The modern methods of reproduction physiology of horses

A.V. Tkachev¹, O.L. Tkacheva², B.V. Gutyj³

¹Belgorod state agricultural university named after V. Gorin, Vavilova Str., 1, Office 306, pos. Mayskiy 308503, Belgorod region, Russia
²Institute of Animal Science of National academy of agrarian sciences of Ukraine, Tvarynnykiv Str. 1-A, Kharkiv, 61026, Ukraine
³Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, Pekarska Str., 50, Lviv, 79010, Ukraine

Abstract

The concept of development of horse breeding in Ukraine until 2020 provides for an increase in the number of horses through the intensive use of modern methods of reproduction biotechnology. However, the imperfection of these methods hinders their widespread use in practice. The aim of the work was to draw attention to the most important problems of the physiology of horse reproduction in Ukraine and to show ways to solve them that have already been proposed by domestic and foreign researchers. The development strategy of the physiology of horse reproduction in order to increase its effectiveness should take into account the least studied aspects that were discussed above. The article shows that taking into account the influence of micromycetes, the absolute number of colony forming units of E. coli in semen of stallions; immunological features; new methods of sanitary preparation of horses for sperm and insemination; the effect of permissible levels of feed mycotoxins on physiological functions improves the efficiency of equine reproduction physiology methods. However, we first discovered new physiological features of the effect of erythrocyte antigens of blood groups of horses of Ukrainian selection on the indicators of their native sperm. In the presence of ad/bcm and dg/cgm alleles of the blood group D system in stallions, sperm motility is on average less than 5 points; alleles ad/cgm, ad/d, ad/de, ad/dk, bcm/d, bcm/de, bcm/dg, bcm/dk, cegm/cgm, cegm/d, cegm/dg, cegm/dk, cegm/dk, cegm/cgm, cegm/cgm, cegm/d, cegm/dk, cegm/dk, dg/di, dk/d, dk/de, dk/dk is accompanied by sperm motility from 5 to 7 points; alleles bcm/cgm, dg/dk, de/cgm, de/dk, dg/di, dk/d, dk/de, dk/dk is observed more than 7 points. The results obtained allowed us to develop for practice ways to increase the efficiency of sperm cryopreservation by immunogenetic parameters. In addition, open physiological correlations can increase the fertility of mares during mating.

Key words: physiology of reproduction, horses, sperm cryopreservation, artificial insemination, methods, perspectives.

1. Introduction

Modern horse breeding in Ukraine is in a difficult condition. The number of horses in 2018 decreased to 300,000, heads, compared to 720000 in 1992. The vast majority of factory breeds of horses, out of 12 officially registered, do not have the minimum required number of breeding stock. Only 3 breeds have the minimum allowable number of breeding stallions and mares. The yield of foals on average in the horse breeding industry of Ukraine does not exceed 50% (Tkachev et al., 2017). Of course, there are separate farms with foals over 80%, but their number is insignificant. The concept of development of horse breeding in Ukraine until 2020 provides for an increase in the number of horses through the intensive use of modern methods of reproduction biotechnology. However, the imperfection of these methods hinders their widespread use in practice. The aim of the work was to draw attention to the most important problems of the physiology of horse reproduction in Ukraine and to show ways to solve them that have already been proposed by domestic and foreign researchers.

2. Results and discussion

One of the directions in the development of methods of physiology of horse reproduction may be the transition from payet and aluminum bags to the use of the syringe tube we offer (Sushko et al., 2010; Tkachov, 2013). In the world there are 3 forms of sperm doses in which stallion sperm is frozen: 1) sequins with a capacity of 0.25–0.5 ml, are used in Western European biotechnology firms.
The concept of the effectiveness of breed-
80–100% of “safe foraging”, because it is calculated as the “mares that have gotten horse”. An analysis of the patterns
in percent; 2) “prosperous foal” in percent; 3) “foaming” or
In practice, the following phrases are used: 1) “yield foals”
chenkov, 2014; Atroshchenko et al., 2016; Naumenkova et al., 2011; Naumenkova et al., 2012; Atroshchenko & Kanashchenkov, 2014; Atroshchenko et al., 2016; Naumenkova et al., 2016; Atroshchenko et al., 2017); 3) open sperm granules of 0.25
ml, which are no longer used in practice, but sometimes the semen of stallions is frozen in the form of open granules
during scientific research (Sushko et al., 2010; Poprasath et al., 2011; Sushko & Tkachov, 2015).

The most common form of sperm in the world are 0.5 ml sequins. Such a volume makes it possible to obtain high
cooling and freezing rates of sperm, which increases their
physiological characteristics after deconservation. A major
practical drawback of payet is the inconvenience of their use
during the artificial insemination of mares. It is necessary
before artificial insemination of each mare: 1) to defrost 8–
10 payet and either to seed 8–10 times in a row, which is
very inconvenient; 2) either drain 8–10 payet into one con-
tainer, put into a syringe, connect it to the instrument and
introduce sperm into the mare's uterus, which contributes to
the contamination of sperm with microorganisms. Another
important practical drawback of payet is the need to pur-
chase special, expensive equipment for freezing them, which
is intended only for stationary work.

The second most common form of stallion sperm doses
is aluminum bags. A large volume of this form of sperm
dose has both advantages and disadvantages. The advantage
of a large sperm dose is the expectation of an increased
likelihood of successful fertilization. An important practical
advantage is that freezing of aluminum bags does not re-
quire special expensive equipment and they are convenient
for expeditionary work. The disadvantage is the inability to
ensure a uniform decrease in temperature throughout the
sperm dose. As a result, the physiological characteristics of
sperm in different places of the sperm dose may vary.

Our studies (Sushko et al., 2010; Tkachov, 2013; Sushko
& Tkachov, 2015) proved that the use of sperm doses in the form
of syringes with a volume of 4–5 ml allows us to cor-
rect the shortcomings of sequins and aluminum bags, to
obtain physiological characteristics of semen stallions after
deconservation on a par with payets. Syringe tubes do not
require special expensive equipment for freezing, do not
require sterilization costs, because already sterile syringes
can be purchased at any pharmacy.

The next important direction in the development of
physiology of horse reproduction in Ukraine may be the
correct timing of artificial insemination and an unambiguous
interpretation of fertilization. Let's start with the need for an
unambiguous interpretation of the effectiveness of mating or
artificial insemination (Naumenkova & Vasilyeva, 2006;
Naumenkova & Vasilyeva, 2007; Atroshchenko & Bragin,
2011; Naumenkova et al., 2012; Atroshchenko & Kanash-
chenkov, 2014; Atroshchenko et al., 2016; Naumenkova et al.,
2016; Tkachov et al., 2016; Atroshchenko et al., 2017).

In practice, the following phrases are used: 1) “yield foals”
in percent; 2) “prosperous foal” in percent; 3) “foaming” or
“mares that have gotten horse”. An analysis of the patterns
of the mating-foal showed that domestic stud farms have
80–100% of “safe foraging”, because it is calculated as the
percentage of foals born from mares that got horse-drawn.
This is a deliberate distortion of the effectiveness of breed-

ing and breeding work, because the diagnosis of pregnancy
in practice is practically not conducted. The stud farms
knowingly do not calculate the “foals yield” – foals that
were born from the number of all the mares that were seeded
naturally or artificially. If you calculate exactly the “yield of
foals”, then it will be at best 50–60%.

For an unambiguous interpretation of the effectiveness
of artificial insemination, we propose the use of such an
expression as “true fertility” and “general fertilization” (Su-
shko & Tkachov, 2015; Tkachov et al., 2016). By “true
fertility” we should understand the percentage of foals born
from those mares in which a full reproductive cycle with
ovulation was observed and inseminated. Only in this way
can one objectively characterize the fertilizing ability of
semen stallions. If the horse was planned to be inseminated
but not inseminated due to the fact that she did not have
ovulation, then they should not be taken into account when
establishing “true fertility”. For example, it was planned to
inseminate 10 mares, only 8 out of which had ovulation
(they were inseminated) from which 5 foals were born.
“True fertility” is 5 × 100/8 = 62.5% – this is an objective
characteristic of the fertilizing ability of semen stallions.
“General fertility” in this case is 5 × 100/10 = 50% – this
characteristic rather characterizes the effectiveness of breed-
ing and breeding work in stud farms and breeding breeders.

We propose the simultaneous use of the expressions “true
fertility” and “general fertility” in scientific papers and offi-
cial statements of the mating-foal of the subjects of breed-

ing. Some foreign researchers are already taking a similar path (Cleys et al., 2003; Morris, 2004).

The next strategic direction in the development of
physiology of horse reproduction is the development
methodological approaches to reduce bacterial (Tkachov
et al., 2011; Atroshchenko & Kanashchenkov, 2014;
Tkachov, 2014; Tkachov & Sheremeta, 2016) and micro-
mycetic (Tkachov et al., 2011; Tkachov & Sheremeta,
2016) semen contamination. Most researchers believe that
it is not even worth investigating the effect of bacterial con-
tamination on the quality of semen stallions, because this
issue has been studied for a long time and no longer has
unexplored aspects. This is a misconception. Let me explain
why. In conditions of prolonged use of antibacterial drugs,
the microflora gets used to them and sanitary and hygienic
measures become ineffective. For more than 40 years, the
veterinary and sanitary instructions for sperm sanitation and
the preparation of stallions have not been reviewed. This
confirms that today it is time for such studies. We noticed
that veterinary-sanitary measures were ineffective when we
observed an increase in the total bacterial contamination of
sperm at various biotechnological stages: obtaining sperm –
cooling – after thawing (Tkachov et al., 2011; Tkachov
& Sheremeta, 2016). We have developed methods to reduce
the contamination of prepuce (Ukrainian patent for the in-
vention No. 109846) and sperm (Ukrainian patent for the in-
vention No. 112473), which can reduce the number of bac-
teria by 56 times, and micromycetes by 40 times due to
the use of Miramistin, Dekasan, Chlorhexedine bigluconate
and Candida.

An unexplored issue remains the effect of the absolute
number of colony forming units of Escherichia coli on the
physiological characteristics of sperm of stallions and other
animals (Tkachov et al., 2011; Tkachov & Sheremeta,
2016). In the semen of stallions and other animals, only the
maximum allowable amount of coli titer is determined (up
The next important direction in the development of physiology of horse reproduction may be an increase in the number of studies on the effect of their cytogenetic profile on reproductive function. The relationship of the cytogenetic profile with the reproductive function of horses is not well studied, both in Ukraine (Tkachova et al., 2014; Rossokha & Tkachova, 2018) and abroad (Baarends et al., 2005; Baumann et al., 2011). It has been established for humans that the physiological level of general chromosomal instability should not exceed 5%. The single physiological level of general chromosomal instability for horses is almost unknown, as different authors give different data, which vary from 3 to 10%. Our studies have shown that for physiology and physiology of horse reproduction, a level of up to 10% of total chromosomal instability can be considered acceptable. Indeed, cryoresistance of semen of stallions with a general chromosomal instability of 5–10% is greater than 3 points (Tkachov & Tkachova, 2017).

The use of cytogenetic studies in reproduction allows to increase the fertility of mares in the conditions of mating and artificial insemination (Ukrainian patent No. 112459). It was found that when using stallions of the Ukrainian horse breed in mating or their semen in artificial insemination, fertility increased only if the level of total chromosomal instability did not exceed 3%, the number of metaphases with aberrations and the total number of aberrations did not exceed 2, the number of single aberrations was not more than 55%, the number of paired aberrations was not more than 45%, there were no ring aberrations and chromatid gaps, the relative length of the fourth auto pair som is not less than 4.4%, the relative length of the eighth pair of autosomes is not more than 3.65%, the relative length of the tenth pair of autosomes is not less than 3.0%, the relative length of the fourteenth pair of autosomes is not less than 4.5%. When using stallions of the Russian trotter, trotter, Belgian, Hano- merian, thoroughbred horse and New Aleksandrovsky heavy-carriage breeds or their sperm, fertility increased only if their level of total chromosomal instability did not exceed 4%. When using stallions of Westphalian, Arabian and Trakenen breeds or their semen, fertility increased only if their level of total chromosomal instability did not exceed 6%.

The proposed method for the first time allows to increase the fertility of mares during mating by 21.76% (P < 0.001), artificial insemination with chilled sperm by 25.05% (P < 0.05), thawed sperm – by 25.13% (P < 0.05) taking into account the total number of aberrations, the number of aberrations per 100 cells, the number of single, paired, androgenic aberrations and the number of gaps in aberrant metaphases for stallions and mares, as well as the relative length of autosomes (Tkachov & Tkachova, 2017).

The level of general chromosomal instability affects the effectiveness of treatment of hypofunction of ovaries of mares (Ukrainian patent No. 109754). The use of the developed method for treating hypofunction of ovaries of mares makes it possible to effectively treat the mild form of severity of ovarian hypofunction from 2.45 days, the moderate form of severity for 6.42 days, the severe form of severity for 17.4 days due to the use of specialized hormonal drugs. It allows to increase true fertility from natural mating to 84.25%, from artificial insemination with chilled sperm to 92.65%, thawed sperm to 74.45%. Such effectiveness in treating hypofunction of ovaries of mares is achieved by dividing mares into three groups depending on the level of...
general chromosomal instability. It is proposed to consider the mild form of the course of ovarian hypofunction with a level of general chromosomal instability of up to 5%; moderate severity of hypofunction – 5–10%; severe mild ovarian hypofunction with more than 10% of total chromosomal instability. This confirms the need for wider use of cytogenetic studies in the reproduction of horses (Tkachov, 2015; Tkachov et al., 2018). However, it should be borne in mind that the cytogenetic profile of horses may depend on the presence of mycotoxins in the feed (Tkachov, 2015). Which, in its turn, can reduce the fertility of mares by 32.8% (P < 0.001), the insemination rate of chilled sperm by 29.5% (P < 0.001), thawed sperm by 25.1% (P < 0.001) when eaten feed with the maximum permissible concentrations of zearalenone, T-2 toxin, deoxynivalenol, aflatoxin for four or more weeks (Tkachov & Zhukova, 2015). One of the revealed mechanisms of the negative influence of permissible levels of mycotoxins in feed for horses is to reduce the resistance (Tkachov, 2014) and the hormonal profile of horses (Tkachov, 2014). If mycotoxins enter the semen, the minimum toxic dose of zearalenone and T-2 toxin is 0.01 mM. At the same time, the biological difference between the semen of stallions and sperm of bulls is shown. The joint presence of zearalenone and T-2 toxin is more toxic to stallion sperm, for bulls only the T-2 toxin is more toxic, and not their combined penetration into semen (Tkachov & Zhukova, 2015). One of the revealed mechanisms of the negative influence of permissible levels of mycotoxins in feed for horses is to reduce the resistance (Tkachov, 2014) and the hormonal profile of horses (Tkachov, 2014). If mycotoxins enter the semen, the minimum toxic dose of zearalenone and T-2 toxin is 0.01 mM. At the same time, the biological difference between the semen of stallions and sperm of bulls is shown. The joint presence of zearalenone and T-2 toxin is more toxic to stallion sperm, for bulls only the T-2 toxin is more toxic, and not their combined penetration into semen (Tkachov & Tkachova, 2017).

Taking into account the abovementioned, the problem arises of increasing the safety of sperm membranes during freezing of semen of stallions. The highest percentage of live intact sperm (about 40%) is observed when IMV, Minitub and the Thinner developed by us are used for freezing, which ensures a foil yield of 80–85%. The use of HCFC provides no more than 19% of sperm with intact membranes, which contributes to a decrease in foals up to 55–60% (Tkachov, 2013).

The insufficiently studied problem of increasing the methods of physiology of horse reproduction is the influence of immunogenetic factors (Khrabrova & Kisilev, 2016; Khrabrova, 2017). A decrease in the yield of foals can be caused by immunological incompatibility in the system of a stallion-mare-fetus similar to a Rhesus conflict in humans. When the foal inherits erythrocyte antigens that are not found in the mare, the body of the latter begins to produce antibodies. The clinical manifestations of such an immunogenetic conflict may be abortion, a decrease in the foal yield and neonatal isoerythrolysis as a result of which the foal may die if it is not separated from the mother in time (Khrabrova et al., 2015; Khrabrova & Alekseeva, 2015; Khrabrova & Trufanov, 2015; Khrabrova & Kisilev, 2016; Khrabrova, 2017).

However, we first discovered new physiological features of the effect of erythrocyte antigens of blood groups of horses of Ukrainian selection on the indicators of their native sperm. In the presence of ad/bcm and dg/cgm alleles of the blood group D system in stallions, sperm motility is on average less than 5 points; alleles ad/cgm, ad/d, ad/de, ad/dk, bcm/d, bcm/de, bcm/dg, bcm/cgm, cegm/d, cegm/dg, cegm/dk, cegm/cgm, cegm/dg, cgm/dk, de/cgm, de/dk, dg/di, dk/dk, dk/dk is accompanied by sperm motility from 5 to 7 points; alleles bcm/cgm, dg/dk, de/d, cgm/d, cgm/de sperm motility is observed more than 7 points (Tkachov et al., 2017). The results obtained allowed us to develop for practical use ways to increase the efficiency of sperm cryopreservation by immunogenetic parameters. In addition, open physiological correlations can increase the fertility of mares during mating.

The insufficiently studied problem of reproduction of horses is the negative effect of helminth invasion. It has been proven that helminthiases are widespread in Europe, Russia, Ukraine and other countries, but studies of their negative effects on reproductive function are not enough. In veterinary medicine, 3 levels of helminth infestation are recognized. Low level (up to 200 eggs/g feces). The average preclinical level (200–500 eggs/g feces). High clinical level (more than 500 eggs/g feces). In this case, helminth invasion is determined for each type of parasite separately. We drew attention to this. For example, finding in the feces of a horse 4 types of parasite of 150 eggs/g, the laboratory concludes that the parasitological situation is preclinical. If 4 × 150 = 600 eggs/g, which is the clinical level of invasion. Having studied the complex effect of the association of three types of intestinal nematodes stronglylidae, parascaris equorum and oxyuris equi, we showed that up to 50 eggs/g of feces should be considered a low level of invasion; medium – 50–300 eggs/g feces; high – more than 300 eggs/g of feces. We proposed such levels of total helminth invasion on the basis that, at a low level (up to 50 eggs/g feces), the foal yield from chilled sperm was 90.3 ± 1.40%; at an average level (50–300 eggs/g feces) – 75.64 ± 1.28%; at a high level (more than 300 eggs/g of feces) – 50.72 ± 1.45%. The yield of foals from frozen sperm was 77, 58, and 42%, respectively (Tkachov, 2014).

3. Conclusions

Thus, the development strategy of the physiology of horse reproduction in order to increase its effectiveness should take into account the least studied aspects that were discussed above. The article shows that taking into account the influence of micromycetes, the absolute number of colony forming units of E. coli in semen of stallions; immunological and cytogenetic features; new methods of sanitary preparation of horses for sperm and insemination; the effect of permissible levels of feed mycotoxins on physiological functions improves the efficiency of equine reproduction physiology methods.

References

Aitken, J.B., Naumovski, N., Curry, B., Grupen, C.G., & Gibb, Z. (2015). Characterization of an L-Amino Acid Oxidase in Equine Spermatozoa. Biology of Reproduction, 92(5), 1–13. doi: 10.1095/biolreprod.114.126052.
Tkachov, A.V. (2011). Vlijanie mikromicetov sperm zherebcov na ee sposobnost’ vyderyzhat’ kriokonservaciju [Influence of micromycetes of sperm of stallions on its ability to withstand cryopreservation]. Scientific and Technical Bulletin Institute of Animal Science NAAN, 105, 172–177 (in Russian).

Tkachov, A.V. (2013). Jefektivnost’ iskusstvennogo osemenenija loshadej v zavisimosti ot stepeni povrezhdenija membran spermatozoidov [Efficiency of artificial insemination of horses depending on the degree of damage to the membranes of spermatozoa]. Fundamental research, 10(1), 145–147. https://www.fundamental-research.ru/ru/article/view?id=32233 (in Russian).

Tkachov, A.V. (2013). Vlijanie k ishechnyh nematod na jeffek- tivnost’ iskusstvennogo osemenenija loshadej v Ukraine [Influence of intestinal nematodes on the efficiency of artificial insemination of horses in Ukraine]. Fundamental research, 10(2), 371–373. https://www.fundamental-research.ru/ru/article/view?id=32286 (in Russian).

Tkachov, A.V. (2014). Citogeneticheskaja i biotehnologicheskaja ocenka zherebcov-pr oizvoditelej zavodskih porod v Ukraine [Cytogenetic and biotechnological estimation of pedigrees of plant breeds of Ukraine]. Livestock and veterinary medicine, 3(14), 3–7 (in Russian).

Tkachov, A.V. (2014). Vzaiemozviazok mikromicetov spermy zherebtsiv-proizvoditelej v Ukraine [Interconnection of micromycetes of sperm of stallions-producers in Ukraine]. Naukovyi visnyk Lvivskoho natsionalnoho universytetu veterinarnoi medytsyny ta biotehnolohii im. S.Z. Gzhytskoho, 16, 3(3), 186–192. https://nvivet.com.ua/index.php/journal/issue/view/22 (in Russian).

Tkachov, A.V. (2015). Citogenetsicheskij status zherebcov pod vlijaniem dopustimyh koncentracij mikotoksyniv korma v Ukrainy [Cytogenetic status of stallions under the influence of permissible concentrations of mycotoxins of fodder]. Bulletin of the Zhytomyr National Agroecological University, 2(56), 126–131. http://nbuv.gov.ua/UJR/Vzhnau_2015_2(1)_.43 (in Ukrainian).

Tkachov, A.V., Tkachova, O.L., & Rossoha, V.I. (2014). Vlijanie k ishechnyh nematod na rezistentnost’ i kontaminaciju spermy zherebtsiv [Influence of intestinal nematodes on the resistance and contamination of sperm of stallions]. Livestock and veterinary medicine, 3(14), 3–7 (in Russian).

Tkachov, A.V., Zhukova, I.O. (2015). Vlijanie iskusstvennogo osemenenija loshadej [Influence of artificial insemination on fertility of mares]. Bulletin of the Sumy National University, 2(1), 178–181. http://nbuv.gov.ua/UJR/Vsna_tvar_2014_2%281%29_44 (in Ukrainian).

Tkachov, A.V. (2015). Vlijanie iskusstvennogo osemenenija [Influence of artificial insemination efficiency]. Bulletin of the Sumy National University, 2(1), 178–181. (in Ukrainian).

Tkachov, A.V., Kalashnikov, V.A., & Sushko, A.B. (2011). Bakte- rial’naja kontaminacija sperm zherebcov-proizvoditelej na raznyh biotekhnologicheskix jetapah kriokonservacii [Bacterial contamination of sperm of stallions-producers at different biotechnological stages of cryopreservation]. Scientific and Technical Bulletin Institute of Animal Science NAAN, 104, 208–212 (in Russian).

Tkachov, A.V., Tkachova, O.L., & Rossoha, V.I. (2018). Citogene- ticheskij status kobyl ukrainskoj verhovoj porody v svjazi s oplodotvorjaemost’ju [Cytogenetic status of mares of Ukrainian upland in connection with fertilization]. Agricultural Biology, 53(2), 302–308. doi: 10.15389/agrobiology.2018.2.302zus (in Russian).

Tkachov, O.V., & Sheremeta, V.I. (2016). Vplyv fiziolochnih kiklasti kshykovoi palychky na efektyvyst kriokonservuvannia sperm zherebtsiv [Influence of physiological quantity of E. coli on the efficiency of cryopreservation of semen of stallions]. Bulletin of Kharkiv National University named after V.N. Karazin, 27, 150–154. https://periodicals.karazin.ua/biology/article/view/8223 (in Ukrainian).

Tkachov, O.V., & Sheremeta, V.I. (2016). Vzaiemovzvazok mikro- biolohichnych chynnykiv z biotekhnolohichnoiu prydatnistiu sperm zherebtsiv do okholodzhennia [Interconnection of microbiological factors with biotechnological suitability of semen of stallions to cooling]. Bulletin of the Zhytomyr National Agroecological University, 2(56), 298–304. http://nbuv.gov.ua/UJR/biv_2015_17_1_19 (in Ukrainian).

Tkachov, O.V., Kalashnikov, V.O., & Sushko, O.B. (2011). Hrybka kovalentziatsiia sperm zherebtsiv-plidnykiv trakenkovi ta arabskoi porid na rynzkyh etapakh biotekhnolohichnoi obrobky [Fungal contamination of semen of pedigrees of Trakhenian and Arab breeds at different stages of biotechnological processing]. Scientific Bulletin of NUIBP series “Tech- nology of production and processing of livestock products”, 160(2), 26–31 (in Ukrainian).

Tkachov, O.V., Sheremeta, V.I., & Tkachova, O.L. (2016). Vplyv chasu shtuchnoho osimeninnia vidnosno ovuliatsii na zaplid- niuvanist kobyl [Influence of artificial insemination time on ovulation time on fertility of mare]. Scientific Messenger LNUVMBT named after S.Z. Gzhytskij, 18, 2(67), 241–244. doi: 10.15421/nlvnet6753 (in Ukrainian).

Tkachov, O.L. Dobrodeva, L.T., Rossoha, V.I., Rossoha, L.V., & Tkachov, A.V. (2014). Citogenetikashecha i biotekhnologicheska ocenenka zherebco-v-proizvoditelej zavodskih porod Ukrainy [Cytogenetic and biotechnological estimation of stallions-producers of plant breeds of Ukraine]. Zootechnical science of Belarus, 49(1), 162–171. https://elibrary.ru/item.asp?id=25051063 (in Russian).