

Innovative method for determining the activity of gonadotropic raw materials in the production of the biological preparation of PMS

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Abstract. The article presents the results of research work to determine the activity of gonadotropic raw materials in the production of the biological preparation of pregnant mare serum (PMS) from mares of the Kazakh breed of horses of the “Zhabe” type of the peasant farm “Sultanbai Ata” of the Turkestan region. An innovative method has been developed based on determining the follicle-stimulating hormone (FSH) content using ELISA, which provides optimal pregnancy periods for mares to obtain gonadotropic raw materials: 50, 60 and 70 days for mares-producers of 3-10 years of age in terms of FSH content. Existing technologies for determining the pregnancy of mares and assessing the activity of gonadotropic hormones in technical and moral aspects are outdated, since diagnostics require a lot of time and the use of laboratory white mice as biological tests is considered ineffective, labor-intensive processes, as well as inappropriate on ethical issues. An express test method has been developed for determining the activity of the gonadotropic hormone PMS in a given donor herd based on the content of the FSH level in the preparation, which makes it possible to subsequently determine the activity in mouse units, bypassing the traditional, labor-intensive, outdated method.

1 Introduction

In intensive livestock farming, various biological preparations are used to stimulate the reproductive functions of animals, and the gonadotropic biological preparation PMS is rightfully considered one of the effective preparations [1].

Currently, in Kazakhstan and in the CIS, biological products that stimulate prolificacy and the ovulatory status of farm animals are not produced, as a result of which they are imported from foreign countries and their prices are very high, which complicates practical use in the technological process of animal reproduction, although there is a huge demand for biological products and there are huge donor resources - the number of mares for the production of the biological product PMS.

The hormonal preparation is prepared from the whole blood serum of healthy pregnant mares during the 45-90 days of their pregnancy [2]. In the technology of preparing a

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biopreparation of PMS, one of the key processes is the determination of activity after the isolation of serum from the blood of pregnant mares.

There are known methods for determining the hormonal activity of gonadotropic raw materials, based on the injection of prepared blood serum in laboratory mice (mouse unit) and rats (international unit) [3].

Meanwhile, existing methods for establishing the activity of a gonadotropic preparation in technical and moral aspects are considered outdated, since diagnostics require a lot of time and their use as biological tests is considered ineffective, labor-intensive processes, as well as inappropriate on ethical issues.

The main component of the biological product PMS, which stimulates and increases the reproductive qualities of animals, is gonadotropic hormones consist of follicle-stimulating (FSH) and luteinizing hormones (LH) [4,5].

The gonadotropic hormone FSH, when introduced to an animal, stimulates the function of the gonads and causes the development of additional follicles in females in the ovaries [6], LH is involved in ovulation and the formation of the corpus luteum [7].

In this regard, the development of an effective method for determining the activity of gonadotropic hormone in the blood serum of pregnant mares and their use in biotechnology for the production of a biological preparation for reproduction are relevant and have practical importance in animal husbandry.

The goal is to develop an innovative method for determining the activity of gonadotropic raw materials in the production of PMS biological preparation.

To achieve the goal, the following tasks were set:

- collection of gonadotropic raw materials and obtaining serum from the blood of pregnant mares.
- improvement of technology for determining the activity of gonadotropic hormones in the serum of pregnant mares
- development of an effective resource-saving technology for determining the activity of the resulting PMS biological preparation.

2 Experimental work. Material.

The object of the study was mares of the Kazakh breed of horses of the “Zhabe” type from the peasant farm “Sultanbai Ata” of the Turkestan region.

Research materials are gonadotropic raw materials: blood of pregnant mares, blood serum of pregnant mares, biological preparation PMS.

To prepare the pregnant mare serum, 2 liters of blood from the jugular vein were taken from 34 donor-producers individually from each mare on 50s, 60s, 70s and 80s days of pregnancy into 5 liter sterilized mounted glass bottles with Bobrov needles. A total of 136 series of blood from pregnant mares were prepared with a total amount of blood of 272 liters.

Isolation of plasma from the blood of pregnant mares was carried out using solutions of sodium citrate and defibrinization was carried out using calcium chloride.

A total of 136 series of serum from pregnant mares were studied in various groups of donor-producer mares using various methods.

Methodology for determining the degree of activity of gonadotropic hormones in the pregnant mareserum using the traditional method. With the traditional method, serum activity (titer) is measured in mouse units, with the injection of 0.2 ml of the prepared solution, capable of causing a clear increase uterus and opening of the vagina in 60% of 5 immature female mice aged 17-23 days with a live weight of 7.5-10g [8,9].

Serum activity was determined in immature laboratory white female mice using standard indicators of gonadotropin hormone activity in the pregnant mare serum [10,11].

If the reaction deviated in one direction or another, new probable dilutions were prepared and the activity test was repeated.

Method for determining the activity of a preparation using ELISA. To determine the activity of PMS, the concentrations of FSH and LH in the prepared 136 batches of the preparation were taken into account, taking 2 ml of samples from each raw material. The contents of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in gonadotropic hormone were determined by ELISA[12,13,14].

The results obtained for the FSH content in the finished biological preparation were divided into the following categories: 4-4.9 mIU/1ml, 5-5.9 mIU/1ml, 6-6.9 mIU/1ml, 7-7.9 mIU/1ml, 8-8, 9 mIU/1ml, 9-9.9 mIU/1ml, 10-10.9 mIU/1ml, 11-11.9 mIU/1ml, 12-12.9 mIU/1ml and 13 more than mIU/1ml.

The obtained results of LH content in the biological preparation were divided into the following categories: 2-2.9 mIU/1ml, 3-3.9 mIU/1ml, 4-4.9 mIU/1ml, 5-5.9 mIU/1ml, 6-6.9 mIU/1ml and 7-7.9 mIU/1ml.

In a comparative aspect, the activity of the resulting 136 series of PMSs was assessed by the content of FSH and LH levels and compared with the known method of determination on immature laboratory white female mice using standard indicators of the activity of gonadotropic hormones in the pregnant mare serum.

The resulting digital materials were processed using the variation statistics method [15].

3 Methods

The content of FSH in the blood serum of mares was studied depending on their timing of the days of their pregnancy (Table 1).

Table 1. Indicators of FSH content in PMS depending on the days of pregnancy of mares, in %

Pregnan cy days for mares	Total samp les	FSH content, mIU/1ml									
		4- 4,9	5- 5,9	6- 6,9	7- 7,9	8- 8,9	9- 9,9	10- 10,9	11- 11,9	12- 12,9	13 and higher
50	34	2,9	11,8	14,7	20,6	35,3	5,9	8,8	-	-	-
60	34	-	-	11,8	11,8	1,9	29,4	32,3	11,8	-	-
70	34	-	-	-	5,9	1,9	2,9	32,3	41,3	5,9	8,8
80	34	-	2,9	5,9	5,9	17,6	29,4	17,6	14,8	5,9	-
On average	136	0,7	3,7	8,1	11,0	14,7	16,9	22,8	16,9	3,0	2,2

On 50s days of pregnancy in mares, the maximum FSH content is at the level of 10-10.9 mIU/1 ml (8.8%) with a minimum level of 4-4.9 mIU/1 ml (2.9%), on 60s days of pregnancy the maximum value reaches the level of 11-11.9 mIU/1ml (11.8%) with an increase in the minimum value to the level of 6-6.9 mIU/1ml (11.8%), on 70s days of pregnancy the maximum content of this hormone reaches 13 mIU/1ml and higher (8.8%) with an increase in the level of its minimum content by 7-7.9 mIU/1ml (5.9%) and on 80s days of pregnancy its maximum value decreases to the level of 12-12.9 mIU/1ml (5.9%) and the minimum content also drops to the level of 5-5.9 mIU/1ml (2.9%).

This indicates that the level of this hormone increases until 70 days of pregnancy and decreases by 80 days of pregnancy. At the same time, the highest specific gravity of FSH content is characteristic on 50s days: 8-8.9 mIU/1 ml -35.3%, on 60s days: 10-10.9 mIU/1 ml -32.3%, on 70s days: 11-11, 9 mIU/1ml-41.3% and on 80s days 9-9.9 mIU/1ml-29.4%.

4 Results and discussion

Thus, the most effective periods of pregnancy for mares to obtain gonadotropic raw materials in terms of FSH content have been established as 50, 60 and 70 days. The influence of donor age on the level of FSH in the blood serum of pregnant mares was studied (Table 2).

Table 2. Indicators of FSH content in PMS depending on the age of mares in %

FSH content , mIU/1 ml	Number of sam ples	Age of donor mares											
		3-4		5-6		7-8		9-10		11-12		13 and older	
		n	%	n	%	n	%	n	%	n	%	n	%
4-4,9	2	-	-	-	-	-	-	-	-	1	5,0	1	8,3
5-5,9	4	-	-	-	-	-	-	-	-	1	5,0	3	25,0
6-6,9	11	-	-	-	-	-	-	4	16,7	5	25,0	2	16,7
7-7,9	16	-	-	2	10,0	4	11,1	5	20,8	3	15,0	2	16,7
8-8,9	18	3	12,5	2	10,0	5	13,9	2	8,3	4	20,0	2	16,7
9-9,9	25	6	25,0	3	15,0	6	16,6	5	20,8	4	20,0	1	8,3
10-10,9	30	9	37,5	4	20,0	1	30,1	4	16,7	1	5,0	1	8,3
11-11,9	23	4	16,6	7	35,0	8	22,2	3	12,5	1	5,0	-	-
12-12,9	4	1	4,2	1	5,0	1	2,8	1	4,2	-	-	-	-
13 and higher	3	1	4,2	1	5,0	1	2,8	-	-	-	-	-	-
total	136	24	17,7	20	14,7	36	26,4	24	17,7	20	14,7	12	8,8

In terms of age, the FSH content in the blood serum of pregnant mares has its own distinctive features. The most effective donor-producers for the production of the PMS preparation are 3-8 years old mares; its level is in the range of 7-7.9 mIU/1ml-13 mIU/1ml and higher. It should be noted that, as noted above, the best option for preparing this preparation is the raw materials obtained from 3 to 8 years old pregnant mares.

In the blood serum of mares obtained from different days of pregnancy, the content of the gonadotropic hormone LH is in the range of 2-2.9 mIU/1 ml and 7-7.9 mIU/1 ml.

In mares of 50 days of pregnancy, the LH content of 2-2.9 mIU/1 ml and 3-3.9 mIU/1 ml was 55.9% and 44.1%, respectively. As pregnancy progresses, the level of the hormone LH in the blood serum increases.

On 60s days of pregnancy in mares, LH levels have the following indicators: 2-2.9 mIU/1 ml-2.9%, 3-3.9 mIU/1 ml-61.8% and 4-4.9 mIU/1 ml-35.3 %. On 70s days of pregnancy in mares, LH levels are characterized by the following indicators: 3-3.9 mIU/1 ml-52.9% and 4-4.9 mIU/1ml-47.1%. On 80s days of pregnancy in mares, LH levels reach the following levels: 4-4.9 mIU/1ml-44.2%, 5-5.9 mIU/1ml-50.0%, 6-6.9 mIU/1ml-2.9% and 7-7.9 mIU/1 ml-2.9%.

Using the traditional method, using standard titer indicators, we determined the biological activity of 136 series of gonadotropic hormonal preparations obtained by us in mouse units

according to the methodology and studied the dynamics of the FSH level depending on the serum activity determined by the traditional method (Table 3).

Table 3. Dynamics of FSH content depending on standard indicators of serum activity of the traditional method.

Serum activity, IU	Series total	%	FSH content, mIU/1ml									
			4-4.9	5-5.9	6-6.9	7-7.9	8-8.9	9-9.9	10-10.9	11-11.9	12-12.9	13 and higher
120	1	0.7	4.1									
140	5	3.7		5.0 - 5.7								
160	11	8.1			6.0 - 6.8							
180	15	11.0				7.2 - 7.9						
200	20	14.7					8.0 - 9.6					
220	25	18.4						9.0-10.3				
240	27	19.9							10-10.8			
260	23	16.9								8.8-11.7		
280	6	4.4									11.2 - 12.7	
300	3	2.2										13.2-13.8
	136	100										

As a result of studies of the activity of 136 series of gonadotropic hormone using the traditional method, it was established that the activity of the preparations ranges from 120 to 300 IU. At the same time, among biological preparations, high activity rates in mouse units are 160, 180, 200, 220, 240, 260, 280 and 300 make up 8.1%, 11%, 14.7%, 18.4%, 19.9%, 16.9% and 4.4%respectively. In the manufactured preparations, the activity indicators 120IU and 140IU are 0.7% and 3.7% respectively.This suggests that the donor-producers of this population have a high potential for hormonal status of reproductive function. The activity of the gonadotropic hormone PMS in mouse units directly depends on the level of FSH, as evidenced by the indicators of its dynamics.Thus, for the resulting preparations, the higher the FSH content and the higher the activity, and the lower the FSH content, the lower the activity. At the same time, each activity of the gonadotropic hormone is characterized by the corresponding content of the FSH level.It should be emphasized that the gradations of FSH levels correspond to the following indicators of biological preparation activity determined by the traditional method: 4-4.9 mIU/1ml-120 IU, 5-5.9 mIU/1ml-140 IU, 6-6.9 mIU/1ml-160 IU, 7-7.9mIU/1ml-180 IU, 8-8.9mIU/1ml-200 IU, 9-9.9mIU/1ml-220 IU, 10-10.9 mIU/1ml-

240 IU, 11-11.9 mIU/1ml-260 IU, 12-12.9 mIU/1ml-280 IU and 13mIU/1ml and higher 300 IU.

Thus, when determining the activity of the gonadotropic hormone FFA, a rapid test method has been developed for a given donor herd based on the content of the FSH level in the preparation, which makes it possible to subsequently determine the activity in mouse units, bypassing the traditional, labor-intensive, outdated method.

5 Conclusions

Conducted research work to determine the activity of gonadotropic raw materials in the production of the biopreparation PMS from mares of the Kazakh breed of horses of the “Zhabe” type at the “Sultanbai Ata” peasant farm in the Turkestan region made it possible to develop an innovative method based on determining the FSH content using ELISA.

At the same time, the most effective periods of pregnancy in mares for obtaining gonadotropic raw materials in terms of FSH content have been established as 50, 60 and 70 days for producer-mares of 3-10 years of age.

The activity of the gonadotropic hormone PMS in mouse units directly depends on the level of FSH, as evidenced by the indicators of its dynamics. Thus, for the resulting preparations, the higher the FSH content and the higher the activity, and the lower the FSH content, the lower the activity. At the same time, each activity of the gonadotropic hormone is characterized by the corresponding content of the FSH level.

Existing technologies for determining the activity of gonadotropic hormones are outdated in technical and moral aspects, since diagnosis requires a lot of time and the use of laboratory white mice as biological tests is considered ineffective, labor-intensive processes, as well as inappropriate for ethical reasons.

An express test method has been developed for determining the activity of the gonadotropic hormone PMS in a given donor herd based on the content of the FSH level in the preparation, which makes it possible to subsequently determine the activity in mouse units, bypassing the traditional, labor-intensive, outdated method.

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