations, our results indicated that the

examined population was relatively uniform and provided a suitable foundation for the ongoing breed reconstruction programme.

We established that no significant conformational differences were found among the three main coat colour varieties (grey, brown and black). However, the place of keeping had a significant influence on body measurements and body indices, reflecting the varying breeding objectives of the farms: smaller-bodied donkeys were kept primarily for children's riding, while larger individuals were maintained for display or meat production purposes. The average withers height of the mare population was 117 cm (**Figure 1**),

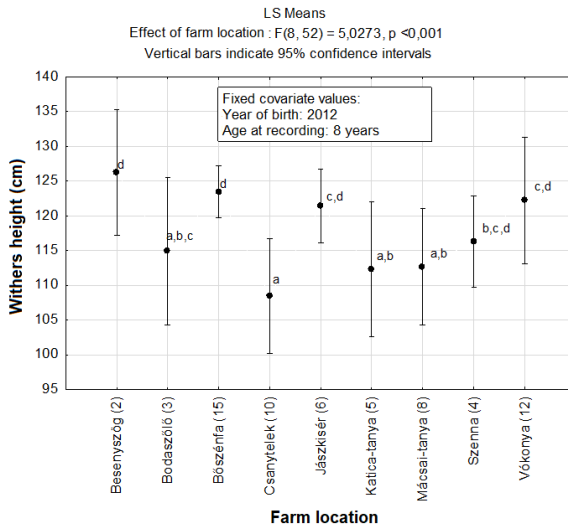


Figure 1. Mean withers height (cm) of Hungarian native donkey mares adjusted to 8 years of age and the 2012 birth year, by farm location. Sample sizes are given in parentheses after each farm location. Different letters (a, b, c, d) indicate significant differences ($p < 0.05$; Fisher's post hoc test).

Their body conformation was characterised as compact, with a shallow chest and relatively long limbs. Commonly identified faults included poorly muscled necks and croups, flat withers, steep shoulder angles, narrow fore hooves, and cow-hocked hind limb conformation.

The results, together with the successful adaptation of the linear conformation assessment system, can be utilised to refine the breed standard and to support the implementation of the reconstruction programme.

Results of the Investigation of Plasma Melatonin Concentration During Pregnancy in Indigenous Donkey Mares

To determine plasma melatonin concentration in the Hungarian Parlagi donkey (*Equus asinus*), blood samples were collected from 15 pregnant and non-pregnant mares at different times of the year, covering the nocturnal phase of the 24-hour daily cycle. Sampling was carried out at Bőszénfa, the only Hungarian Parlagi donkey nucleus herd in the country.

Following radioimmunoassay (RIA) measurements and subsequent data correction, plasma melatonin concentrations ranged between 10 and 50 pg mL⁻¹. Mean values measured at midnight (36.6 pg mL⁻¹) were significantly higher than those recorded in the early morning (06:00, 24.6 pg mL⁻¹) and evening (18:00, 24.1 pg mL⁻¹).

Within the examined mare population (**Figure 2**), the highest melatonin concentrations were observed during the winter solstice (approximately 32–33 pg mL⁻¹), whereas the lowest concentrations were typical at the

summer solstice (around 20 pg mL⁻¹). At the autumn and spring equinoxes, melatonin concentrations showed approximately equal values (27 and 25 pg mL⁻¹, respectively), positioned proportionally between the values measured at the two solstices.

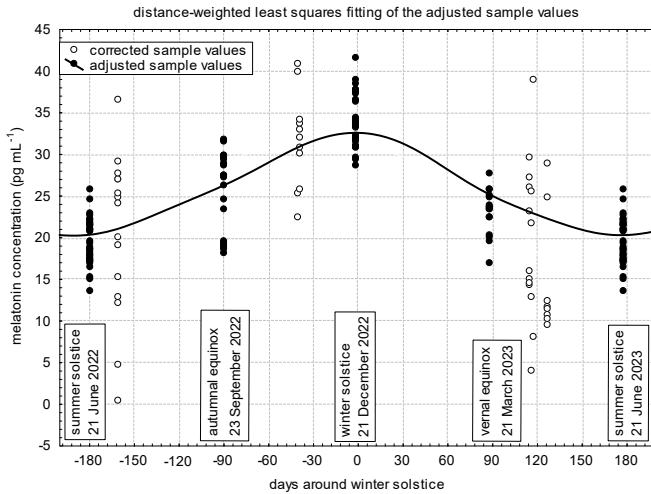


Figure 2. Annual distribution of plasma melatonin concentrations in the examined donkey mares (pregnant and non-pregnant individuals) (pg mL⁻¹).

In our study, the melatonin concentration measured at midnight during the winter solstice (45.2 pg mL⁻¹) was the highest, being significantly greater ($p < 0.05$) than the values obtained at the summer solstice (34.2 pg mL⁻¹) and the spring equinox (30.7 pg mL⁻¹), while remaining at a nearly identical level to the concentration measured at midnight during the autumnal equinox (36.3 pg mL⁻¹). As the examined population was kept under stabled conditions, where daytime light

intensity did not exceed 100 lux, it was also possible to measure daytime melatonin concentrations (**Figure 3**).

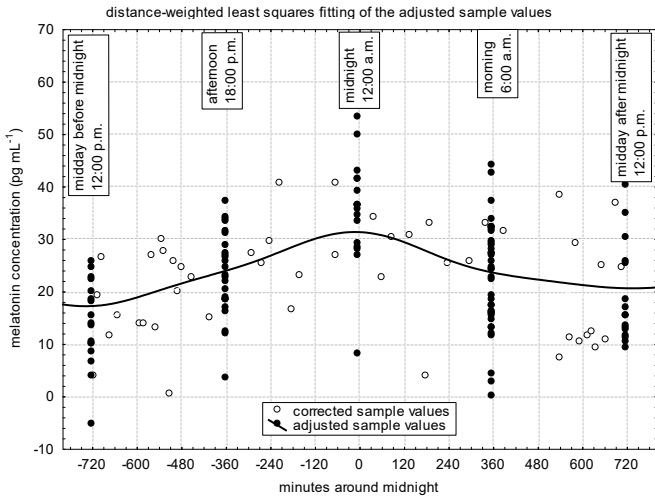


Figure 3. Daily distribution of plasma melatonin concentrations in the examined donkey mares (based on combined data of pregnant and non-pregnant individuals) (pg mL⁻¹).

In the examined donkey mares, the highest melatonin concentration was observed at midnight (approximately 32 pg mL⁻¹), while the lowest values were recorded at noon and immediately before and after midnight (± 720 minutes from midnight; approximately 17 and 20 pg mL⁻¹, respectively). In contrast, at ± 360 minutes (6 hours before and after midnight), concentrations were similar, showing values of 24 and 23 pg mL⁻¹, respectively.

As a consequence, circadian and circannual rhythms appeared within a narrower range; however, it is likely that under natural light conditions broader differences in melatonin levels would have been observed. The sampling time-corrected and gestational day-adjusted data, together with the estimated distribution of melatonin concentrations during the 300 days preceding parturition, are presented in **Figure 4**. During this period, plasma melatonin levels in pregnant donkey mares remained relatively constant ($\sim 25 \text{ pg mL}^{-1}$), which corresponded closely to the control values measured in non-pregnant animals.

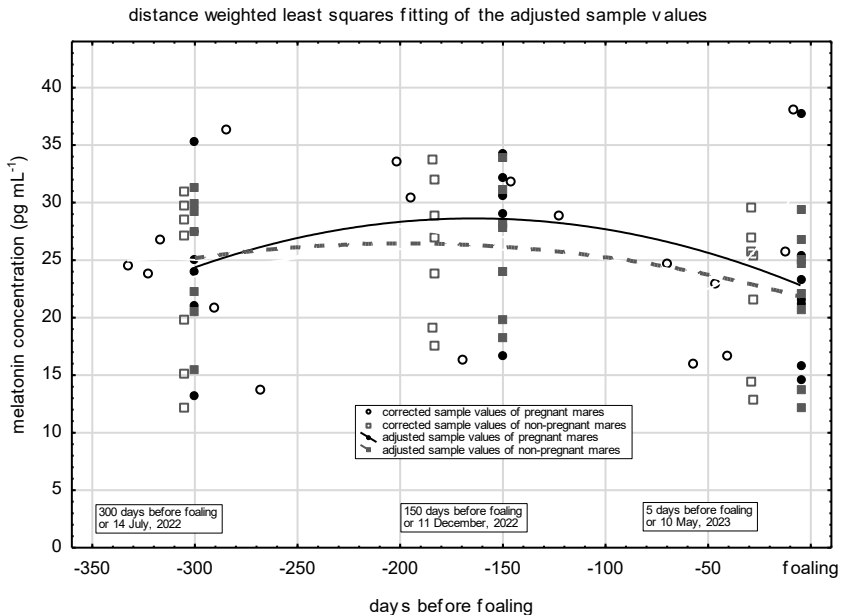


Figure 4. Plasma melatonin concentrations at different stages of pregnancy in donkey mares, compared with the non-pregnant control group (pg mL^{-1})

Our results confirmed the presence of circadian and circannual melatonin rhythms in the domestic donkey, which are well comparable to the hormonal cyclicality previously described in the horse (*Equus caballus*). An important novel finding was that plasma melatonin concentration did not change with the progression of pregnancy, a pattern that contradicts observations reported in human reproductive biology. Thus, the data obtained in this study substantially contribute to the expansion of the currently very limited knowledge concerning seasonal hormonal changes in the domestic donkey.

Results of the Investigation of Plasma Melatonin Concentration During Pregnancy in Indigenous Ewes

In the study conducted on Tsigai ewes at the Experimental Farm of the University of Veterinary Medicine Budapest, blood samples were collected from 16 pregnant individuals during the night hours, at different stages of pregnancy, as well as in the days following parturition. Plasma melatonin levels were determined using the radioimmunoassay (RIA) method, and population genetic statistical analysis was applied to evaluate the observed changes.

The mean nocturnal melatonin concentration characteristic of the study period was 134 pg mL⁻¹, which showed an increase from the autumn equinox to the winter solstice (**Figure 5**). The average value corrected for midnight sampling was 162.4 pg mL⁻¹, with moderate nocturnal fluctuations observed.

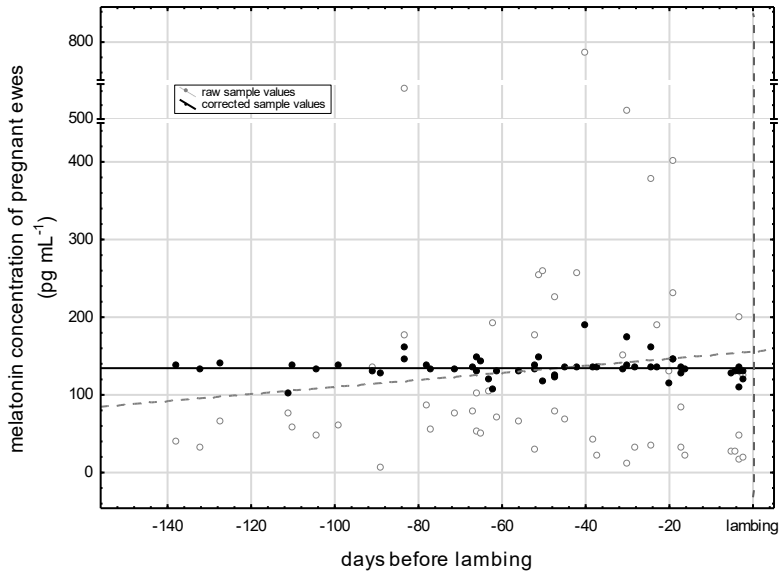


Figure 5. Changes in plasma melatonin concentration in pregnant ewes, based on sampling, from the autumn equinox to the winter solstice (pg mL^{-1})

In the investigation related to a potential increase in melatonin levels with advancing gestational age, the fitted linear function did not confirm such an association ($p = 0.442$). After correction, the curve showed a horizontal course, and statistical evaluation demonstrated that plasma melatonin concentration did not change significantly during the progression of pregnancy ($p = 0.998$). The mean value of the adjusted samples was approximately 134 pg mL^{-1} . When plasma melatonin concentrations were corrected to midnight values on specific gestational days (-98 , -49 , and -7 days), the mean melatonin level was 162 pg mL^{-1} .

¹, but no statistically significant differences were detected between the different time points ($p = 0.783$).

By the end of the study period, 12 ewes had lambed, and thus raw and corrected values from 12 ewe–offspring pairs were evaluated during the days following parturition (**Figure 6**).

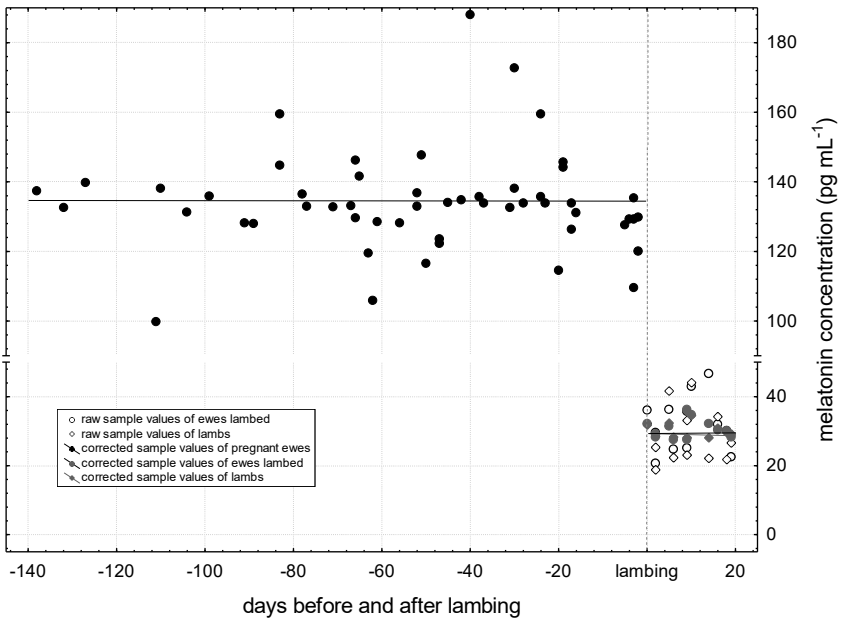


Figure 6. Distribution of plasma melatonin concentration before and after parturition (pg mL^{-1}).

Both raw and corrected (adjusted) values showed similarly shaped, nearly linear curves, and the high p -values obtained for the linear regressions (0.995 and 0.771) indicated that the time elapsed since

parturition had no statistically demonstrable effect on changes in melatonin production.

Plasma melatonin concentrations measured after parturition were significantly lower compared with gestational values, stabilising at an average of approximately 30 pg mL⁻¹. The plasma melatonin levels of newborn lambs were of the same order of magnitude as those of their mothers.

The melatonin concentration estimated for midnight on the second day before parturition was 160.2 pg mL⁻¹. By contrast, the midnight-corrected plasma melatonin concentrations measured on the first day after lambing were significantly different ($p < 0.001$) in both ewes and lambs, showing uniformly lower values (36.18 and 35.92 pg mL⁻¹, respectively). The low melatonin levels measured in the postpartum period exhibited only a one-fifth reduction in nocturnal elevation compared with values recorded during pregnancy (approximately 6 pg mL⁻¹ versus 28 pg mL⁻¹).

Our results therefore confirm that plasma melatonin concentration in Tsigai ewes remains essentially constant throughout pregnancy. Furthermore, no statistically significant association could be established between plasma melatonin levels and gestational age in Central European Tsigai ewes.

We were the first to describe that, in the early postpartum period, plasma melatonin concentration decreases to low levels (30 pg mL⁻¹) in both ewes and their lambs, without nocturnal fluctuation and independently of

the daily rhythm. This phenomenon may plausibly be explained by placental expulsion, increased maternal vigilance in the ewe–lamb bond, and the antagonistic interaction between melatonin and prolactin.

Summary of new scientific results

Conformation and morphological studies in the Hungarian native donkey

a) We were the first to describe the conformation of the reconstructed population of Hungarian native donkey mares using body measurements and body size indices, and we found that its coat-colour variants do not differ substantially in conformation.

b) We were the first to adapt the linear conformation scoring system for donkeys and to demonstrate its applicability in this species; therefore, we recommend its implementation in the breeding of the indigenous Hungarian native donkey.

Pregnancy-related blood plasma melatonin concentration in native donkey mares

a) The circannual and circadian rhythms of blood plasma melatonin concentration were determined for the first time in donkey mares (*Equus asinus*). Maximum melatonin concentration (45 pg mL^{-1}) was recorded at the winter solstice. Both daily and annual rhythms were detectable, with lower values measured in other seasons and times of day.

b) We were the first to demonstrate that the midnight melatonin concentration of donkey mares (approximately 38 pg mL⁻¹) does not change significantly as a function of either gestational age or pregnancy status.

Pregnancy-related blood plasma melatonin concentration in native ewes

a) We were the first to demonstrate that changes (i.e., an increase) in melatonin concentration, when evaluated across the entire gestation period under natural light conditions, are not associated with the progression of pregnancy but are instead attributable to changes in daytime length.

b) Following parturition, the nocturnal blood plasma melatonin level decreases to the same low concentration in both ewes and their newborn lambs, irrespective of the time of day.

Own Publications Forming the Basis of the Dissertation

In peer-reviewed scientific journals indexed with impact factor

Bartha, B., Harmat, L., Somoskői, B., Cseh, S., Fekete, S. Gy. & Gáspárdy, A. (2021): A melatonin szerepe a ló és a szamár szaporodásában – irodalmi összefoglaló. *Magyar Állatorvosok Lapja*, 143, 599–608.

Harmat, L., Nagy, J., Somoskői, B., Alpár, A., Fekete, S. G., & Gáspárdy, A. (2024). Determination of rhythmicity and gestational stage-related distribution of blood plasma melatonin concentrations in donkey mares. *Veterinary Sciences*, 11(7), 310.

Harmat, L., Chandran, A. N., Nagy, J., Alpár, A., Somoskői, B., Fekete, S. G., Becskei, Zs., & Gáspárdy, A. (2025). Seasonal development of nocturnal blood plasma melatonin concentration in the Hungarian native donkey. *Veterinarski Glasnik*, 79(1), 38–48.

Gáspárdy, A., Gulyás, L., Polland, I., Alpár, A., Fekete, S. Gy. & Harmat, L. (2025): Determination of Natural Blood Plasma Melatonin Concentration of Tsigai Ewes Characteristic for Gestation and Early Postpartum Period Between Autumnal Equinox and Winter Solstice. *Veterinary Sciences*, 12(4), 336.

Full text papers in peer-reviewed journals

Harmat L, Kuncicky A, Lénárt Z, Ernst M, Nagy J, Gáspárdy A (2022):
Conformation traits of Hungarian Fallow Donkey mares according to
their basic colour. ***Danubian Animal Genetic Resources***, 7(2): 17-21

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