AGRICULTURAL BIOLOGY,
2018, V. 53, № 4, pp. 753-761
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.2:636.082:618

doi: 10.15389/agrobiology.2018.4.753eng
doi: 10.15389/agrobiology.2018.4.753rus

FORECASTING THE EMBRYO PRODUCTIVITY OF DONOR COWS ON THE BASIS OF ECHOGRAPHIC CHARACTERISTICS OF THE OVARIES

A.V. BRIGIDA[1], V.I. SOROKIN[2], S.N. KOVALCHUK[1], K.S. PANTIUKH[3], I.V. RUKIN[3, 4], K.A. ROZHIN[2]

[1]*Center for Experimental Embryology and Reproductive Biotechnologies,* Federal Agency of Scientific Organizations, 12/4, ul. Kostyakova, Moscow, 127422 Russia, e-mail brigida_86@mail.ru, s.n.kovalchuk@mial.ru;
[2]*Orenburg State Agrarian University,* 18, ul. Cheluskintsev, Orenburg, 460014, Russia, e-mail vis5256@yandex.ru, info-ceerb@mail.ru;
[3]*ZAO Genoanalitika,* 77/vl. 1, ul. Leninskie gori, Moscow, 119991 Russia, e-mail pantiukh@i-gene.ru;
[4]*Lomonosov Moscow State University,* 1, ul. Leninskie gori, Moscow, 119991 Russia, e-mail ruki.n.ilya@gmail.com
(✉ corresponding author)
ORCID:
Brigida A.V. orcid.org/0000-0002-0139-8087
Sorokin V.I. orcid.org/0000-0003-0057-2976
Kovalchuk S.N. orcid.org/0000-0002-5029-0750
Pantiukh K.S. orcid.org/0000-0002-2595-0673
Rukin I.V. orcid.org/0000-0003-4093-3254
Rozhin K.A. orcid.org/0000-0002-4034-7434
The authors declare no conflict of interests
Acknowledgements:
Authors thank employees of the farm Plemzavod Dimitrovsky (Orenburg region) for their assistance in the research. Supported financially by Ministry of Education and Science of the Russian Federation for applied projects, Subsidy Agreement № 14.579.21.0147, UID RFMEFI57917X0147
Received December 25, 2017

Abstract

The main problem restricting the wide use of reproductive biotechnology in animal husbandry is insufficiently developed methods for selection of donor cows for embryo transfer. The objective reason is the variability of the ovarian response to gonadotropins injections. Until now, there is no reliable information about possibility of forecasting the embryo productivity of donor cows before gonadotropin stimulation, which affects substantively the economic feasibility of embryo transfer as a method of accelerated cattle reproduction. We have applied the post-pressing analysis of ovaries echograms for forecasting the embryo productivity of donor cows on the basis of comparisons of quantitative and qualitative indicators of ovaries after induced superovulation and its' echographic characteristics. We carried out morphofunctional study of ovaries in donor cows (n = 30) on day 10 of estrous cycle, before artificial insemination (estrus) and on day 7 of the inducted estrous cycle, immediately before the extraction of embryos using data on post-pressing ovarian morphometry. Animals were divided into three groups (I, II и III, n = 10 for each group) with yellow body length of 2.5 cm, 1.5-2.5 cm and 1.5 cm, respectively. Echographic visualization of the ovaries was performed using endorectal ultrasonography. Polyovulatory response of ovaries was induced with FSH-super (Russia) injected eight times, with 12 h interval, at decreasing doses. The embryos were recovered on day 7 after artificial insemination. Optimal criteria for predicting the polyovulatory response of ovaries and the quantity of embryos were determined on the basis of the ovarian morphometry. Statistically significant differences with the control were assessed by the Student's t-test. It was found that the average areas of the ovaries on the echograms were 7.9±0.94, 5.7±0.78 and 3.5±0.06 cm^2 for group I, group II and group III, respectively. The area of the yellow body in group I averaged 4.5±1.21 cm^2, was 2.08 cm^2 higher ($P \leq 0.05$) than in group II, and exceeded the corresponding parameter in group III by 3.43 cm^2 ($P \leq 0.05$). A comparative evaluation of the ratio of the yellow body areas to the ovaries area of each animal and on average along the groups showed that in group I with the ratio of 57.1±3.01 % the number of yellow bodies was 11.6±1.26 and the average yield of the embryos was 9.3±1.23 per animal. In group II with the ratio of the areas of yellow bodies and ovaries of 42.1±2.9 % the number of yellow bodies before embryos recovery was 5.7±1.24, and 4.6±1.01 embryos were recovered per procedure. The lowest embryo recovery (less than one embryo per procedure) was observed in group III with the relative area of yellow bodies of 30.2±2.56 % and the average number of the yellow bodies of 1.8±0.18. Comparison of the size of the yellow bodies before the induction of polyovulation and data characterizing the efficiency of induction of superovulation and recovery of embryos showed that in animals with a ratio of the areas of the yellow body and ovary more than 50 %, high response can be obtained resulting in 11.6±1.26 yellow bodies and 9.3±1.23 embryos per extraction.

Keywords: cow, ovaries, estrus, echography, morphometric parameters, polyovulation, embryos, embryo productivity

Increase of cattle livestock severely curtailed by extremely low frequency of tweens and long pregnancy. Though the female generative potential in cattle is enormous, i.e. hundreds of thousands of ova, with only a small portion used before the end of the economic use of the animal [1].

Embryo transfer biotechnology of accelerated reproduction opens huge opportunities in the realization of reproductive and biological potential of the animals with the given phenotypic and genotypic characteristics and for its subsequent reproduction in the least valuable recipient herds [2-6]. Despite the advances in technology, the search is relevant for methods to select donors and recipients, to induce superovulation with a higher output of embryos, to decrease the process efforts, and to lower animal stress [7-9].

Embryo transfer technology includes many stages [10]. The first requires the selection of donor cows [11] for the subsequent induction of the polyovulatory response of ovaries [12, 13]. The focus at this point is usually put on the breeding value and the gynaecological health of the animal, but not to the prediction of individual ovarian response on the gonadotropins injected. At the same time, the donor embryos selection is critical for the effectiveness of the following stages and economic expediency of the transfer procedure in general [14].

Variability of cows' ovaries response to exogenous gonadotropins leads to exclusion of approximately 30% of the treated animals from the donor group because of lack of the polyovulatory response to gonadotropins [15]. It is the high variability that makes the polyovulatory response unpredictable [16, 17] and serves as the main limitation of the practical application of the embryo transfer technology. Currently, no reliable information is still present on the possibility of predicting embryo productivity in donor cows prior to the introduction of gonadotropins that significantly affects the cost-effectiveness of such an expedited reproduction of the cattle.

Analysis of reported dataindicates that the effectiveness of the response of the ovaries of embryo donor cows to the exogenous gonadotropin is influenced by many factors [18]. The major are the breed and physiological state of the animals [19, 20], especially metabolic [21] and hormonal status [22-25], genetic predisposition [26-30], ecological and climatic conditions [31, 32]. Given the multiplicity of reasons, the ovaries are the most convenient object to predict embryo productivity of a cow [33-35].

Ultrasonograhy (US) is considered as a promising method to evaluate the ovarian morphofunctional state for embryo transfer [36]. The examination is easy to reproduce in a production environment, it is minimally invasive, and allows one to get the information, complete and objective enough, in real time.

We were the first to use the postpressing morphometry of the ovarian structures for prediction of superovulatory response prior to the introduction of gonadotropins and therefore provided a fast method of selection of cows for embryos recovery. The method testing showed its perspectivity for the cows' embryo productivity estimation.

The aim of the study was to compare the ovarian US study data, embryo productivity, and embryo recovery to develop ways to assess the expected superovulatory reaction and identify the promising donors of embryos.

Techniques. A study is made on the cows from breed Kazakh white-headed (LLC Plemzavod Dimitrovskij, Ileksky region, Orenburg Province). As donors, clinically healthy animals were selected (n = 30) with no signs of metabolic disorders (obesity, dystrophy, etc.) with data on the origin of at least three rows of ancestors, with a strong constitution and the exterior score no less than 8

points, body weighing not below the standard of the breed, aged 3-6 calvings, with certainty of origin (by blood groups), with the first manifestations of estrus within 50 days after calving, easy calving and uncomplicated postpartum period, insemination index of 1.2-1.5, and normal condition of the uterus and ovaries (according to rectogenital US as a real-time visualization, and to the endorectal palpation of reproductive organs). All cows have been screened for infectious diseases (brucellosis, tuberculosis, viral respiratory infections, leukemia, trichomoniasis, including foot and mouth disease, etc.). According to the results, the animals were divided into three groups (n = 10 each) depending on the length of the yellow body (I, II and III, respectively from 2.5; 1.5-2.5; and less than 1.5 cm).

Morphofunctional evaluation of ovaries of the cows was conducted with the follow-up (considering also the postpressing ovaries morphometry) on day 10 of the sexual cycle prior to the introduction of follicular stimulators, prior to insemination if multiple mature dominant follicles were present, and at day 7 of the induced sexual cycle with the polyovulatory response of ovaries and the presence of multiple yellow bodies on the surface of the ovaries (directly before removing the embryos).

For endorektal sonogaphic ovarian visualization, the Tringa Linear (ESA OTES p. a., Italy) and Kaixin KX5200 (Xuzhou Kaixin Electronic Instrument Co., Ltd, China) ultrasound scanners were used with linear sensors (frequency 7.5 MHz); the black and white US images of reproductive organs were recorded. When postpressing morphometry, the US images were treated using the ImageJ graphical editor (National Institute of Health, USA). The length, width, and size of ovaries and yellow bodies as parameters of functional activity were determined. The values were calculated taking into account the length of the straight line, polyline, irregular circumference line, the area of geometric figure, and the angle of projection of the two sections. The projections of the structures at the US image were displayed schematically. Ovarian and yellow body areas were determined by the formula for the ellipsoid: $S = \pi Rr$, where R and r are, respectively, the major and minor semiaxis.

The sexual cycle of cows was induced by Estrofan (Bioveta, Czech Republic), a prostaglandin F2a drug, 2 ml per animal (one intramuscular injection), polyovulation — by the FSH-super (Russia) injected eight times with 12 hours intervals (morning-evening) in decreasing doses (50 AE, 3; 3; 2.5; 2.5; 2; 2; 1.5; 1.5 ml per animal).

For artificial insemination the fresh semen of outstanding bulls was used, tested for offspring quality and recognized as grades based on such factors as ease of calving and milk yield. Semen was consistent with RF Standard GOST 26030-2015 (main indices: the percentage of sperm with straight-forward movement is not less than 40%, their number in a single dose is 15 mln; the amount of doses for artificial insemination is not less than 0.2 cm^3; the viability of sperm at 38 °C is no less than 5 hours). Selection of sires and donor cows was in accordance with the plan of breeding work at the farm.

Embryos were harvested using the extrusion method (liquid injection in an isolated part of the uterine horn with a syringe connected to a 2-channel catheter previously entered in the uterus horn) on day 7 after insemination. Flexible cleaning catheters Neustadt/Aisch Rusch SN18 were used with four ports (Minitube, Germany). Fluid filtration and collection of embryos, the filters for cattle Em Guard (Minitube, Germany) were used.

To define the morphological condition of the embryos, they were examined under a Biolam microscope (JSC LOMO, Russia, × 60-100 magnification). The following was considered: the compliance with stages of development, the

shape of the zone pellucida and its integrity, the uniformity of crushing blastomeres, and the general condition of the cytoplasm.

When aggregating results, the medium (*M*) and their standard errors (± SEM) were calculated. The data was processed using Microsoft Excel 2010 and Statistica 6 (StatSoft, Inc., USA). Reliability of differences was assessed by *t*-test, deeming them statistically significant at $p \leq 0.05$.

Results. An example of echographic images of the cow ovarian morphometry is provided on Figure 1.

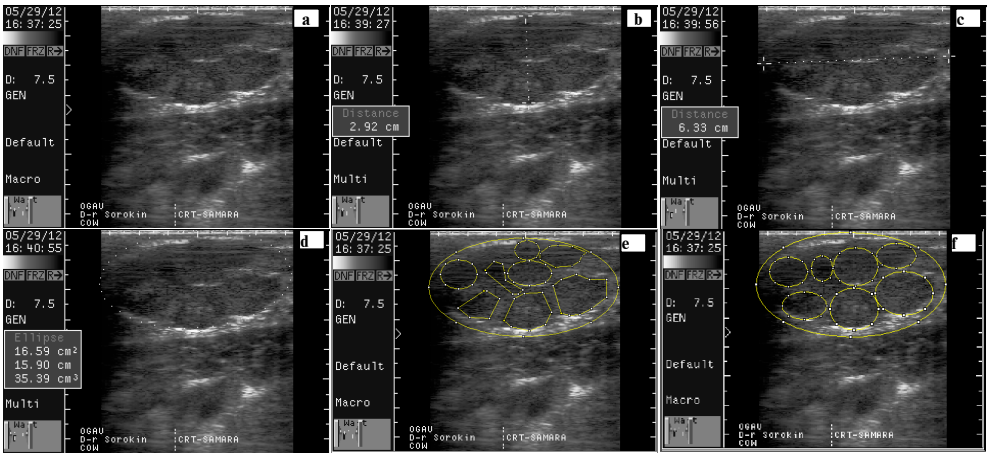

Fig 1. An example of US image processing of the embryo donor cow ovary No. 12764 (Kazakh white-headed breed) when assessing the polyovulatory response: a — original image; b— the height of the ovary, c — the width of the ovary, d — the circumference of the ovary, e — the border of the structures depicted expressed by irregular circlular polylines, f — the projection of the structures shown on the US image (sagittal scanning plane, b-f are image processing with graphics processing ImageJ editor).

For the prediction of ovarian response to gonadotropin drugs, we evaluated the embryo productivity of the donor cows by analyzing the morphometric indices of ovaries and yellow bodies with subsequent postpressing processing of US images of reproductive organs of the cows at day 10 of the sexual cycle. Measuring the length and width of the ovaries and yellow bodies allowed us to calculate their area, the ratio of the size of the yellow body and the ovary.

The results of the morphometry of the linear dimensions and area of ovaries and yellow bodies in donor cows are presented in Table 1.

1. Morphometric indices of ovaries and yellow body in embryo donor cows at day 10 of sexual cycle without polyovulation induction (*M*±SEM, Kazakh white-headed breed, LLC Plemzavod Dimitrovskij, Orenburg Province, 2017)

Average for group	Ovaries			Yellow body		
	length, cm	width, cm	area, cm^2	length, cm	width, cm	area, cm^2
I (*n* = 10)	4.0±0.09	2.5±0.11	7.9±0.94	2.7±0.02	2.1±0.14	4.5±1.21
II (*n* = 10)	3.2±0.04	2.3±0.34	5.7±0.78*	2.0±0.90	1.5±0.08	2.4±0.24*
III (*n* = 10)	2.4±0.12	1.8±0.04	3.5±0.06**	1.4±0.08	1.0±0.00	1.1±0.25*

N o t e. Groups I, II, and III are animals with the yellow body length, respectively, up to 2.5, 1.5-2.5, and less than 1.5 cm.

*, * Differences in ovarian area in groups II and III as compared to group I are statistically significant at $p \leq 0.05$ and $p \leq 0.001$, respectively.

The largest ovarian area was in group I, the lowest was in group III. This rate in cows of group I exceeded that of individuals from group II (2.2 cm^2, $p \leq 0.05$) and group III (4.3 cm^2, $p \leq 0.001$). The average size of yellow bodies also varies by group. Thus, in the group I the value was higher than in the group II by 2.08 cm^2 ($p \leq 0.05$) and group III by 3.43 cm^2 ($p \leq 0.05$).

2. Areas of ovaries and yellow body in embryo donor cows at day 10 of sexual cycle without polyovulation induction (*M*± SEM, Kazakh white-headed breed, LLC Plemzavod Dimitrovskij, Orenburg Province, 2017)

Average for group	Area, cm^2		Area of the yellow body of the ovary area, %
	ovary	yellow body	
I (*n* = 10)	7.9±0.94	4.5±1.21	57.1±3.01**
II (*n* = 10)	5.7±0.78	2.4±0.24	42.1±2.95*
III (*n* = 10)	3.5±0.06	1.1±0.25	30.2±2.56

Note. Groups I, II, and III are animals with the yellow body length, respectively, up to 2.5, 1.5-2.5, and less than 1.5 cm.
*, ** Differences of ratios and areas of yellow bodies and ovaries in groups I and II as compared to group III are statistically significant at $p \leq 0.01$ and $p \leq 0.001$, respectively.

We compared the ratio of areas of yellow bodies and the ovaries of each animal and the average for the groups (Table 2). This estimate, characterizing the size of the functionally active yellow body before induction of polyovulation, shows the size of luteal tissue being the determinator of the size of the ovaries. In animals with the smallest size of yellow body its lutein structures amounted to 30.2% of the total area of the ovaries that is reliably less than the relevant indices in animals in groups II and III by 12.0 ($p \leq 0.01$) and 27.0 cm^2 ($p \leq 0.001$), respectively.

Further ovarian US visualization conducted during estrus before insemination (0 day of sexual cycle) and before removing embryos at day 7 after insemination. The postpressing US images received were processed to determine the changes in the ovaries in response to FSH stimulation and exogenous luteolytic drugs of the F2a prostaglandin type. In doing so, we considered also the ovaries morphometry and the quantitative and qualitative composition of follicles and yellow bodies, reflecting the polyovulatory response of ovaries (Fig. 2).

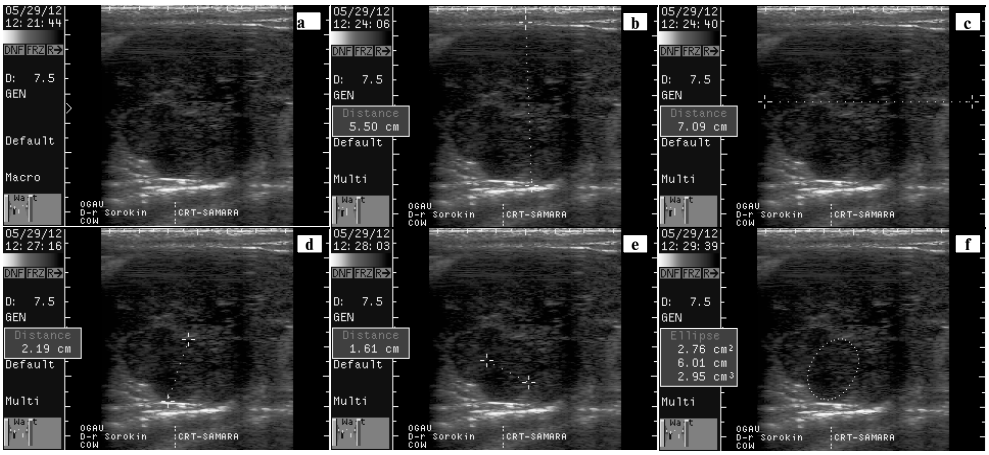

Fig. 2. The US display of polyovulatory response of ovaries of embryo donor cows (Kazakh white-headed breed) with a length of yellow body of 1.5-2.5 cm: a — the original image; b — the height of an ovary; c — the width of the ovary; d — the height of the yellow body; e — the width of the yellow body; f — the circumference of the yellow body (sagittal plane scan, image processing with graphics processing ImageJ editor).

The results of the rectopalpatory ovaries exam, as well as the data obtained by ultrasound scanning and postpressing US processing, demonstrate that the polyovulatory response of ovaries and conception oocyte fertilization depend on the quality of the yellow body in the middle of L-phase (during the period prior to the beginning of the gonadotrophic stimulation of the donor cows) (Table 3). In groups I and II the number of yellow bodies and resultant embryos differed nearly twice, while in the group III were almost an order of magnitude lower (see Table 3).

We used the postpressing US images processing for the morphometric evaluation of ovaries and structures involved in folliculogenesis and luteogenesis in

cows. By the report of V. Kayacik et. al. [36], postpressing US images processing indicates high efficiency of echography method in the morphometric studies.

3. Stimulation of polyovulation and embryo productivity in embryo donor cows, depending on the ratio of the yellow body and ovary area (*M*±SEM, Kazakh white-headed breed, LLC Plemzavod Dimitrovskij, Orenburg Province, 2017)

Average for group	Area of the yellow body of the ovary area, %	Number of	
		yellow bodies	embryos
I (*n* = 10)	57.1±3.01	11.6±1.26	9.3±1.23
II (*n* = 10)	42.1±2.95	5.7±1.24	4.6±1.01
III (*n* = 10)	30.2±2.56	1.8±0.18	0.5±0.02

Note. Groups I, II, and III are animals with the yellow body length, respectively, up to 2.5, 1.5-2.5, and less than 1.5 cm.

The comparison of data on the size of yellow bodies before induction of polyovulation and the results characterizing the efficiency of superovulation and embryos recovery show that cows with the more than 50% ratio of the yellow body to ovary area are appropriate for ovarian induction to get the number of yellow bodies 11.6±1.26 on average and embryos 9.3±1.23 per extraction. Note, the recovery of average 5-6 embryos per one handled animal is usually reported [3, 8, 15, 34].

Thus, the US ovarian morphometry is highly informative and allows breeder to objectively evaluate ovarian functional status and predict the embryo productivity in cattle. Processing of US images in ImageJ editor significantly improves the performance of US imaging. It is shown that the response to the FSH stimulation and embryo recovery depend on the size of the yellow body in the potential donors at day 10 of sexual cycle (without the induction of polyovulation). This can be used as a criterion in the selection of animals to a group of potential donors of embryos. So, when the ratio of the yellow body and the ovary area is more than 50%, the numbers of ovulated follicles and quality embryos are, respectively, 11.6±1.26 and 9.3±1.23 per cow.

REFERENCES

1. Anzorov V.A., Shiriev V.M., Sergeev N.I., Titov V.A., Nasibov F.N., Baitlesov E.U. Comparative characteristic and estimation of viability in embryos obtained from healthy and problem cows-donors with various level of dairy productivity. *Sel'skokhozyaistvennaya Biologiya* [*Agricultural Biology*], 2005, 6: 37-41 (in Russ.).
2. Kononov V.P., Chernykh V.Ya. *Biotekhnika reproduktsii v molochnom skotovodstve* [Biotechnics of reproductions in dairy cattle breeding]. Moscow, 2009 (in Russ.).
3. Baruselli P.S., Vieira1 L.M., Batista E.O.S., Ferreira R.M., Sales J.N.S., Gimenes L.U., Torres-Junior J.R.S., Martins C.M., SáFilho M.F., Bo G.A. Updates on embryo production strategies. *Anim. Reprod.*, 2015, 12(3): 375-382.
4. Hopper R.M. *Bovine reproduction.* Wiley Blackwell, 2015: 696-733.
5. Kugonza D.R., Kayitesi A., Semahoro F., Ingabire D., Manzi M., Hirwa C.A., Gahakwa D. Factors affecting suitability of surrogate dams for embryo transfer in cattle. *Journal of Animal Science Advances*, 2013, 3(4): 203-210 (doi: 10.5455/jasa.20130430123202).
6. Vasconcelos J.L.M., Dementrio D.G.B., Santos R.M., Chiari J.R., Rodrigues C.A., Filho O.G. Factors potentially affecting fertility of lactating dairy cow recipients. *Therlogenology*, 2006, 65: 192-200 (doi: 10.1016/j.theriogenology.2005.09.030).
7. Ptashinskaya M. *Kratkoe rukovodstvo po reproduktsii zhivotnykh* [A brief guide to animal reproduction]. Intervet International BV, 2009 (in Russ.).
8. Ernst L.K., Varnavskii A.N. *Reproduktsiya zhivotnykh* [Animal reproduction]. Moscow, 2007 (in Russ.).
9. Mapletoft J., Garcia Guerra A., Dias F.C.F., Singh J., Adams G.P. In vitro *and* in vivo embryo production in cattle superstimulated with FSH for 7 days. *Anim. Reprod.,* 2015, 12 (3): 758-388.
10. Petroman I., Pacala N., Petroman C., Bencsik I., Orboi M.D., Dronca D., Băneş A. Utilization of gestagen hormones and pituitary FSH extracts in inducing the superovulation at embryo donor cows. *Journal of Food Agriculture & Environment,* 2009, 7(2): 193-195.
11. Voronin E.S., Petrov A.M., Serykh M.M., Devrishov D.A. *Immunologiya* [Immunology]. Moscow, 2002 (in Russ.).
12. Kosovskii G.Yu., Popov D.V., Brigida A.V. *Veterinariya i kormlenie*, 2016, 5: 29-31 (in Russ.).
13. Kosovskii G.Yu., Popov D.V., Brigida A.V., Volkolupov G.V. *Izvestiya Nizhnevolzhskogo agrouniversitetskogo kompleksa: nauka i vysshee professional'noe obrazovanie*, 2015, 3(39): 106-108 (in Russ.).
14. Sanchez Z., Lammoglia M.A., Alarcon M.A., Romero J.J., Galina C.S. Is the production of

embryos in small-scale farming an economically feasible enterprise? *Reprod. Domest. Anim.*, 2015, 50(4): 574-579 (doi: 10.1111/rda.12526).
15. Kosovskii G.Yu., Popov D.V., Brigida A.V., Volkolupov G.V. *Izvestiya Nizhnevolzhskogo agrouniversitetskogo kompleksa: nauka i vysshee professional'noe obrazovanie*, 2015, 2(38): 148-152 (in Russ.).
16. Liang A., Salzano A., D'Esposito M., Comin A., Montillo M., Yang L., Campanile G., Gasparrini B. Anti-Mullerian hormone (AMH) concentration in follicular fluid and mRNA expression of AMH receptor in granulose cell as predictive markers of good buffalo (*Bubalus bubalis*) donors. *Theriogenology*, 2016, 86(4): 963-970 (doi: 10.1016/j.theriogenology.2016.03.020).
17. Rao M.M., Uma Mahesh Y., Misra A.K. Evaluation of ovarian response and embryo production pattern in Ongole cows. *Indian Journal of Animal Science*, 2010, 80(10): 973-975.
18. Baruselli P.S., Batista E.O.S., Vieira L.M., Sales J.N.S., Gimenes L.U., Ferreira R.M. Intrinsic and extrinsic factors that influence ovarian environment and efficiency of reproduction in cattle. *Anim. Reprod.*, 2017, 14(1): 48-60 (doi: 10.21451/1984-3143-AR907).
19. Ali M.S., Khandoker M.A.M.Y., Afroz M.A., Bhuiyan A.K.F.H. Ovarian response to different dose levels of follicle stimulating hormone (FSH) in different genotypes of Bangladeshi cattle. *Asian-Australasian Journal of Animal Science,* 2012, 25(1): 52-58 (doi: 10.5713/ajas.2011.11167).
20. Villaseron Gonzaleza F., de la Torre Sanchez J.F., Martinez Velazques G., Gallardo H.A., Reynozo S.P., Fránquez J.A.P., Sojo R.P., Bermúdez M.M. Characterization of the ovarian response to superovulation in Creole Coreno cattle using reduced doses of FSH. *Revista Mexicana De Ciencias Pecuarias*, 2017, 8(3): 225-232.
21. Sartori R., Monteiro P.L.J. Jr., Wiltbank M.C. Endocrine and metabolic differences between *Bos taurus* and *Bos indicus* cows and implications for reproductive management. *Anim. Reprod.*, 2016, 13(3): 168-181 (doi: 10.21451/1984-3143-AR868).
22. Aziz R.L.A., Khalil A.A.Y., Abdel-Wahab A., Hassan N.Y., Abdel-Hamied E., Kasimanickam R. Relationship among circulating anti-Mullerian hormone, insulin like growth factor 1, cadmium and superovulatory response in dairy cows. *Theriogenology*, 2017, 100: 72-79 (doi: 10.1016/j.theriogenology.2017.06.007).
23. Baruselli P.S., Batista E.O.S., Vieira L.M., Souza A.H. Relationship between follicle population, AMH concentration and fertility in cattle. *Anim. Reprod.*, 2015, 12(3): 487-497.
24. Mossa F., Jimenez-Krassel F., Scheetz D., Weber-Nielsen M., Evans A.C.O., Ireland J.J. Anti-Mullerian Hormont (AMH) and fertility management in agricultural species. *Reproduction,* 2017, 154(1): R1-R11 (doi: 10.1530/REP-17-0104).
25. Redhead A.K., Siew N., Lambie N., Carnarvon D., Ramgattie R., Knights M. The relationship between circulating conception of AMH and LH content in the follicle stimulating hormone (FSH) preparations on follicular growth and ovulatory response to superovulation in water buffaloes. *Animal Reproduction Science*, 2018, 188: 66-73 (doi: 10.1016/j.anireprosci.2017.11.010).
26. Yang W.C., Tang K.Q., Li S.J., Yang L. Association analysis between variants in bovine progesterone receptor gene and superovulation traits in Chinese Holstein cows. *Reprod. Domest. Anim.*, 2011, 46(6): 1029-1034 (doi: 10.1111/j.1439-0531.2011.01780.x).
27. Yang W.C., Li S.J., Tang K.Q., Hua G.H., Zhang C.Y., Yu J.N., Han L., Yang L.G. Polymorphisms in 5′ upstream region of the FSH receptor gene, and their association with superovulation traits in Chinese Holstein cows. *Animal Reproduction Science*, 2010, 119(3-4): 172-177 (doi: 10.1016/j.anireprosci.2010.02.004).
28. Yang W.C., Tang K.Q., Li S.J., Chao L.M., Yang L.M. Polymorphisms of the bovine luteinizing hormone/choriogonadotropin receptor (*LHCGR*) gene and its association with superovulation traits. *Molecular Biology Reports,* 2012, 39(3): 2481-2487 (doi: 10.1007/s11033-011-0999-4).
29. Yang W.C. Li S.J., Xie Y.H., Tang K.Q., Hua G.H., Zhang C., Yang L.G. Two novel SNPs of the type I gonadotropin releasing hormone receptor gene and their associations with superovulation traits in Chinese Holstein cows. *Livestock Science*, 2011, 136: 164-168 (doi: 10.1016/j.livsci.2010.08.012).
30. Yu Y., Pang Y., Zhao H., Xu X., Wu Z., An L., Tian J. Association of a missense mutation in the luteinizing hormone/choriogonadotropin receptor gene (*LHCGR*) with superovulation trains in Chinese Holstein heifers. *Journal of Animal Science and Biotechnology*, 2012, 3(1): 35 (doi: 10.1186/2049-1891-3-35).
31. Bezdicek J., Makarevich A., Stadnik A., Kubovičová E., Louda F., Hegedüšová Z., Holásek R., Ducháček J., Stupka R. Analysis of factors affecting the quantity and quality of embryo production in superovulated cows. *Zuchtungskunde*, 2015, 87(4): 249-264.
32. Mehmood M.U., Mehmood S., Riaz A., Ahmad N., Sattar A. Superovulatory response in Summer anestrus Buffaloes and cattle treated with estrus synchronization protocol. *Journal of Animal and Plant Science*, 2012, 22(4): 888-893.
33. Glazko T.T., Kosovskii, G.Yu., Popov D.V., Brigida A.V. *Veterinariya Kubani*, 2015, 6: 9-11 (in Russ.).
34. Kosovskii, G.Yu., Popov D.V., Brigida A.V. *Veterinariya Kubani*, 2015, 5: 15-17 (in Russ.).
35. Tomac J., Cekinovich D., Arapovic J. Biology of the corpus luteum. *Periodicum Biologorum*, 2011, 113(1): 43-49.
36. Kayacik V., Salmanoglu M.R., Polat B., Ozluer A. Evaluations of the corpus luteum size throughout the cycle by ultrasonography and progesterone assay in cows. *Turk. J. Vet Anim Sci.*, 2005, 29: 1311-1316.