

Hormonal activity of pregnant mare serum of different age and pregnancy terms at donors

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Abstract. The effective way of selection of pregnant mares as producers of the gonadotropic hormones, promoting improvement of quality of preparation of a preparation of pregnant mare serum with high hormonal activity and an optimum ratio of FSH/LH is developed. Biological qualities of pregnant mare serum of different age and pregnancy terms - hormonal activity, a ratio of follicle-stimulating and luteinizing hormones for preparation of a hormonal biological product of PMS (pregnant mare serum) with the use of methods of diagnostics of a pregnancy of mares on mature males of lake frogs, and the immunoferrmental analysis for definition of a ratio of hormones in blood plasma are investigated. On a variety of activity of PMS and FSH and LH ratio in blood serum essential influence renders to the age of donor mares and their terms of a pregnancy. The influence of age of pregnant mares on FSH/LH ratio in blood serum, received from them is 0.107 ± 0.046 with reliability at level of the first probability threshold ($P < 0.05$), and influence terms of a pregnancy of donors on level of concentration of gonadotropic hormones in serum is authentic above at the significance of $P < 0.001$ (0.447 ± 0.033). The influence of age of donors on activity of PMS and on FSH/LH ratio in serum is not more than 9.9 % and 20.4 % respectively, and influence of FSH/LH ratio on activity of PMS is not less than 63.6 % and not more than 73.2 %. On the basis of the carried-out pilot studies the new way of selection of pregnant mares as producers of gonadotropic hormones is developed for production of PMS with the high activity. Thus, the optimum day for blood draw from 3-5 year donor mares is considered between 65-80 days of pregnancy. For donors of 6-8 years the best term of the period is from 60 to 75 day and for advanced age of 9 and 10 years is from 55 to 65th day of pregnancy.

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Introduction

Animal husbandry is one of leading branches of agrarian sector of the Republic of Kazakhstan (Umbitaliev A.D., 2013). In the report of FAO "Animal Genetic Resources for Food and Agriculture, Rome, Italy" (2007) it is noted that animal husbandry is a dynamic branch of agriculture which 40 % of gross output of agriculture are the share and provides means of livelihood and food security for nearly billion people on a planet (FAO Guidelines for the In Vivo Conservation of Animal Genetic Resources, 2007).

Currently used traditional methods of reproduction and selection of animals are not completely open the potential of possibilities of breeding animals that essentially constrains genetic improvement of domestic breeds and creation of highly productive competitive animals (Olliver L., 1999). Therefore for the purpose of the more large-scale use of their genetic potential in selection and breeding programs of improvement of planted breeds in modern conditions of intensive maintaining animal husbandry the special urgency is got by bioprocessing methods and methods of receiving descendants from

high-valuable animals. The most progressive contribution to the solution of problems of a sustainable development of animal husbandry at the present stage is a creation of complex system of agricultural biotechnology and their introduction in production (Rege JEO, 1994).

In biotechnology of the accelerated reproduction of high-valuable genotypes and preservation of disappearing, rare populations by superbasis of technological processes is causing polyovulation of potential donor females the with use of hormonal preparations (Urov I., 2001).

Now in this regard application of hormones and hormonal and active preparations for the purpose of the directed regulation of process of reproduction and fertility of animal of different types of efficiency is extremely important (Jensen P., Andersson L., 2005). It is especially necessary and perspective in connection with an increasing tendency in our republic to large-scale animal industry. However, today in the republic own hormonal preparation for induction of polyovulation and increase of fertility of agricultural animals are not made. The use of hormonal preparations in biotechnology of

reproduction of animals is generally delivered from the foreign countries which introduction and is complicated with acquisition and the high price of these preparations. Therefore there is a possibility to adjust production and release the quality of domestic gonadotropic preparations as the alternative for import. Especially, without hormonal preparations introduction of biotechnological methods in the accelerated reproduction of highly productive agricultural animals is impossible. Therefore, the production of hormonal preparations and their use in the accelerated reproduction of agricultural animals is a topical issue (Svitoyus A.G., 1989; Ashimova I.D., 2012).

The use of gonadotropic hormones for agricultural animals promotes the formation of additional follicles (Jamalova G.A., 2004), maturing of two, three and more ova capable to fertilization since its glycoprotein unites properties of luteinizing and follicle-stimulating hormones (William T. Moore, Jr. and D.N. Ward, 1980).

The main source of production of gonadotropic hormones is the pregnant mare serum representing sterile serum (Allen W.R., 1970), received of blood of healthy mares during the period from the 40th day to the 100th day of pregnancy (Cole H.H., Hart G.H. 1930).

Pregnant mare serum as the basis of a hormonal method applied in animal husbandry for many years, allowing to operate sexual processes in an organism of an animal to strengthen rates of expanded reproduction (Murphy B.D., 2012) and to increase the efficiency of animal husbandry (Ian R. Gordon, 2004).

However, variability of exit of gonadotropic hormones and its activity in the preparations received from blood serum at the same pregnant mares is very high and not always promotes receiving positive results (Licht P., Gallo A.B., Aggarwal B.B., Farmer S.W., Castelino J.B., Papkoff H., 1979; Paducheva AL, Boyko D.F., 1965).

This factor considerably interferes with effective production of GPMS and management of reproduction process at high-valuable genotypes of agricultural animals by a hormonal method (Fedorov, 2005).

Proceeding from the foregoing, it is necessary to study and improve technologies of selection of pregnant mares as PMS donor producers. As concentration of a hormone in blood of pregnant mares in steps raises or decreases from 40th day to the 100th day of pregnancy (Cole H.H., F.J. Saunders, Published Online: July 01, 2013).

In this regard production of a gonadotropic hormone of PMS with high activity is actual.

Material and Methods

The work has been carried out on Biotechnology department of the M. Auezov Southern Kazakhstan state university, in Biotechnology laboratories of Southwest research institute of animal husbandry.

As an object of research donor mares of breed "jabe" in number of 16 heads, different age (3-10 years) and in different terms of a pregnancy (40-80 days) have been served.

As material of research pregnant mare serum (PMS) has served.

The studied animals have been selected in private country enterprises "Zhantore" and "Zhaylau" of the Southern Kazakhstan region.

The terms of a pregnancy of donor mares were determined by copulation date. In 40-45 days after copulation the diagnostics of a pregnancy of mares on mature males of lake frogs has been carried out.

For carrying out diagnostics of a pregnancy of mares from each donor mare 10 ml of blood have been taken (Shatalov P.I., 1975).

Hormonal diagnostics of pregnancy of mares was carried out on mature males of lake frogs in length 10-17 cm. Males differ from females by callosities of dark grey color on internal fingers of forward extremities and voice bags, resonators of dark grey color which are in mouth corners; in the inflated condition they light grey. From above of dense green, olive, grey or brown colour of various shades. On a back at many individuals are located large, small spots and/or a light longitudinal strip, sometimes greenish are much rarer.

Frogs are contained in the artificial reservoirs having sites of a land. Before statement of reactions check, that there is no sperm in their urine. Four this males were wrapped up in a wet gauze, into a fowl place on depth of 0.5 cm enter a Pasteur pipette, take a drop of urine and put it on subject glass, impose integumentary glass and view under a microscope. The males in which urine had no spermium were selected.

The selected males are introduced into a back lymphatic bag 2 ml of fresh not concentrated blood serum of donor mares. After they were placed in glasswares, contained without water and in 2-4 hours after preparation introduction from frogs take urine and investigate it under a microscope.

The gonadotrophin of blood of pregnant mares causes emergence of sperms in urine of males of lake frogs. The reaction is considered positive if in sight of a microscope sperms are found in two of three experimental males; negative, if sperms were not present at all three frogs.

After establishment of a pregnancy the donor mares proceed for a blood draw.

Determination of hormonal activity of PMS was carried out in laboratory on transplantation of embryos of RSE "South-western scientific production center of agriculture", on a white laboratory mouse according to the standard technique.

Definition of follicle-stimulating and luteinizing hormones in plasma of blood of pregnant mares made on a microstrip photometer "Stat Fax 2100" intended for the immunofluorescent analysis in the following order:

The ready calibration test and control serum was used.

1. Strips. Before opening a package maintained in room temperature within 30 minutes.

2. Flushing buffer. For this purpose native serum was diluted with 10-fold volume of the distilled water, carefully mixing, avoiding foaming.

3. Anti-FSHLH-peroxidase conjugate was used.

4. TMB solution and stop reagent was used.

Carrying out analysis

1. All reagents before carrying out the analysis are led to room temperature (20-25°C).

1.1. In all holes except for A1 A2 have brought on 100 µl of solution of anti-FSHLH-peroxidase.

1.2. Then in the corresponding holes on 50 µl calibration tests and control serum, and in the remained holes on 50 µl studied tests (in two parallels). Thus, introduction of tests is necessary for carrying out within 2-3 minutes. Afterwards stirred up a tablet and were convinced that the bottom of each hole is covered with a sample.

1.3. Incubated strips of 60 minutes when stirring in a thermostate shaker at room temperature, with a speed 500-800 rpm.

1.4. Upon termination of incubation have removed contents from holes and have washed a tablet.

1.5. Brought in all holes 100 µl of working solution of conjugate and again incubated strips within 60 minutes on a shaker at room temperature.

1.6. Brought in all holes 100 µl of TMB substratum (tetramethylbenzidine) by means of a multichannel pipette and have put on a tablet for 10-20 minutes at room temperature while fermentative reaction has not given dark blue coloring. After that reaction was stopped by addition in each hole of stop reagent (10 µl 5 % of solution of sulfuric acid). Thus contents of holes were painted in brightly yellow color. Then at once measured optical density in holes on a photometer.

The calculation of results of concentration of follicle-stimulating and luteinizing hormones in serum

of a surveyed sample made by means of multichannel platespectrophotometer at length of a wave 450 nanometers.

The results of researches received in the course of test works, were processed by a method of variation statistics (N.A. Plokhinsky, 1980).

Results and discussion

The important theoretical and practical value has the determination of gonadotropic activity and the maintenance of follicle-stimulating and luteinizing hormones in pregnant mare serum (*Farmer S.W., Parkoff H.* 1979; *Papkoff H.* 1981).

Hormonal activity of blood serum taken from pregnant mares of different age and in various terms of a pregnancy is defined.

Table 1. Hormonal activity of PMS at donor mares of different age

| Age of donors | Number of blood samples | Indicators | |
|---------------|-------------------------|---------------|-----------|
| | | M±m | Cv±mq |
| 3 | 18 | 184.17±18.671 | 43.0±7.17 |
| 4 | 18 | 213.88±16.851 | 33.4±5.17 |
| 5 | 18 | 218.05±17.828 | 34.7±5.78 |
| 6 | 18 | 237.78±16.871 | 30.1±5.02 |
| 7 | 18 | 238.61±15.304 | 27.2±4.53 |
| 8 | 18 | 236.39±15.849 | 28.4±4.73 |
| 9 | 18 | 225.55±14.833 | 27.9±4.65 |
| 10 | 18 | 221.11±14.214 | 27.2±4.53 |

Apparently from data from table 1, the lowest activity of blood serum was observed at the 3-year donor mares and was 184.17±18.671 m.e./ml., high activity is established in serum of the blood, received from donor mares at the age of 6-8 years. Reliability of difference by activity of pregnant mare serum between the 3-year and 6-8 year donors corresponds to the first threshold of probability ($P < 0.05$). Between other groups of animals by activity of a gonadotrophin in serum of authentic distinction is not revealed ($P > 0.05$).

It should be also noted, with increase in age of mares of donors, that the factor of variability of activity of gonadotropic hormones essentially decreases by 15.8 %.

From this it is possible to conclude that the fluctuation and variability of activity of a

gonadotrophin in blood serum at pregnant mares is stabilized with the age.

The minimum activity of gonadotropic hormones is established in serum of donor mares with term of a pregnancy of 40 days. Serums of donor mares received on 45-55 days of pregnancy were intermediate by activity. The samples of blood received from mares - donors for 60-65 days of a pregnancy difined high activity, and have made in limits from 265.0±10.68 m.e./ml. to 284.4±6.31 m.e./ml. On the average on groups of donors this data was 221.9±5.95 m e., with average factor of variability 32.1±1.89 %. As a whole, accumulation of gonadotropic hormones rises till 65th day of a pregnancy and after that gradual decrease in hormonal activity begins. It should be noted also that with the increase of concentration of gonadotropic hormones in blood serum factors of variability of their activity decreases.

During researches the hormonal status of donor mares of different age has been defined.

Table 2. Hormonal status of donor mares of different age

| Age of donors | The number of samples | Activity of PMS | | |
|---------------|-----------------------|--------------------|------------------------|--------------------------|
| | | High (250-320m.e.) | Average (160-240 m.e.) | Low (150 m e. and lower) |
| 3 | 18 | 38.9 | 22.2 | 38.9 |
| 4 | 18 | 50.0 | 22.2 | 27.8 |
| 5 | 18 | 38.9 | 38.9 | 22.2 |
| 6 | 18 | 61.1 | 27.8 | 11.1 |
| 7 | 18 | 55.6 | 33,3 | 11.1 |
| 8 | 18 | 61.1 | 27.8 | 11.1 |
| 9 | 18 | 50.0 | 38.9 | 22.2 |
| 10 | 18 | 38.9 | 44.4 | 16.7 |
| Total | 144 | 49.3 | 31.9 | 11.8 |

It is established that the hormonal status of the studied donor mares among themselves slightly differs. Apparently from the data from table # 3, the 6-8 year donor mares had the high hormonal status, and was within the limits from 55.6 % to 61.1 %. The hormonal status of the 3, 5 and 10 year donor mares was approximately identical, and was 38.9 %. In serums, received from 4 and 9 year donor mares the hormonal status was 50.0 %.

The obtained data allow to make the important conclusion, variability of gonadotropic

activity in serum of the pregnant mares received from identical donor mares by age is very essential. Therefore when manufacturing native PMS it will not always be effective to adhere to the certain age of donor mares, because of high variability of a studied sign.

Hormonal preparations received from donor mares with different terms of a pregnancy on the status among themselves essentially differed. (Table 3)

The pregnant mare serum received for the 40th day of a pregnancy had only the low hormonal status 100 %, for the 45th day of a pregnancy the average status was 56.2 % and the low status was 43.8 %. From donors with term of a pregnancy of 50-55 days preparations with the average status of 62.5 % and 56.2 % are generally received.

Table 3. Hormonal status of donor mares depending on terms of their pregnancy

| Timing of foals of donors | The number of donors | Activity of PMS | | |
|---------------------------|----------------------|--------------------|------------------------|--------------------------|
| | | High (250-320m.e.) | Average (160-240 m e.) | Low (150 m e. and lower) |
| 40 | 16 | - | - | 100.0 |
| 45 | 16 | - | 56.2 | 43.8 |
| 50 | 16 | 18.7 | 62.5 | 18.7 |
| 55 | 16 | 37.5 | 56.2 | 6.3 |
| 60 | 16 | 68.8 | 31.2 | - |
| 65 | 16 | 93.7 | 6.3 | - |
| 70 | 16 | 81.3 | 18.7 | - |
| 75 | 16 | 81.3 | 18.7 | - |
| 80 | 16 | 75.0 | 12.5 | 12.5 |
| Total | 144 | 50.7 | 29.2 | 20.1 |

In a preparation of PMS donors from the 65th to the 80th day of a pregnancy, from 75.0 % to 93.7 % had the highest hormonal status. From donor mares with term of a pregnancy of 60 days, preparations with the high hormonal status of 68.8 % and with the average status of 31.2 % were received.

Thus, it is established that the activity of a gonadotrophin in blood serum of donor mares of different age essential influences renders a hormonal background of an individual and their terms of a pregnancy.

The specific hormonal feature is necessary for using as criterion of the hormonal characteristic of donor mares at a blood draw. Thus the maximum peak of activity was observed from the 60th to the 75th day of a pregnancy.

The age of pregnant mares does not render essential influence on activity in blood serum. Thus with increase in the age tendency to decrease the activity of blood was observed.

It is known that follicle-stimulating and luteinizing hormones belong to gonadotrophins and directly participate in regulation of reproductive ability at females of agricultural animals. In this regard we have defined a ratio of follicle-stimulating(FSH) and luteinizing (LH) hormones in serums of pregnant mares of different age and the pregnancy period.

During the research influence of age of pregnant mares on ratio FSH/LH is studied.

Table 4. FSH/LH ratio in blood samples at pregnant mares of different age

| Age of donors | Number of samples | Indicators | | |
|---------------|-------------------|------------|----------------|--------------------|
| | | M±m | Fluctuation | Cv±m _{cv} |
| 3 | 18 | 3.01±0.266 | 1.11/1-5.19/1 | 37.7±8.983 |
| 4 | 18 | 3.03±0.289 | 1.28/1-5.61/1 | 39.6±7.60 |
| 5 | 18 | 3.36±0.239 | 1.37/-5.80/1 | 37.8±6.783 |
| 6 | 18 | 3.73±0.359 | 1.55/1-7.17/1 | 40.7±6.183 |
| 7 | 18 | 3.89±0.305 | 1.40/1-7.61/1 | 40.4±5.90 |
| 8 | 18 | 4.10±0.448 | 1.70/1-9.16/1 | 46.3±7.50 |
| 9 | 18 | 5.54±1.019 | 1.34/1-11.35/1 | 77.9±12.850 |
| 10 | 18 | 5.62±1.095 | 1.23/1-10.04/1 | 82.0±14.25 |
| Total | 144 | 4.06±0.227 | 1.11/1-11.35/1 | 67.2±3.959 |

The data from table 6 show that the average ratio FSH/LH of hormones in blood serum, taken from the 3 and 7 year donor mares was 3.0/1 with fluctuations from 1.11/1 to 7.61/1. Serums received from 8 summer donor mares had a ratio of hormones on the average 4/1±0.45 with variations from 1.7/1 to 9.1/1. The ratio of hormones FSH/LH in serum of pregnant mares, received from donors of more advanced age of 9-10 years the studied indicator on the average was 5/1 with high fluctuations from 1.3/1 to 11,3/1.

It should be noted that with the age the index of a standard deviation and factors of variability of ratio FSH/LH essentially increases within the limits from 1.13 to 4.61 and from 37.7 % to 82.0 % respectively.

The difference between 3-5 year and 9-10 year donors in FSH/LH ratio in blood serum is statistically authentic (P<0.05) and between other groups of authentic distinctions it is not revealed.

The contents of FSH/LH in serum is studied, depending on terms of a pregnancy of donors of PMS producers (Table 5).

Table 5. Variability ratio of FSH/LH in blood of mares depending on terms of their pregnancy

| Timing of foals of donors | The number of samples | Indicators | | |
|---------------------------|-----------------------|--------------|----------------|--------------------|
| | | M±m | Fluctuation | Cv±m _{cv} |
| 40 | 16 | 1.85/1±0.101 | 1.11/1-2.65/1 | 22.0±3.887 |
| 45 | 16 | 2.06/1±0.130 | 1.23/1-3.22/1 | 25.4±4.487 |
| 50 | 16 | 2.66/1±0.125 | 1.84/1-3.39/1 | 18.8±2.138 |
| 55 | 16 | 3.13/1±0.177 | 1.69/1-4.83/1 | 22.6±3.639 |
| 60 | 16 | 3.73/1±0.222 | 2.53/1-4.96/1 | 23.8±3.904 |
| 65 | 16 | 4.07/1±0.135 | 3.10/1-4.90/1 | 13.3±3.215 |
| 70 | 16 | 6.30/1±1.152 | 3.31/1-19.27/1 | 9.2±1.625 |
| 75 | 16 | 5.98/1±1.795 | 3.30/1-11.35/1 | 48.8±8.462 |
| 80 | 16 | 6.87/1±0.605 | 4.29/1-16.14/1 | 35.2±10.388 |
| Total | 144 | 3.82±0.154 | 1.11/1-11.35/1 | 48.0±3.958 |

The influence of terms of a pregnancy of donor mares on FSH/LH ratio is essential and is not identical. For example, FSH/LH ratio for the 40th day of a pregnancy was averaged 1.85/1 with fluctuations within limits from 1.11/1 to 2.65/1 and on 45 and 50th day 2.06/1-2.66/1, with fluctuations within the limits from 1.23/1 to 3.39/17 between 55-60 days this indicator was 3/1 and their considerable variations 1.69/1-4.96/1 were observed. FSH/LH ratio in PMS of donor mares with term of a pregnancy of 65 days fluctuated slightly within the limits from 3.1/1 to 4.9/1 with an average ratio 4.07/1. The highest ratio was observed in a preparation, received from donors with the term of a pregnancy of 70-80 days and was on the average from 5.98/1 to 6.87/1 with the maximum fluctuations from 3.30/1 to 11.35/1.

The analysis of pilot studies after studying of biological quality of PMS received from donors with different term of a pregnancy shown a big variability of sizes of FSH/LH ratios in biological liquids. The evidence to that are indicators of a standard deviation and factor of variability of this indicator where it hesitates within the range 0.41-2.17 and 13.3 %-40 % respectively.

Thus, in researches it is revealed that the size FSH/LH value in biological liquids depends generally on pregnancy terms, than the age of donors.

It is established that the difference between FSH/LH ratio at investigated between a pregnancy

considerable and authentically fluctuations from $P<0.05$ to $P<0.001$.

It is known that FSH/LH ratios in serum are an important biological criterion in the selection of pregnant mares as donors of PMS producers.

Therefore depending on level of concentration of FSH in relation to LH, the studied preparations have been divided into three groups: with low (1.1/1 – 1.9/1) average (2.0/1 – 3.9/1) and high (4.0/1 and higher) FSH/LH ratio.

The research has shown that the status by FSH/LH ratio at donors of different age did not differ essentially. (Table 5)

Table 6. Status of mares of donors of different age by FSH/LH ratio in blood samples

| Age of donors | Number of donors | FSH/LH ratio | | |
|---------------|------------------|------------------------|-----------------------|-------------------|
| | | High (4.01 and higher) | Average (2.0/1-3.9/1) | Low (1.1/1-1.9/1) |
| 3 | 18 | 27.8 | 50.0 | 22.2 |
| 4 | 18 | 33.3 | 50.0 | 16.7 |
| 5 | 18 | 33.3 | 55.6 | 11.1 |
| 6 | 18 | 33.3 | 61.1 | 5.6 |
| 7 | 18 | 38.9 | 56.6 | 5.6 |
| 8 | 18 | 44.4 | 38.9 | 16.7 |
| 9 | 18 | 50.0 | 33.3 | 16.7 |
| 10 | 18 | 50.0 | 33.3 | 16.7 |
| Total | 144 | 38.9±4.06 | 47.2±4.16 | 13.9±2.88 |

The majority of donors from 3 to 7 years had the average status 50.0 – 61.1 % and in group of 8-10 year donors a share of donors with the high status by FSH/LH ratio was within 44.4-50.0 %.

Follicle-stimulating the status of donors with different term of a pregnancy among themselves essentially differed (table 9). For example, the follicle-stimulating status of donors with term of a pregnancy of 40 days generally were low (62.5 %) and a number of the animals, having the average status was more in group of donors with the term of a pregnancy of 45-60 days and was 62.5 – 81.2 %. The share of animals with the high status was more (56.3-100 %) in group of donors for pregnancy of 70-80 days.

It should be noted that in group of donors for pregnancy of 60-80 days was not revealed donors with the low status of FSH/LH ratio.

On the average in the studied preparations of PMS 38.9±4.06 % was high, 47.2±4.16 %, average and 13.9±2.90 % the low follicle-stimulating status.

Table 7. Follicle-stimulating status of PMS received from donors with different term of pregnancy

| Timing of foals of donors | Number of donors | FSH/LH ratio | | |
|---------------------------|------------------|-----------------------|----------------------|--------------------|
| | | High(4,01 and higher) | Average(2,0/1-3,9/1) | Low (1,01/1-1,9/1) |
| 40 | 16 | - | 37.5 | 62.5 |
| 45 | 16 | - | 62.5 | 37.5 |
| 50 | 16 | - | 81.2 | 18.8 |
| 55 | 16 | 12.5 | 81.2 | 6.2 |
| 60 | 16 | 37.5 | 62.5 | - |
| 65 | 16 | 50.0 | 50.0 | - |
| 70 | 16 | 56.3 | 43.7 | - |
| 75 | 16 | 87.5 | 12.5 | - |
| 80 | 16 | 100.0 | - | - |
| On the average | 144 | 38.9±4.06 | 47.2±4.16 | 13.9±2.90 |

Discussion

The research of biological quality of pregnant mare serum has shown variability of sizes of concentration of gonadotropic hormones in biological liquids of an organism of pregnant mares of different age and pregnancy term. Thus it may be noted that by the variety of activity of PMS and FSH/LH ratio in blood serum age and terms of a pregnancy of donors has essential influence.

The provided data allow to draw a conclusion that efficiency of use of pregnant mare serum depends on a compatibility combination of activity and FSH/LH ratio in preparations. Therefore the obtained data have special values when developing complex indexes of selection PMS combining their biological qualities and FSH/LH ratio in preparations which are necessary for modulation of the production technology of gonadotropic hormones.

As a whole, the received positive and authentic indicators, characterizing correlative communication between the considered signs, we consider natural. Since the indicators of biological quality of pregnant mare serum depending on their age and pregnancy term essentially changes that confirms the obtained data.

Therefore, this correlative dependence of registration signs of donors and a PMS preparation are of great importance for correction of a method of selection of pregnant mares as a producer of gonadotropic hormones.

The results of the dispersive analysis are necessary at identification of a share of variability, at observable biological objects.

In this regard we have studied a share of influence of separate factors on variability of a biological sign of a preparation of PMS by application of a method of the dispersive analysis.

On the basis of the dispersive analysis it is established that the variation of endocrine indicators at

pregnant mares is caused by action of different factors.

For example the influence of age of pregnant mares on FSH/LH ratio in serum of the blood, received from them was 0.107 ± 0.046 with reliability at level of the first probability threshold ($P < 0.05$) and influence terms of a pregnancy of donors on level of concentration of gonadotropic hormones in serum is authentic above at the significance of $P < 0.001$ (0.447 ± 0.033).

Share of influence of FSH/LH ratio in serum and terms of a pregnancy of donors on gonadotropic activity of PMS was within the limits of 0.684 ± 0.024 and 0.687 ± 0.016 at reliability of a difference respectively from 28.5 to 42.9 ($P < 0.001$).

The influence of age of donors by level of activity of PMS was very low and was 7.5 %.

As a whole, influence of the considered factors authentically, except a factor – “age of donors – activity of PMS”. Thus, influence terms of a pregnancy of donors on FSH/LH condition has made not less than 36.6 % and not more than 51.3 % and for activity of PMS within the limits from 65.5 % to 71.9 %.

The influence of age of donors on activity of PMS and on FSH/LH ratio in serum is not more than 9.9 % and 20.4 % respectively, and influence of SH/LH ratio on activity of PMS is not less than 63.6 % and not more than 73.2 %.

Thus, the terms of a pregnancy of donors have a direct effect on changes of concentration of gonadotrophins and a ratio of follicle-stimulating and luteinizing activity in blood serum of pregnant mares.

These received results about high variability of concentration of a gonadotrophin in pregnant mare serum of different age and with different terms of a pregnancy, characterize their specific features and are an informative indicator of their endocrine characteristics.

Conclusions

On the basis of results of these researches the new way of selection of pregnant mares as donors of producers of gonadotropic hormones is developed. Thus, the optimum day for a blood draw from 3-4-5 year donors we consider between 65-80 days of pregnancy. For donors of 6-7-8 years the best term of the period from 60 to 75 day and for advanced age of 9 and 10 years - from 55 to 65th day of a pregnancy.

The advantage of this way in comparison with the traditional consists the following:

- to reveal highly productive days of a pregnancy of donors

- allows to use rationally the high-valuable genotypes which have been selected as donors in horse breeding

- allows to make PMS preparation only with high activity

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