Theses of Doctoral (PhD) Dissertation

Study of ovarian patterns in mares and the role of anti-Müllerian hormone in different types of donor mares

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1. Introduction and aim of the thesis

Ultrasound-guided transvaginal oocyte pick-up (OPU) in cattle has proved to be a very successful and important technique, allowing repeated oocyte collection from heifers and cows of high genetic value. The technique is also highly relevant in horses as a solution to reduced fertility. In addition, ovum pick-up - *in vitro* embryo production procedures can be used to obtain embryos from mares actively involved in competitive sport, whose peak of their sporting career overlaps with the most fertile period in terms of reproductive biology. Furthermore, in many cases, there is a need to breed older mares for their genetic value and/or performance (BOLS and STOUT, 2018). However unfortunately mares of significant genetic value may die suddenly or be euthanised for health reasons. Thus, over the last few years, there has been increasing interest from horse owners in the use of Assisted Reproductive Techniques (ART) to produce foals from the oocytes of mares that have died unexpectedly or been euthanised. For these animals, *post mortem* OPU is the last and only option to gain oocytes. In these cases, it is crucial to retrieve the oocytes as efficiently and accurately as possible, a procedure that can be prepared for by practising on ovaries retrieved at the slaughterhouse. In most species of domestic animals, a large number of oocytes are collected from ovaries from slaughterhouses. In cattle, sheep and pig species, this has greatly facilitated research on *in vitro* maturation, fertilisation and embryo production. However, progress in this area has been much slower in horses, where large numbers of oocytes cannot be collected (SHABPAREH et al., 1993).

The importance of measuring anti-Mullerian hormone (AMH) levels in infertility screening is becoming increasingly important as women tend to have children later in life. It provides information on the size of the ovarian reserve, that is the available follicular and oocyte reserves, and can also help to estimate the likelihood of early menopause. However, much less research has been done in mares and cows, although these investigations are crucial for efficient reproduction. Assisted reproductive techniques can be used to optimise reproductive performance, but their effectiveness is highly dependent on the characteristics of the individual, so rigorous selection of animals prior to their use is of paramount importance. As a potential reproductive biomarker, knowledge of AMH levels can be of considerable help in the latter selection process (REDHEAD, 2017).

Aim of the thesis

In the first phase of my PhD research, my goal was to optimise *post-mortem* oocyte processing and retrieval techniques. Developing the most efficient method would greatly facilitate the production of foals from dead mares of high genetic value. In the second phase of the research, follicle analysis was performed on Lipizzan, Welsh pony and Hungarian Coldblood mares using ultrasound and the results were compared with AMH levels to gain a broader picture of the importance and potential use of AMH in mares.

In my research I was looking for answers to the following questions:

1. What correlations can be established between AMH levels and the number of small, medium, large and total follicles?

2. What is the relationship between AMH levels and the age of the mares?

3. Is there a significant difference in AMH levels and regarding the previous two questions between the 3 breeds (Lipizzaner, Welsh pony, Hungarian Coldblood)?

4. Is it possible that the seasons have a significant influence on AMH levels?

5. Is there a significant correlation between AMH levels and certain blood biochemical parameters (alkaline phosphatase, gamma-glutamyl transferase, phosphorus, urea, creatinine, cholesterol, triglyceride, magnesium, potassium)?

2. Materials and methods

2.1 *Post mortem* follicle and COC examination

2.1.1. *Post mortem* ovary collection

The mare ovaries for our experiment were provided by Pásztor-Hús Kft. in Eger. After the animals were slaughtered, the ovaries were retrieved within 20 minutes. The ovaries were placed in separate zip lock bags per mare. Then I placed the sealed bags containing the ovaries in heat-retaining styrofoam boxes. The transport time between the slaughterhouse and the laboratory was 2 hours each time, during which time the ovaries were kept at a temperature of 20-25 ºC. The temperature inside the styrofoam box was controlled by a digital thermometer with a wired sensor.

2.1.2. *Post mortem* oocyte retrieval

From the abattoir, the ovaries were transported to the Laboratory of Andrology and Assisted Reproduction at the University of Veterinary Medicine in Budapest, where the oocytes were retrieved. In the first place, the connective tissue surrounding the ovaries and the *tunica albuginea* were removed by dissection and then rinsed with sterile saline. Then all visible follicles were manually aspirated with a 20 gauge needle and a 10 ml syringe. The follicular fluid was collected in a Petri dish. Next, the follicles were opened with a scalpel blade and the inner surface was scraped several times (6-8 times) with a small bone curette. The granulosa cell layer extracted with the bone curette was washed into the Petri dish with PBS solution. Then the ovaries were sliced with a scalpel blade in order to perform the previous two steps, follicle aspiration and dissection, on the follicles found in the inner ovarian lining. Next, the contents of the Petri dishes were examined by stereomicroscopy and COCs were detected. They were evaluated according to their structural integrity and morphology and classified as compact or expanded. An ovarian examination protocol was completed for each case. The visible follicles were counted and classified according to size. The extracted oocytes were grouped according to the number and quality of the surrounding follicular epithelial cell layers. COCs with a dense cumulus cell layer closely surrounding the oocyte were classified as compact. In contrast, the expanded COCs had loose cumulus cells, radially arranged around the *zona* *pellucida* and a slightly "cloudy" structure. Denuded oocytes were not surrounded by cumulus cells.

2.1.3. *In vitro* maturation

After the oocytes were retrieved, they were matured *in vitro*. This operation was also carried out at the Laboratory of Andrology and Assisted Reproduction of the University of Veterinary Medicine in Budapest. The culture medium used for maturation was a serum-reduced medium supplemented with antibiotics (penicillin, streptomycin) and hormones (FSH, LH). Two staining procedures were used to evaluate the *in vitro* maturation results. For oocytes and cumulus cells, the ratio of live to dead cells was assessed using a double staining procedure with propidium iodide and SYBR green. Propidium iodide penetrates into necrotic cells with damaged membranes, allowing apoptotic cells to be separated by this staining procedure. SYBR green is a fluorescent dye that is incorporated into the double-stranded DNA chain and emits green light upon excitation. In summary, the green colour indicates living cells, while the red colour, characteristic of propidium iodide, indicates dead cells.
An important step after *in vitro* maturation is the stripping of the oocytes, the dissolution of the cumulus cells. These were removed in several steps using chemical and physical methods. Basically, a chemical method of digestion in medium containing 0,1% hyaluronidase enzyme was used to remove the cumulus cells surrounding the oocyte, but this method alone was not successful. Therefore, we supplemented this procedure with two physical methods. One of these physical methods was vortexing. The cumulus cells that were still present were removed using a denuding pipette with a very small internal diameter (130/140 µm). The oocytes were moved in and out of the pipette until the cumulus cells were mechanically removed from the oocyte.

2.2. OPU performed on mares

These studies have been performed at the Embryo Transfer Station of the Department of Obstetrics and Food Animal Medicine Clinic of the University of Veterinary Medicine in Üllő, 3 times so far (25.10.2022; 28.11.2022; 12.12.2022). These investigations are still ongoing. OPU was performed on the same 3 mares each time. The procedure was performed under standing sedation, using detomidine hydrochloride (Domosedan 10 mg/ml injection A.U.V., Orion Pharma) and butorphanol (Alvegesic Vet 10 mg/ml, Alpha-Vet Kft, Budapest). A smooth muscle relaxant (Buscopan compositum injection A.U.V., Boehringer Ingelheim Vetmedica

GmbH, Ingelheim am Rhein, Germany) was also used to mobilise the rectum and genital tract. After removal of the feces from the rectum, the mare's tail was bandaged and tied to the side. The perineal region was washed thoroughly with lukewarm water. Before insertion into the vagina, the OPU unit was smeared with sterile lubricant and my supervisor, Dr. Boglárka Vincze, started the manipulation in the rectum after inserting the adapter. The ovary should be oriented towards the vaginal vault on the same side in order to ensure that the follicles can be properly imaged in the field of view of the ultrasound machine. Once the correct position was achieved, the follicular fluid was aspirated using the aspiration pump, which could be operated by foot pedal. Depending on the size of the follicle, the lavage fluid was dosed and the number of irrigations was determined. The tube system opened into a container, into which the aspirated oocytes arrived together with the medium. After the OPU was finished, the contents of the collection vessel were poured into Petri dishes and the COC units were searched for using a stereomicroscope.

2.3. Analysis of AMH levels in 3 horse breeds

2.3.1. The animals examined during the experiment

A total of 23 Lipizzaner, 10 Hungarian Coldblood and 15 Welsh ponies were examined and blood samples were taken from them. The mares were aged between 3–24 years (mean: 14,4 ± 5,5 years). The Lipizzaner mares were examined at the National Stud Farm Szilvásvárad, with a total of four sampling dates scheduled (24. 01. 2022, 30. 03. 2022, 13. 04. 2022, 22. 06. 2022). The 10 Hungarian Coldblood mares all originated from the same private stud. The first sampling took place on 30th November 2022, the second on 26th June 2023. In both cases the sampling was carried out at the Riding Stables of the Mátyás family. The Welsh pony mares were tested both in the Honvéd Riding-Hall in Pasarét and in a privately owned stud in Inárcs. The samples were taken on 3 dates: 06.12.2022 (Honvéd Riding-Hall, Inárcs), 15.06.2023 (Honvéd Riding-Hall), 27.06.2023 (Inárcs).

2.3.2. Procedure of the ultrasound examination and blood sampling

The genital examination was performed by visual inspection of the vulva and the udder, the genital conformation and the presence of vaginal discharge in the mares. This was followed by a rectal reproductive biology examination using an ultrasound machine, performed by Dr. Gabriella Kútvölgyi, veterinarian. She first examined the reproductive organs (cervix, uterus, uterine horns, right and left ovaries) by palpation and then by ultrasound. The follicles in both ovaries were counted and classified according to their size. Blood was taken from the mares by Dr. Boglárka Vincze, veterinarian, from the external jugular vein. Blood was drawn using a sterile 20G needle (BD Vacutainer® Precisionglide™ Cannulas 20G 0.9 x 38 mm, BD Medical, USA), an one-use tube holder (BD Vacutainer® Holder BD Medical, USA) and serum blood collection tubes (Vacutainer® Serum Tube, BD Medical, USA). Immediately after sampling, the collected blood samples were transported to the Laboratory of Andrology and Assisted Reproduction at the University of Veterinary Medicine in Budapest, where they were centrifuged at 3000 x g for 10 minutes. After centrifugation, serum was separated into labeled sampling tubes and samples were frozen.

2.3.3. Measurement of AMH levels and biochemical tests

Measurement of anti-Mullerian hormone levels in blood samples was performed using the Equine AMH ELISA kit manufactured by AnshLabs (Webster, Texas, USA) at the Laboratory of Andrology and Assisted Reproduction, University of Veterinary Medicine in Budapest. Measurements were performed using a 96-well ELISA plate and a sample volume of 50 µL was required from each animal. All samples were thawed prior to testing and enzyme-linked immunosorbent assay was performed as described in the manufacturer's manual.

The biochemical tests were carried out at the Faculty of Agriculture, Food Science and Environmental Management, Department of Animal Husbandry, University of Debrecen, using a LAB-ANALYSE Practical-Vet clinical chemistry semi-automated system (Orvostechnika Kft., Hungary). From serum samples, the following parameters were analysed: alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), phosphorus (PHO), urea (UREA), creatinine (CREA), cholesterol (CHOL), triglyceride (TG), magnesium (MG), potassium (K). These parameters were analysed by implementing kinetic, end-point and two-point measurements. After thawing the serum samples, the measurements were performed according to the manufacturer's instructions, which differed from parameter to parameter. The amount of serum sample required for the measurements varied from 5 to 50 microlitres depending on the parameter.

3. Results

3.1. *Post mortem* ovarian examination

3.1.1. *Post mortem* oocyte retrieval results

During my PhD work, a total of 50 ovaries were processed. In total, 268 follicles were aspirated and 103 oocytes were retrieved. The distribution of follicles by size is shown in Table 1. It can be seen that the largest number of follicles extracted were small (less than 10 mm in diameter).

Table 1

Size distribution of follicles

Follicle size	Number	Percentage
<10 mm	106	39,5%
10–20 mm	90	33,6%
> 20 mm	72	26,9%

The success of OPU and, more generally, of oocyte retrieval procedures can be quantified by the so-called oocyte recovery rate (RR). This can be calculated using the following equation:

$$RR = \frac{\text{number of COCs}}{\text{number of follicles punctured}} \times 100$$

In my own study, the recovery rate was 38.4%.

When classifying the COCs according to quality, I found that the highest number (51%) of oocytes that I was able to extract were those that were surrounded by compact cumulus cells in more than 4 cell lines. The classification of COCs by quality is shown in Figure 1.

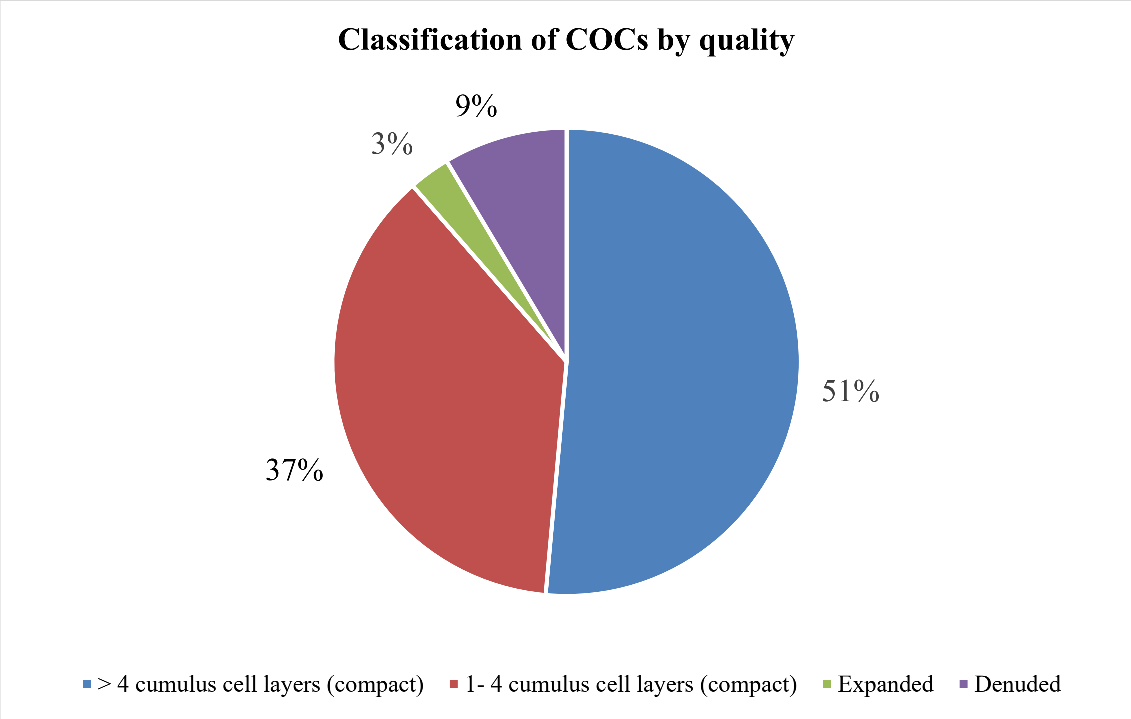

Figure 1: **Classification of COCs by quality**

3.2. Results of AMH level measurements

3.2.1. Relationship between AMH levels and follicle count

a) For all animals

A total of 83 samples were measured for AMH levels during my PhD studies. For all animals, I investigated whether AMH concentration was related to the number of small (<10 mm), medium (10–20 mm), large follicles (>20 mm) and total follicle number. A significant correlation was found between the total number of large follicles and AMH levels (p = 0,009), the data were plotted on a dot plot (*Figure 2*). The higher the number of large follicles present in the ovary, the higher the concentration of AMH. In contrast, there was no statistically significant correlation between the small, medium and total follicle counts and AMH levels (p = 0,13; p = 0,2; p = 0,48).

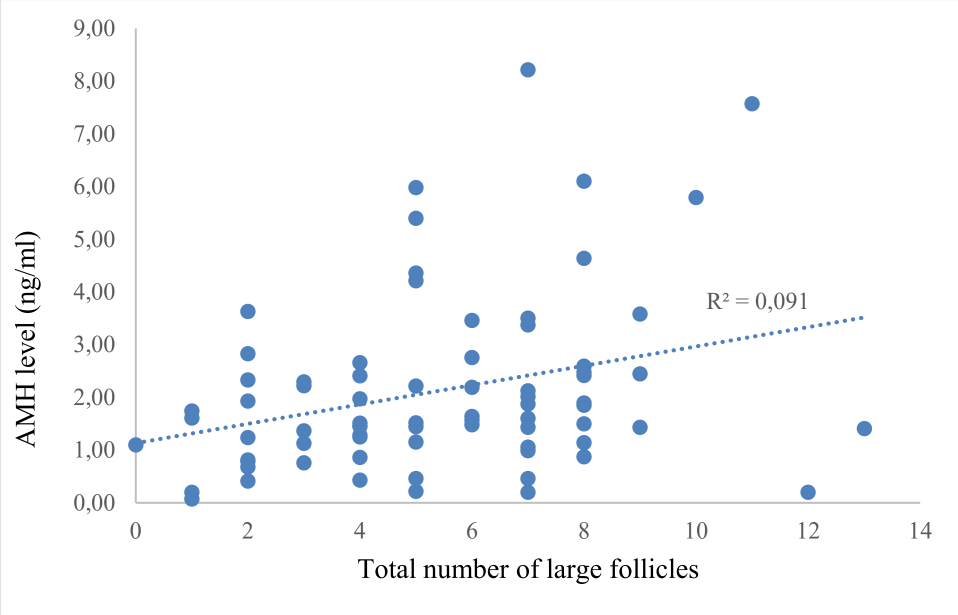

Figure 2: **Relationship between AMH levels and the total number of large follicles**

($y = 1,13 + 0,18*x$; $R^2 = 0,09$)

b) By age group

Next, all mares were grouped into three age groups. Young mares are those aged 1 to 5 years, middle-aged mares are those aged 6 to 15 years, and old mares are those older than 15 years. Only 2 animals were included in the young mares group, so there was little data available for correlation analyses for this group. In the middle-aged group, no significant correlation between AMH level and follicle number was observed. The strongest correlation in this group was observed between the total number of large follicles and AMH level (*Figure 3*), but this correlation was not confirmed statistically ($p = 0,15$). Data were plotted on a dot plot and a trend line was fitted. The resulting function equation was: $y = 1,46 + 0,16*x$. The correlation between total follicle count and AMH level in middle-aged mares was not confirmed statistically ($p = 0,79$).

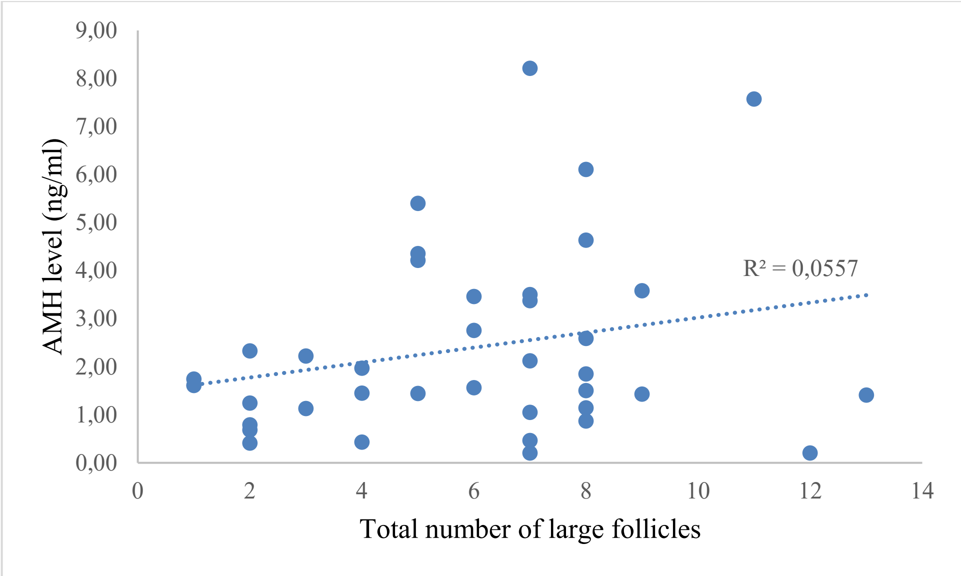

Figure 3: **Relationship between AMH levels and total number of large follicles in middle-aged mares**

(y = 1,46 + 0,16*x; R^2 = 0,06)

However, in the group of old mares, a statistically proven correlation between the total number of large follicles and AMH levels was obtained (p = 0,031). The data were again plotted on a dot plot and a trend line was drawn (*Figure 4*), which can be described by y = 1 + 0,19*x. However, for this age group, the correlation between total follicle count and AMH concentration was not confirmed statistically (p = 0,39).

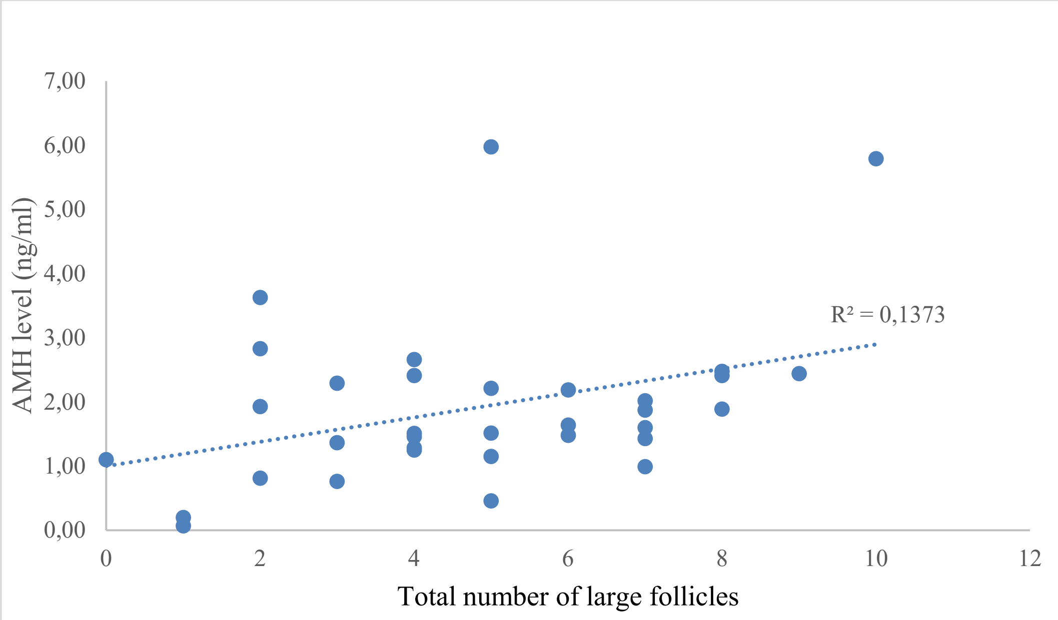

Figure 4: **Relationship between AMH levels and total number of large follicles in old mares**

(y = 1 + 0,19*x; R^2 = 0,14)

c) By breeds

I also analysed whether, if the 3 different breeds were examined separately, any correlation between hormone concentration and follicle number could be found. I could not find a significant correlation in the Hungarian Coldblood breed. However, in the ponies, I found a statistically proven correlation between the total number of large follicles and AMH concentration (p = 0,04). The resulting data are plotted on a dot plot (*Figure 5*), with the trend line drawn as y = 0,85 + 0,13*x.

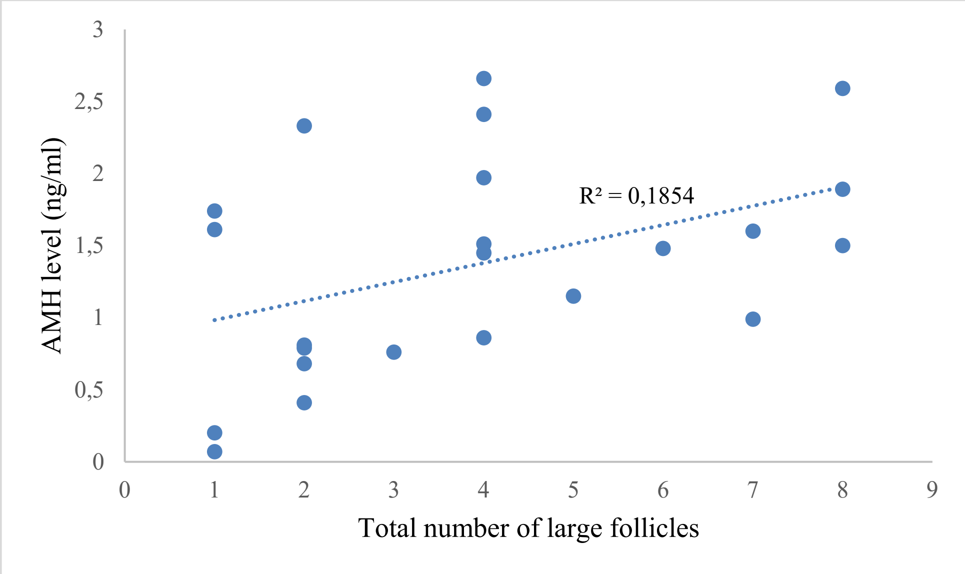

Figure 5: **Relationship between AMH levels and total number of large follicles in pony mares**

(y = 0,85 + 0,13*x; R^2 = 0,19)

In Lipizzaner mares, I found significant correlation in three cases: between the total number of medium follicles and AMH levels (p = 0,02), between the total number of large follicles and AMH concentration (p = 0,02) (*Figure 6*), and between total follicle count and hormone levels (p = 0,03) (*Figure 7*). The latter two relationships are plotted as a dot plot, with the two trend lines drawn. The trend line is described by y = 1,05 + 0,32*x for large follicles and y = 1,1 + 0,12*x for all follicles.

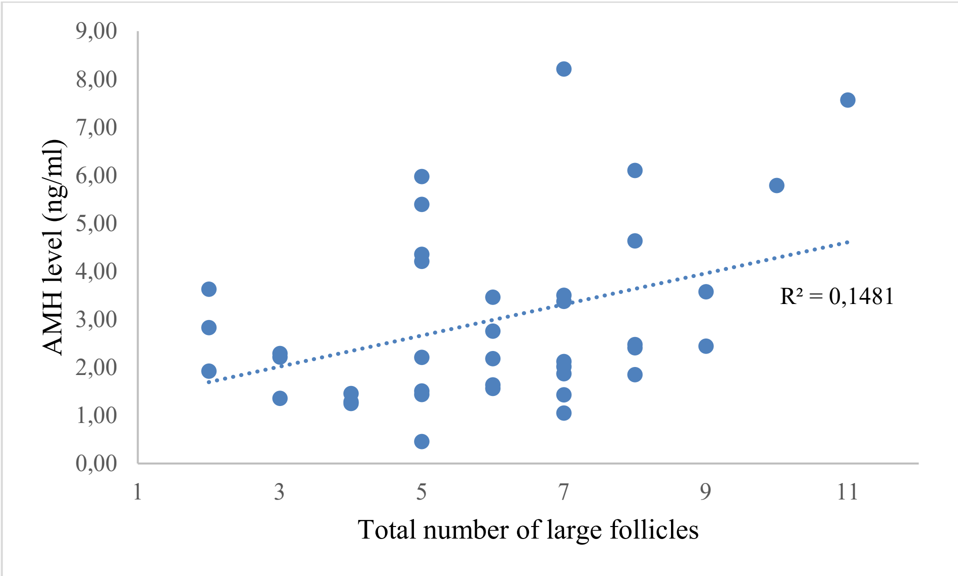

Figure 6: **Correlation between AMH levels and total number of large follicles in Lipizzaner mares**

(y = 1,05 + 0,32*x; R^2 = 0,15)

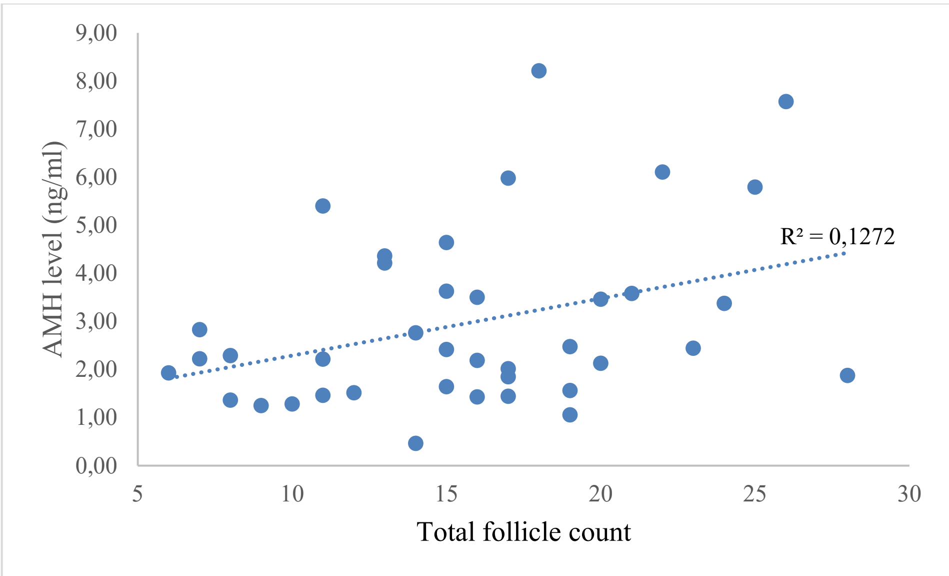

Figure 7: **Relationship between AMH levels and total follicle count in Lipizzaner mares**

(y = 1,1 + 0,12*x; R^2 = 0,13)

3.2.2. Relationships between AMH levels and age

a) For all animals

When evaluating AMH concentrations in all mares (n = 83), I could not detect a significant relationship between hormone concentration and age of the mares (p = 0,77). These data were plotted on a dot plot with age groups (*Figure 8*). It can be seen that the highest values were measured in middle-aged mares, while the hormone concentration decreased with increasing age.

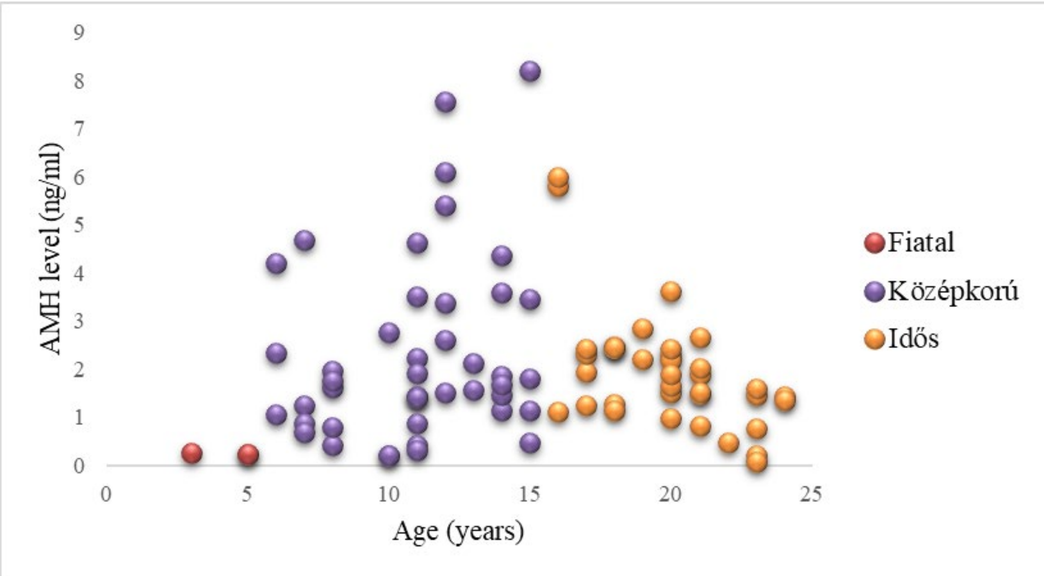

Figure 8: **Relationship between age and AMH levels**

The AMH concentration varied between 0,07 and 8,21 ng/ml, with a median value of 1,64 ng/ml. The age of the mares varied from 3 to 24 years, with a mean age of 14,6 years. The mean AMH concentration was 0,45 ng/ml for young animals, 2,32 ng/ml for middle-aged animals, 1,94 ng/ml for old mares and 2,09 ng/ml for all animals.

b) By age group

In the case where only middle-aged mares were examined, there was no statistically significant correlation between AMH levels and age (p = 0,17) (*Figure 9*). While a significant correlation was found in the group of older mares (p = 0,001) (*Figure 10*). For both groups, I plotted the data on a dot plot with trend lines (*Figures 9* and *10*). The function equation obtained in this way was y = 0,75 + 0,14*x for the middle-aged group and y = 7,66 – 0,29*x for the old mares.

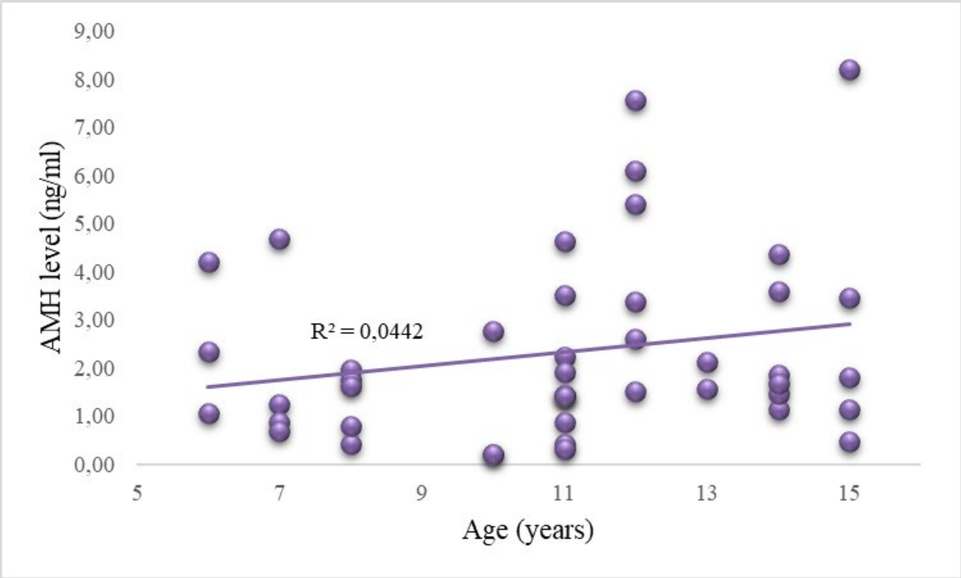

Figure 9: **Relationship between AMH levels and age in middle-aged mares**

(y = 0,75 + 0,14*x; R^2 = 0,04)

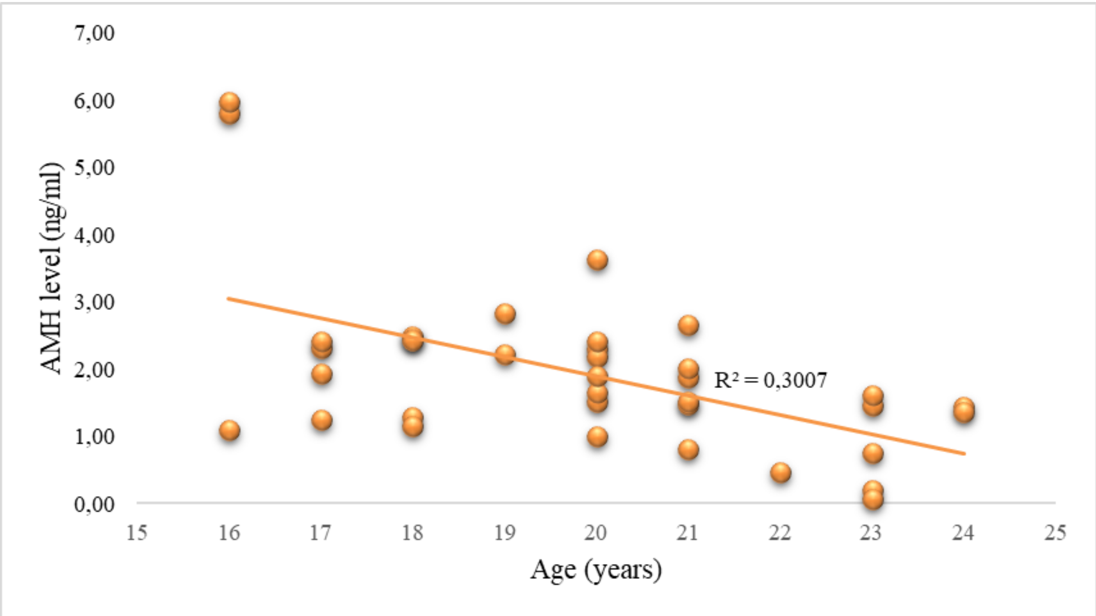

Figure 10: **Relationship between AMH levels and age in older mares**

(y = 7,66 – 0,29*x; R^2 = 0,3)

In the middle-aged mares, AMH concentrations increased with age, while the opposite result was obtained in the group of older mares, where a decrease in AMH levels was observed.

c) By species

I studied the breed composition within each age group. For the middle-aged mares, I examined Lipizzaner (40,9%), Hungarian Coldblood (34,1%) and Welsh Pony (25%) mares (*Figure 11*). However, within the older age group, I analysed data mainly from Lipizzaner (58,3%) and Welsh Pony (38,9%) mares, with only one Hungarian Coldblood (2,8%) mare belonging to this age group (*Figure 12*).

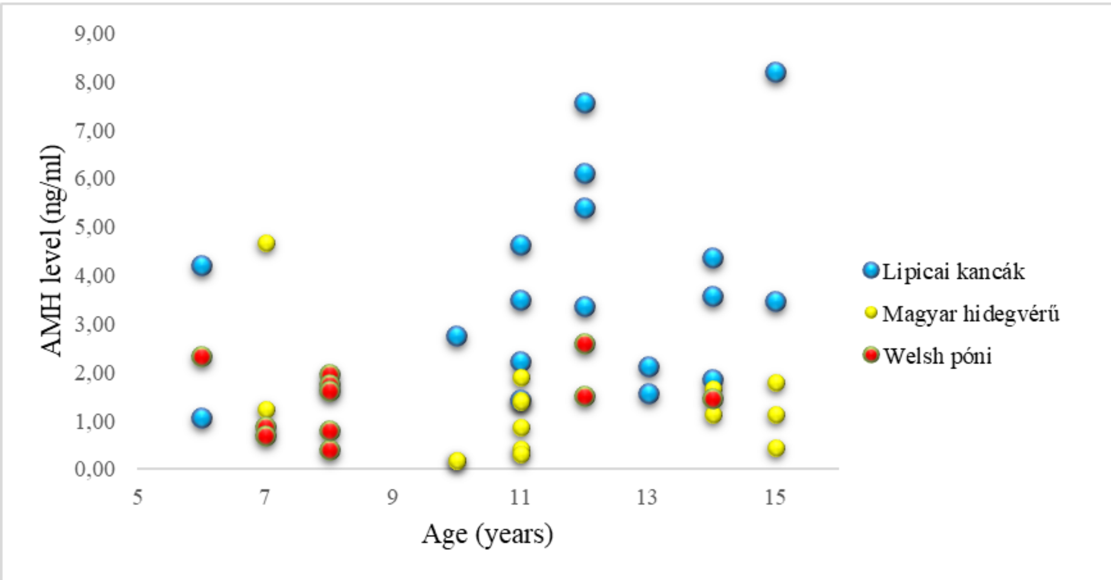

Figure 11: **Relationship between AMH levels and age in the middle-aged group with breeds indicated**

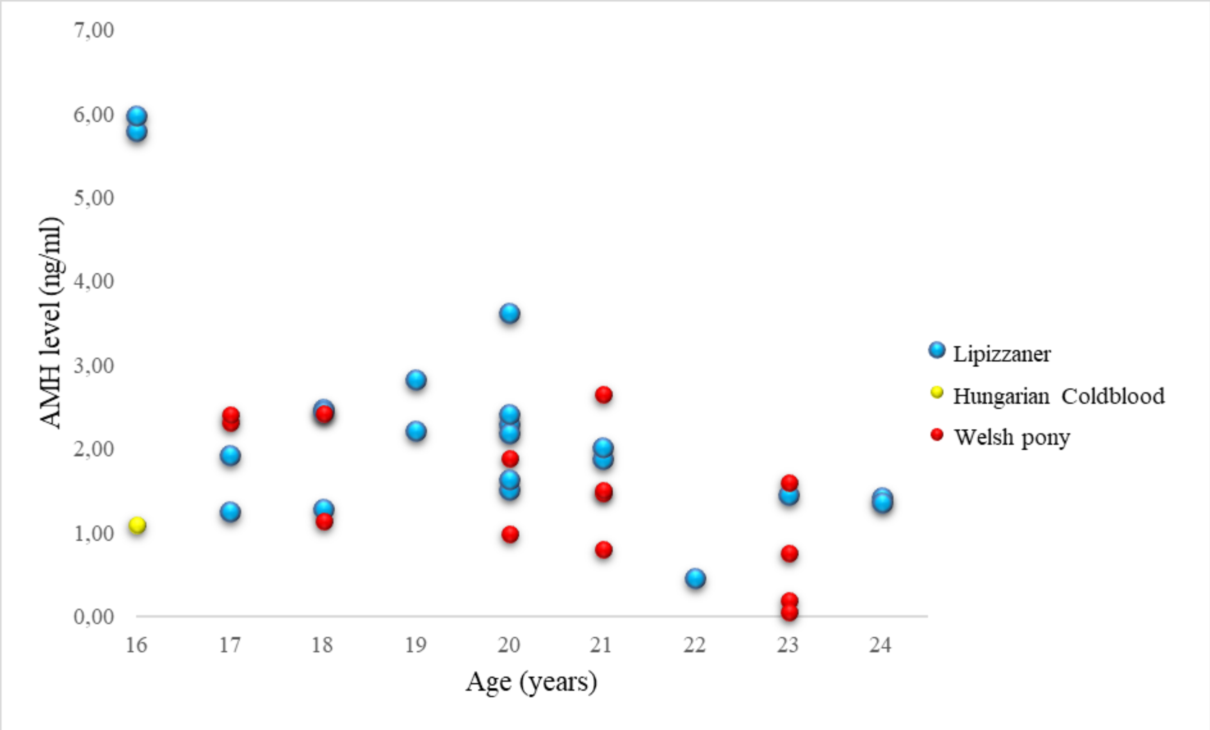

Figure 12: **Relationship between AMH levels and age in older mares with breeds indicated**

Correlations in the group of older mares were therefore only analysed for Lipizzaner and Welsh pony mares. In this age group, the correlation between age and AMH levels was statistically confirmed for both ponies and Lipizzaner mares. As before, the relationship between AMH levels and age was was plotted on a dot plot (*Figures 13* and *14*). The trend line is described by y = 6,5 – 0,25*x for Welsh ponies and y = 9,1 – 0,35*x for Lipizzaner mares.

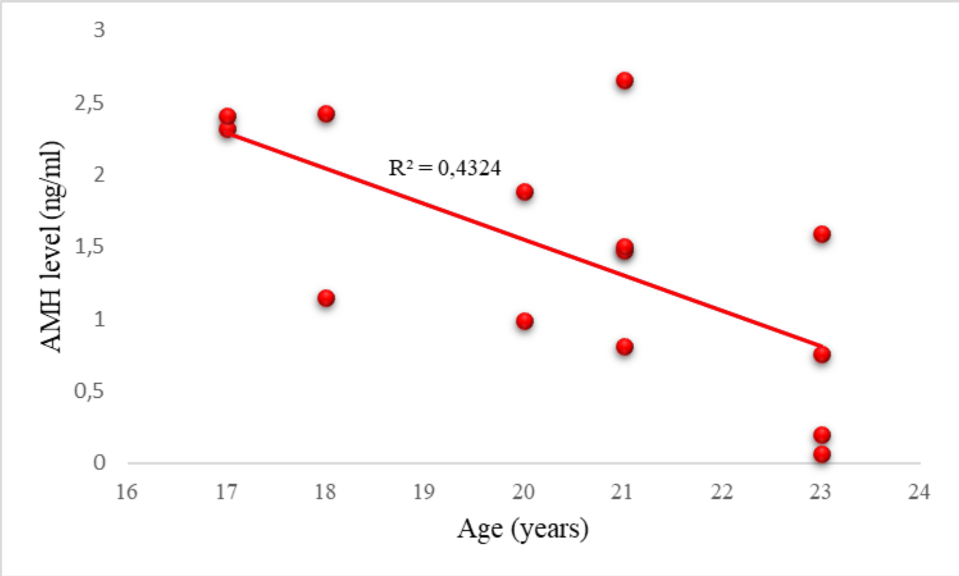

Figure 13: **Relationship between AMH levels and age in aged Welsh pony mares**

(y = 6,5 – 0,25*x; R^2 = 0,43)

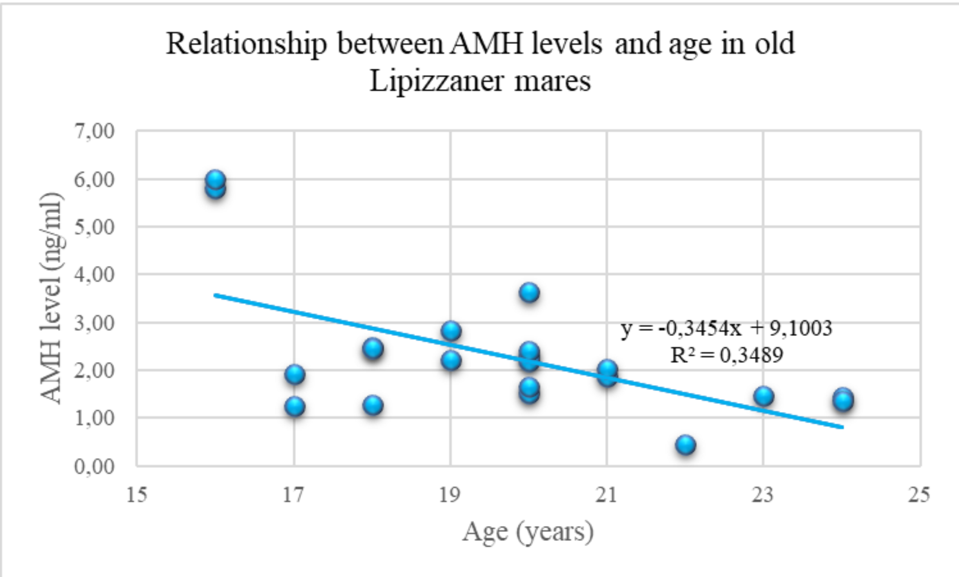

Figure 14: **Correlation diagram of AMH levels and age in old Lipizzaner mares**

(y=9.1-0.35*x; R2 = 0.349)

A negative correlation was also observed in the older Welsh pony and Lipizzaner mares, so in general, the higher the age of the animals, the lower their AMH levels.

3.2.3. Examining the differences between the first and second measurements for the three horse breeds

I investigated the mean AMH levels in each breed at the two measurement times and whether there was a significant difference in hormone concentration between the two measurement times using a paired t-test. *Table 3* shows that the highest mean value was measured in Lipizzaner mares and the lowest in Hungarian Coldblood mares. There was no significant difference between the results of the two measurements for either breed.

Table 3

Average AMH levels at the two measurement times and analysis of the difference between measurements

	Measurement	Number of mares examined	Average of AMH levels (ng/ml)	Significance value of the difference between means
Lipizzaner	1.	12	3,09	0,43
	2.	12	3,48	
Hungarian Coldblood	1.	8	0,75	0,1
	2.	8	1,53	
Welsh pony	1.	11	1,3	0,57
	2.	11	1,47	

3.2.4. Examining the impact of the seasons

a) Analysis of AMH levels in the two different seasons

For Lipizzaner mares, samples were taken in January, March, April and June 2022, for this breed the samples for the autumn-winter season are from January and the samples for the spring-summer season are from March and April. For the Hungarian Coldblood mares, the November 2022 samples were included in the autumn-winter samples, while the June 2023 samples were included in the spring-summer season. For Welsh pony mares, the December

2022 samples were placed in the autumn-winter group, while the June 2023 samples were placed in the spring-summer season samples.

Table 4

Mean AMH levels for the three breeds in the autumn-winter season

	Autumn-winter season			
	Lipizzaner (n = 14)	Hungarian Coldblood (n = 10)	Welsh pony (n = 14)	Total (n = 38)
Average AMH levels (ng/ml)	3,00	0,82	1,44	1,85
Minimum value of AMH levels (ng/ml)	1,28	0,20 ✰	0,20 ✰	0,20
Maximum value of AMH levels (ng/ml)	7,57 ✪	1,43	2,59	7,57

✰: *lowest value*

✪: *highest value*

As shown in Table 4, the lowest value (0,20 ng/ml, indicated in the table as ✰) was measured in two mares during the autumn-winter period, a Hungarian Coldblood and a Welsh pony mare. In contrast, the highest value (7,57 ng/ml, marked ✪ in Table 4) was measured in a 12-year-old Lipizzaner mare. During this period, the average AMH levels were highest in Lipizzaner mares (3,00 ng/ml) and lowest in Hungarian Coldblood mares (0,82 ng/ml).

Table 5

Mean AMH levels for the three breeds in the spring-summer season

	Spring-summer season			
	Lipizzaner (n = 21)	Hungarian Coldblood (n = 8)	Welsh pony (n = 12)	Total (n = 41)
Average AMH levels (ng/ml)	3,04	1,53	1,42	2,27
Minimum value of AMH levels (ng/ml)	0,46	0,20	0,07 ✰	0,07
Maximum value of AMH levels (ng/ml)	8,21 ✪	4,69	2,66	8,21

✰*: lowest value*

✪*: highest value*

The lowest AMH concentration in the spring-summer period was 0,07 ng/ml. It was interesting for us that this value was measured in the same 23 year old Welsh pony mare that had the lowest value during the autumn-winter period. However, this minimum value was lower than the minimum value for the autumn-winter period (0,20 ng/ml) and the highest AMH concentration was 8,21 ng/ml during the spring-summer period and throughout the study. In Lipizzaner and Hungarian Coldblood mares, the mean AMH levels during this period were higher than the mean levels in the autumn-winter period.

I performed a homogeneity test, which showed a large difference between the variance of the three breeds for both the spring-summer and autumn-winter periods ($p = 0{,}04$; $p = 0{,}005$). Therefore, I did not use ANOVA, but a more robust test, Welch's test, which showed a difference between the means of the groups for both periods. In order to analyse the differences between breeds more precisely, I performed a Post Hoc test. In the autumn-winter period, I found a significant difference (p = 0,002) between the AMH levels of Lipizzaner mares and Hungarian Coldblood mares. There was no significant difference between the AMH levels of Hungarian Coldblood and Welsh pony mares and between Lipizzaner and pony mares in this period. A significant difference was found between AMH levels in Lipizzaner and Welsh pony mares during the spring-summer period (p = 0,006).

3.2.5. Results of biochemical laboratory tests

Biochemical analyses were performed on the Hungarian Coldblood and Welsh pony mares, with a total of 9 parameters (alkaline phosphatase, gamma-glutamyl transferase, phosphorus, urea, creatinine, cholesterol, triglyceride, magnesium, potassium). As two samples were taken from each of the two breeds, we were able to measure the biochemical parameters in addition to AMH levels on two occasions. I investigated whether a correlation between biochemical parameter values and AMH levels could be observed, but found that no statistically proven correlation with hormone concentrations could be found for any of the parameters. However, significant differences between the mean values of the two breeds were found for the following parameters: UREA1 (p = 0,02), P1 (p = 0,01), CHOL1 (p = 0,002), KREA2 (p = 0,04), TG1 (p = 0,02), TG2 (p = 0,05), GGT2 (p = 0,02).

4. New scientific results

1. For anti-Müllerian hormone (AMH) tests, there was a significant correlation between the total number of large follicles (>20 mm) and AMH levels in all mares (Lipizzan, Welsh pony, Hungarian cold-blooded), in the group of older mares (over 15 years), in Welsh ponies and in Lipizzaner mares (p = 0,01; p = 0,03; p = 0,04; p = 0,02).

2. In Lipizzaner mares, a significant correlation was found between the total number of medium (10–20 mm) follicles and anti-Müllerian hormone levels, and between total follicle number and hormone levels (p = 0,02; p = 0,03). However, in Hungarian Coldblood mares, no significant correlation was found in any case.

3. The concentration of anti-Müllerian hormone varied between 0,07 and 8,21 ng/ml for all mares tested, with a median value of 1,64 ng/ml. The mean AMH concentration was 2,32 ng/ml for middle-aged mares (6–15 years), 1,94 ng/ml for old mares (over 15 years) and 2,09 ng/ml for all animals. In the group of old mares, old Welsh ponies and old Lipizzaner mares, there was a significant correlation between age and AMH levels (p = 0,001; p = 0,01; p = 0,01).

4. A significant difference was found between the anti-Müllerian hormone concentrations of Lipizzaner mares and Hungarian Coldblood mares during the autumn-winter period and between Lipizzaner and Welsh pony mares during the spring-summer period (p = 0,002; p = 0,01).

5. There was no significant correlation between any of the biochemical parameters studied (alkaline phosphatase, gamma-glutamyl transferase, phosphorus, urea, creatinine, cholesterol, triglyceride, magnesium, potassium) and AMH levels. However, there was a statistically significant difference between the mean values of urea, phosphorus, cholesterol, triglyceride in the first sampling of Welsh ponies and Hungarian Coldblood mares (p = 0,02; p = 0,01; p = 0,002; p = 0,02) and creatinine, triglyceride and gamma-glutamine transferase in the second sampling (p = 0,04; p = 0,05; p = 0,02).

5. Important results of the thesis for practice

1. The oocyte recovery rate (38,4%) in the processing of abattoir ovaries was lower than reported in the literature. However, the practical experience gained from the development of this combined method may help to improve the efficiency of oocyte retrieval from the ovary after the sudden death of a mare.

2. In the populations (Lipizzaner, Hungarian Coldblood, Welsh pony) we studied, it was basically the large follicles (>20 mm) that determined anti-Mullerian hormone levels, not the small (<10 mm) and medium-sized (10–20 mm) ones.

3. Blood concentrations of AMH showed differences between different types of horses (warmblood, coldblood, pony), so this should be taken into account when designing future experiments.

4. AMH concentrations showed no correlation with blood biochemical parameters.

5. The season of the year had different effects on AMH levels in different types of horses.

The aforementioned correlations may help to better map the function of AMH and its usefulness in reproductive biology work in mares.

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7. List of publication

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Subject: PhD Publication List

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Doctoral School: Doctoral School of Animal Husbandry
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List of publications related to the dissertation

Hungarian scientific articles in Hungarian journals (3)

1. **Angyal, E.**, Somoskői, B., Török, D., Bordás, L., Cseh, S., Novotniné Dankó, G., Vincze, B.: Az anti-Müller-hormon (AMH) mennyiségének összefüggése a petefészekképletekkel és a petesejttartalék kimerülésével kancában és tehénben.
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6. **Angyal, E.**, Vincze, B., Novotniné Dankó, G., Cseh, S., Somoskői, B., Török, D., Bordás, L., Kútvölgyi, G.: Az Anti-Müller hormon (AMH) mennyiségének összefüggése a petefészek-képletek előfordulásával és az életkorral lipicai kancáknál.
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Animals (Basel). 12 (15), 1-11, 2022. EISSN: 2076-2615.
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