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Research Notes

Advance in the Diagnostics of Mare Pregnancy and Assessing the Activity of Gonadotropin in Serum of in-Foal Mare

[1]Kaliya Kudaikulovna Yerkekulova, [1]Nuradin Alibayev, [2]Yusupzhan Artykovich Yuldashbayev, [1]Oralbek Alikhanov, [1]Orynbek Beketauov and [3]Dastanbek Asylbekovich Baimukanov

[1]Agricultural Biotechnology Research Laboratory, M. Auezov South Kazakhstan University, Shymkent, Kazakhstan
[2]Department of Private Animal Science, Russian State Agrarian University-Moscow Timiryazev Agricultural Academy, Moscow, Russia
[3]Department of Animal Husbandry, Veterinary Medicine and Feed and Milk Quality Assessment, Scientific and Production Center for Animal Husbandry and Veterinary, Astana, Kazakhstan

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Corresponding Author:
Dastanbek Asylbekovich Baimukanov
Department of Animal Husbandry, Veterinary Medicine and Feed and Milk Quality Assessment, Scientific and Production Center for Animal Husbandry and Veterinary, Astana, Kazakhstan
Email: dastanbekbaimukanov@gmail.com

Abstract: Research works on the improvement of mare pregnancy diagnostics and evaluation of biological activity of gonadotropic preparation based on the use of enzyme-linked immunosorbent assay were carried out. As the result of diagnostics and evaluation, effective producers as donors for obtaining gonadotropic preparation among the Kazakh population of mares with type “zhabe”, the animal units of 60 days with their 5-6 year pregnancy containing FSH -10,38±0,65 mIU/1 mL and 7-8-year-old with FSH content -10,50±0,32 mIU/1 mL; 70 days of their 3-4-year-old pregnancy with FSH content -10,52±0,21 mIU/1 mL, 5-6-year-old with FSH content -11,34±0,45 mIU/1mL, 7-8-year-old with FSH content -11,34±0,32 mIU/1 mL, 9-10-year-old with FSH content -11,06±0,29 mIU/1 mL are identified. A new method for the selection of a drug by biological activity based on the content of follicle-stimulating hormone level for a given herd was developed, which allows to determination of the activity in international units, without the traditional, labor-consuming, outdated method for determination using laboratory white mice, addressing ethical issues in research.

Keywords: Mare, Producer, Blood Sample, Mare Age, Gonadotropic Hormone Activity, Follicle-Stimulating Hormone, Luteinizing Hormone, Gonadotropic Hormone

Introduction

The animal husbandry of Kazakhstan is a traditional branch of agricultural production and the intensive reproduction of livestock with high-value animals is of key importance in the technology for the production of raw animal material (Richardovich *et al*., 2019; Iskhan *et al*., 2019a-b; Abugaliyev *et al*., 2019; Kargaeyeva *et al*., 2020; Baimukanov *et al*., 2022; Nikolaevich *et al*., 2021; Temirbekovich *et al*., 2021).

A special importance is emphasized on developing productive horse breeding (Zhaleluly *et al*., 2019; Iskhan, 2020; Kargaeva *et al*., 2022; Kargayeva, 2020; Baimukanov *et al*., 2023; Baymukanov and Aubakirov, 2023; Assanbayev *et al*., 2019; Sharapatov *et al*., 2023; Kargaeyeva *et al*., 2023).

Under modern conditions of livestock development, one of the priority areas for the dynamic growth of livestock is the reproduction of farm animals (Abay *et al*., 2014).

When regulating the sexual function of farm animals, gonadotropic preparations are considered to be one of the effective biological stimulators in animal husbandry (Stewart *et al*., 1976).

A major achievement in zootechnical and biological sciences is the discovery of a hormonal stimulation method by using hormonal preparation PMS (elixir of multiple pregnancies), which has been used for many years to increase multiple pregnancies of animals and to get two or more animals from a uterus instead of one (Abay *et al*., 2014).

By using this method, it is possible to control the reproduction processes: To regulate the service-period duration, cyclicity, and synchronization of females' arrival

in season, to reduce second breeding, and to get more offspring from each uterine (Licht *et al*., 1979).

The productivity potentials of farm animals are quite high and should be fully implemented (Baimukanov *et al*., 2021; 2024).

Pregnant mare serum is used in farm animals for induction of superovulation and synchronization of hunting. Pregnant mare serum induces the most effective multiple pregnancies and does not interfere with insemination, unlike prolan (Stewart *et al*.,1977).

The gonadotropin produces a uterine structure known as an endometrial bowl, which is formed around the developing fetal bag. These limited areas of decidual tissue exist only in pregnant animals; these are most developed until 70 days of pregnancy and then degenerate quickly. For a long time, their nature and the factors that stimulate the development of endometrial bowls remained unstudied (Tourkova *et al*., 2015; Hashem and Gonzalez-Bulnes, 2021).

Currently, Kazakhstan, including the CIS, does not produce biogenic stimulants for animal reproduction gonadotropic hormones.

There is demand for biogenic stimulants in the area of reproduction of farm animals in Kazakhstan and the market capacity is estimated at least 25-135 mLn doses. Domestic and foreign agricultural producers of livestock breeding, sheep breeding, and pig breeding areas are considered potential consumers. Kazakhstan has a huge donor resource for the production of the gonadotropic hormone Preganat Mare Serum (PMS) the number of mares with more than 1.5 million.

The raw sources for hormonal preparations are donor-producing mares during the period of 45-90 days of foaling. Different technologies exist for the production of the gonadotropic hormone of pregnant mare serum, which is based on a collection of the blood of pregnant mares, extraction of serum from blood, determination of serum activity, and preservation of finished pregnant mare serum. Determination of pregnancy in mares and the activity of obtained bioproducts are the key activities in the production process of gonadotropic hormone.

Having analyzed the available literature data on PMS preparation biological properties, it can be noted that they have significant differences in absolute values, which, apparently, can be explained by purification methods, serum separation, species characteristics of animals, and conditions of hormone metabolism in the organism of pregnant mares.

At present, the well-known technologies, as per our studies, do not meet the modern requirements for the production of gonadotropic preparation and also considering the ethical issues of the Universal Declaration on Bioethics and Human Rights adopted at the 33rd session of the general conference of UNESCO, we consider this method to be inapplicable.

In the meantime, in order to address the ethical side of technology, we have conducted research work on developing alternative methods excluding the use of mice and frogs as biological tests, where the criteria of FSH content for each activity of gonadotropic hormone were identified.

Based on this, the actual focus of research work aimed at improving the diagnosis of mare pregnancy and evaluation of the gonadotropic hormone activity of Pregnant Mare Serum (PMS) in biotechnology is a selection of effective gonadotropic hormone producers among the Kazakh population of mares, type “zhabe”, in different periods of their pregnancy in the age aspect for the formation of a valuable donor herd producing mares as a raw source for gonadotropic preparations.

A scientific novelty is the method of establishing mares pregnancy, which is based on studying the content of follicle-stimulating hormone in blood of donors not lower than 4-4,9 mIU/1 mL and luteinizing hormone not lower than 2-2,9 mIU/1 mL, which allows to select donor-producers with accuracy in the amount of 34 heads, that matches the final result of mare foaling by 100%, as well as a new express method developed, of identification the use of laboratory white mice and addresses ethical issues in research and selection methodology of producers’ valuable donors with FSH level not lower than 10 mIU/1mL, as raw sources of gonadotropic preparation in the age aspect and their foaling days.

Aim of the Study

Upgrading of pregnancy mare diagnostics and selection of effective producers as donors among mares of Kazakh population “zhabe” in biotechnology of gonadotropic preparation production.

Formation of mare donor producers, a record of coupling days, and blood collection.

Materials and Methods

The object of research is a horse “zhabe” of the South Kazakhstan population bred in the Arys-Turkistan area of the Republic of Kazakhstan.

Research material: Mares, donor horses, pregnant mare serum, foaling mares, blood serum activity, gonadotropic hormone PMS (Yerkekulova *et al*., 2024).

Three groups of mares were formed based on their age: Group I young mares at the age of 3-4 years in a number of 5 headsbay with an average live weight of 371.4±1.2 kg and 5-6 years old in the number of 6 heads, bay with an average live weight of 491.0±1.3 kg, group II mares at the age of 7-8 years in the number of 9 heads, bay with an average live weight of 520,7±1,3 kg and 9-10 years in number of 7 heads, bay with an average live weight of 502,14±1,8 kg, group III mares aged 11-12 years in number of 9 head, bay with an average live weight of

486.8±1.9 kg, as well as 13 years and older in number of 5 head, bay with an average live weight of 481.8±0.7 kg, where mares were individually coupled by stallions-producers attached separately for each group in the period from May 1-10 with fixation of microchip numbers and recorded in the register of the microchip database containing detailed information about the coupling.

A blood sample was collected from 39 mares after foaling 7-10 days from the jugular vein individually from each mare 10 mL per EDTA tube, after filling the tube with blood up to the mark, within ±10% of specified volume and to avoid hemolysis, the tube with blood was carefully turned 4-6 times by 180° to mix the sample with a filler. Blood samples from all 39 mares were analyzed for FSH and LH levels by Enzyme-Linked Immunosorbent Assay (ELISA).

Between June 19 and June 29, given the date of hand mare coupling after 50 days, their foaling was determined by ELISA, which was compared with traditional methods based on using white mice, and external and rectal ultrasound.

To determine mare foaling by ELISA, blood samples were collected from the jugular vein of 39 mares individually from each mare, 10 mL per EDTA tube. After the tube was filled with blood up to the mark, within ±10% of the specified volume and to avoid hemolysis, the tube with blood was carefully inverted 4-6 times by 180° to mix the sample with a filler.

To determine Follicle-Stimulating (FSH) and Luteinizing (LH) hormones in the plasma of in-foal mares the following were carried out: Awareness technology of semi-automatic photometer tablet (USA), "STAT FAXR 2100", designated for immunoenzymatic analysis, using the reagent kit "Gonadotropin ELISA-FSH" and "Gonadotropin ELISA-LH", designated for quantitative determination of FSH and LH hormone concentrations as per the instructions for use.

The calculation of FSH and LH concentration results in the plasma of a tested sample was done using a multichannel tablet spectrophotometer of accuracy ±1%, at a wavelength of 450 nm.

Prior to analysis, all reagents were thoroughly mixed and brought to room temperature (20/125°C).

FSH levels in the blood of yield mares of different ages are divided into four groups: 0.1-0.9, 1-1.9, 2-2.9, and 3-3.9 mIU/1 mL.

LH levels in the blood of yield mares of different ages were divided into four groups: From 0.1 0.9 mIU/1 mL and from 1-1.9 mIU/1 mL.

To determine mares' pregnancy, ultrasonic diagnostics was carried out 50 days after their coupling by means of MyLab™ Delta VET device by "Esaote" company using external and rectal methods, as well as mares pregnancy was determined by traditional methods on laboratory white mice in 39 mares by a known method.

Finally, the results of different methods for determining mare foaling were compared with mare foaling and foal production.

In order to get serum from the blood of donor mares 50-60-70-80 days of pregnancy in age aspects, blood was taken from five heads 3-4 years of age 40 L, six heads 5-6 years of age 48 L, nine heads 7-8 years of age 72 L, seven heads 9-10 years of age 56 L, seven heads 11-12 years of age 56 L, five heads 13 and above years of age 40 L and PMS was prepared individually from each mare as per the age and days of their pregnancy.

The FSH and LH concentrations were identified of the produced preparation from gonadotropic raw animal material of different aged mares from 3-4, 5-6, 7-8, 9-10, 11-12, 13, and older at 50, 60, 70 and 80 days of foaling on the basis of ELISA method (Aggarwal *et al*., 1980).

FSH content in preparation for the level of its activity with an average value of 9.05 ±0.16 mIU/1 mL was divided into three groups: Low-less from the average value, medium, high - more from the average value.

The materials obtained in the research work process were subjected to processing by the statistical method of variation.

Results and Discussion

The experimental studies complied with the ethical standards accepted in horse breeding (Kargaeyeva *et al*., 2023).

The contents of FSH and LH in the blood serum were studied in mature single mares for selection as a donor herd (Aggarwal *et al*., 1980).

The research results showed that the serum levels of FSH within 0.1-2.9 mIU/1 mL and LH within 0.1-1.9 mIU/1 mL are characteristic of yield mares. Besides, the mares at the age of 7-8 and 9-10 years have the maximum FSH level. By contrast, the minimum FSH levels are found in young 3-4-year-old and 13 and older mares (Table 1).

Total FSH content in the herd was distributed as follows: 0.1-0.9 mIU/1 mL 18%, 1-1.9 mIU/1 mL 61.5%, and 2-2.9 mIU/1 mL 20.5%.

The LH level in serum samples from 39 non-pregnant mares was 48.7% had 0.1-0.9 mIU/1 mL and 51.3% of individuals had 1-1.9 mIU/1 mL.

Therefore, specific features of FSH and LH content during the service period from pregnancy to 7-10 days have been determined for this herd of mares.

In a comparative aspect, the research work was conducted to determine the effectiveness of different methods for diagnosing mare pregnancy using traditional (hormonal), rectal ultrasound, external ultrasound, and immune-enzyme analysis.

The analysis results for diagnostics of mare pregnancy in three groups of Kazakh horse breed of "zhabe" type in the amount of 39 heads by various methods demonstrated a different efficiency (Table 2).

When using a traditional method on laboratory mice, 82.4% of the total mares were found to be in foal, while

rectal and external ultrasound diagnostic methods were able to identify foals in 94.1 and 20.6% of the mares, or respectively 17.6, 5.9 and 79.4% lower from the final result of mare foaling.

ELISA method used for diagnosis of mare pregnancy with setting the criteria of FSH levels not lower than 4-4,9 mIU/1 mL and LH levels in blood serum not lower than 2-2,9 mIU/1 mL enabled to select donor-producers in the amount of 34 heads with accuracy that corresponds to the final result of mare foaling for 100%.

Determination of mare foaling using a traditional method is based on outdated technology, which is supported by using at least 5 experimental lab mice for one sample, and 195 white mice were used to determine the pregnancy of 39 mares (Aggarwal *et al*., 1980).

Performing the rectal ultrasound to diagnose the mare pregnancy is a time-consuming process, which is not practiced in PMS biopreparation production because it leads to animal stress and fetal miscarriages.

Diagnosis of mare pregnancy with external ultrasound showed ineffectiveness as the fetus was still in the pelvic cavity during this period and it was virtually impossible to detect it.

The research results demonstrated that the ELISA method based on FSH and LH hormone levels has a reliable resolving capacity among the methods used by us in a comparative aspect.

Integrated studies were performed to develop a method of selecting mares as producers, including the age of mares, days of pregnancy, and FSH level, ensuring a reliable selection of high-value donors of producers (Table 3).

Table 1: Contents of FSH and LH in blood serum in single mares (in percent)

		FSH content, mIU/1 mL			LH content, mIU/1 mL	
Age of mares	Number of mares	0.1-0.9	1-1.9	2-2.9	0.1-0.9	1-1.9
3-4	5	40.0	60.0	-	40.0	60.0
5-6	6	17.0	66.0	17.0	33.3	66.7
7-8	9	11.1	55.6	33.3	44.4	55.6
9-10	7	14.3	57.1	28.6	42.9	57.1
11-12	7	14.3	71.4	14.3	71.4	28.6
13 and older	5	20.0	60.0	20.0	60.0	40.0
Total	39	18.0	61.5	20.5	48.7	51.3

Table 2: Results of mare pregnancy diagnostics at different evaluation methods

			Methods for determining the foal rate of mares												Criteria for evaluating mares by foaling Obtained foals	
			Traditional (control) In-foal			Ultrasound (experimental)						ELISA In-foal				
						Rectal In-foal			External In-foal							
Age of mares	Mare group	Total mares	n	%	Difference %	n	%	Difference %	n	%	Difference %	n	%	Difference %	n	%
3-4	I	5	2	50												
5-6		6	4	80												
7-8	II	9	8	100												
9-10		7	5	71,4												
11-12	III	7	6	100												
13 and over		5	3	75												

Table 3: Results of producer donor evaluation

			FSH level, mIU/1 mL	
Mare foaling days	Age of mares	Number of samples	M ± m	Evaluation indicators
50	3-4	4	8,27±0,08	Low
	5-6	5	9,12±0,47	Medium
	7-8	8	9,02±0,36	Medium
	9-10	7	7,23±0,17	Low
	11-12	6	6,02±0,27	Very low
	13 and over	4	5,42±0,11	Very low
On an average		34	7,63±0,27	
60	3-4	4	9,77±0,27	Medium
	5-6	5	10,38±0,65	High
	7-8	8	10,50±0,32	High
	9-10	7	8,91±0,60	Low
	11-12	6	8,27±0,56	Low

Table 3: Continue

	13 and over	4	7,05±0,20	Low
On an average		34	9,27±0,29	
70	3-4	4	10,52±0,21	High
	5-6	5	11,34±0,45	High
	7-8	8	11,34±0,32	High
	9-10	7	11,06±0,29	High
	11-12	6	9,15±070	Medium
	13 and over	4	9,07±0,45	Medium
On an average		34	10,54±0,23	
80	3-4	4	9,35±0,19	Medium
	5-6	5	10,74±0,48	High
	7-8	8	9,80±0,34	Medium
	9-10	7	8,40±0,40	Low
	11-12	6	6,85±0,56	Low
	13 and over	4	7,0±0,42	Low
On an average		34	8,75±0,29	
Overall average		136	9,05±0,16	

Table 4: Dynamics of LH level in donor producers

		LH level, mIU/1mL							
		Days of Pregnancy							
		50		60		70		80	
Age of mares, years old	Number of samples	N	M ± m	n	M ± m	n	M ± m	n	M ± m
3-4	16	4	2,82±0,30	4	3,73±0,21	4	4,25±0,20	4	5,0±0,22
5-6	20	5	3,12±0,18	5	4,0±0,23	5	3,80±0,25	5	3,70±0,16
7-8	32	8	2,82±0,16	8	3,71±0,14	8	4,0±0,15	8	4,74±0,17
9-10	28	7	2,46±0,14	7	3,21±0,21	7	3,58±0,16	7	5,27±0,35
11-12	24	6	2,25±0,08	6	3,80±0,19	6	3,98±0,23	6	5,33±0,28
13 and over	16	4	3,30±0,24	4	3,82±0,23	4	4,0±0,17	4	4,77±0,24

The most unreasonable for use as donor-producers are mares with low and very low FSH levels at 50 days of foaling pregnancy: 3-4-year-old -8.27±0.08 mIU/1 mL, 9-10-year-old -7.23±0.17 mIU/1 mL, 11-12-year-old 6.02±0.27 mIU/1 mL and 13 and over year old -5.42±0.11 mIU/1 mL, on 60 days of pregnancy: 9-10-year-old -8.91±0.60 mIU/1 mL, 11-12-year-old -8.27±0.56 mIU/1 mL, 13 and over year old -7.05±0.20 mIU/1 mL, on 80 days of pregnancy: 9-10-year-old -8.40±0.40 mIU/1 mL, 11-12-year-old -6.85±0.56 mIU/1 mL, 13 and over year old -7.0±0.42 mIU/1 mL.

The indicators between high and low levels of FSH evaluation are statistically of high reliability ($p<0,001$).

Consequently, for effective gonadotropic preparation, one should use mares with high FSH levels and, in addition, with average FSH evaluation values ±9.0 mIU/1 mL: At 50 days of pregnancy 5-6, 7-8-year-old, at 60 days of pregnancy 3-4-year-old, at 70 days of pregnancy 11-12, 13 and over a year old, at 80 days of pregnancy 3-4, 7-8-year-old.

The research on LH level dynamics of producer mares has shown that there are no significant differences between their ages and the content of this hormone increases with increasing pregnancy days (Table 4).

It indicates that the luteinizing hormone, which is a stimulator for follicle ovulation, cannot act as an indicator for evaluating the biological activity in the production of a gonadotropic preparation. IU. and 13 mIU/1mL and above 300 IU.

Summary

In a comparative aspect the resolving capability of different methods for diagnosing the mare pregnancy using traditional (hormonal), rectal ultrasound, external ultrasound, and enzyme-linked immunosorbent assay were identified. The use of the ELISA method in diagnostics of mare pregnancy is highly effective among these methods with setting FSH levels in blood serum within limits not lower than 4-4,9 mIU/1 mL and LH levels in blood serum within the limits not lower than 2-2,9 mIU/1 mL allowed selecting donor-producers with accuracy in the amount of 34 heads, which complies with the final result of mare pregnancy by 100%.

A new method of selection on the biological activity of preparation was developed based on the content of follicle-stimulating hormone level for a given herd, which allows determining the activity in international units,

without traditional, time-consuming, outdated method for identification using laboratory white mice, addressing ethical issues in research, selection of valuable producer donors, with FSH level not lower than 10 mIU/1 mL, as a gonadotropic preparation sources in the age aspect and their days of pregnancy.

Conclusion

Integrated scientific research of improving the diagnosis of mare pregnancy and evaluation of gonadotropic hormone activity of Pregnant Mare Serum (PMS) allowed us to formulate the following findings:

1. The hormonal status of mares of the Kazakh breed of "zhabe" type in the South Kazakhstan population in the farm "Sultanbay ata" in non-coupling season is by FSH level content within 0.1-2.9 mIU/1 mL and LH content within 0.1-1.9 mIU/1 mL
2. For expanded production of gonadotropic preparations, it is allowed to use producers with average values of FSH evaluation ≥9.0 mIU/1mL: At 50 days of pregnancy 5-6, 7-8-year-olds, at 60 days of pregnancy 3-4-year-olds, at 70 days of pregnancy 11-12, 13 and over year olds, at 80 days of pregnancy 3-4, 7-8-year-old individuals

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Author's Contributions

Kaliya Kudaikulovna Yerkekulova: Responsible executor, experimental part of the research. corresponding author, preparation of the manuscript. Share of implementation and contribution to the preparation of the article.

Nuradin Alibayev: Author of the idea, analysis, and generalization of the obtained data, Share of implementation, and contribution to the preparation of the article.

Oralbek Alikhanov and Orynbek Beketauov: Share of implementation and contribution to the preparation of the article.

Yusupzhan Artykovich Yuldashbayev: Performer, analysis of experimental data. Share of implementation and contribution to the preparation of the article.

Dastanbek Asylbekovich Baimukanov: Executor. Performer, analysis of research results. Share of implementation and contribution to the preparation of the article.

Ethics

During the research, ethics were observed in the process of studying the milk productivity of experimental animals of the studied breed. The authors of the article confirm the absence of a conflict of interest with third-party organizations.

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