

Functional asymmetry in cattle ovaries and donor-recipients embryo

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The results of scientific research of the functional asymmetry of paired gonads of dairy cows had been studied. Statistical analysis of the lateral localization of morphofunctional ovarian state of cows during the different phases of the sexual cycle confirmed the conclusions of the evolutionary concept of the relevance of functional asymmetry of paired organs of bilateral organisms. The adaptive relevance is displayed by organizing the lateral structure on principle of enantiomeric domination. The quantitative ratio of the activity of the left or right ovaries in cows from different populations had been confirmed for the first time. According to our research, the abovementioned ratio was close to universal harmonic proportion of L/R as 1.000/1.612-1.622. We had revealed that environmental change impact the universal biological mechanism of self-regulation of functions in complex multicomponent living systems. The relative dominance of the right gonad in cows and the inversion phenomenon of dominance under the influence of hormonal stimulation of multiple folliculogenesis by the injection of gonadotropic drugs (FSH) had been proved for the first time. Experimentally established fundamental laws of the structural organization of the functional asymmetry of the of cows paired gonads allowed to the development of applied ways of improving the methods of stimulation and optimization of reproductive function of lactating dairy cows and donor- recipients of pre-implantation cattle embryos.

Keywords: Functional asymmetry; Cows; Evolution; Adaptation; Ovaries; Palpation diagnostics; Laterality

Introduction

Biological features of the farm animal reproductive system are the basic components of the production processes of all branches of animal husbandry. Breeding of cattle is crucial for the food security of any country, since livestock products not only provide a substantial part of the protein in the diet of the population, but also solve important social employment issues of the rural regions. Modern biotechnologies of processing waste of cattle-breeding farms contribute to the preservation of the environment and develop the production of alternative energy resources (bioenergy, bio-fertilizers, etc.). Recently in the developed countries an in-depth study of the biological laws of the reproductive function of cows and heifers has become relevant, which gives an additional impetus to a more efficient use of this type of farm animals (Bomko et al., 2018; Mazur et al., 2020; Roman et al., 2020; Borshch et al., 2020; Grymak et al., 2020).

All mammals have a bilateral body structure. Most farm animals, including cattle, belong to the mammals, therefore the study of the patterns of bilateral dimorphism of different systems and organs of their life-support has a great theoretical and practical importance. For more than 150 years, the researchers have been documenting the facts of functional asymmetry in animals of different species as well as in people, but up until now, the discussions are taking place among the biologist the origin of this phenomenon (Martynenko, 1956; Denenberg, 1983; Bianki, 1985; Selk, 2014). Over the past decades, the controversy towards biological significance of functional asymmetry in bilateral organisms have intensified as a result of the emergence of new and controversial experimental data on the laterality of the neurohumoral regulation of the body, chemical asymmetry and of brain hemispheric asymmetry testing (Bianki, 1985; Nikitin, 1991; Geodakyan, 2005; Ljashhenko & Bessalova, 2006; Klimentenko, 2008).

The review of the literature indicates that there are the open questions on the origin and significance of the functional asymmetry of bilateral organisms. The most research focused on study individual physiological processes and do not cover the activities of the organism, the species or the population in general. Therefore experimental data on laboratory animals, the results of field studies of different species of wild fauna or the analysis of testing of left-right-handed groups of people can't be extrapolated due to of common methodological basis (Denenberg, 1983). The data obtained using different research methods are disproportionate and cannot be systematically summarized. The generally accepted conclusions about inter-hemispheric functional asymmetry in humans remain debatable regarding different animal species (Denenberg, 1983; Klement'ev, 1995; Orlov et al., 2002; Ioffe & Pletnev, 2002; Dominguez, 2003; Klimentenko, 2008; Ljashhenko & Bessalova, 2006; Petrosienko & Cheremushnikva, 2010).

Intersectoral and interdisciplinary barriers characteristic of the current state of science hinder the consideration of complex fundamental related problems of various mammals species in the modern constantly changing world. This includes productive animals reproduction of which takes place in artificial conditions of industrial enterprises. Paired gonads of cows and heifers are the main organs of the reproductive system. These organs are the anatomical and physiological basis of technological processes in dairy and beef cattle breeding. Therefore, the depth of understanding of ovarian morphogenesis and ways of its regulation determines the efficiency of the industry and profitability of enterprises.

Now days the study of the morphology and ovarian function in cows is carried out by comparing of morphometric parameters of the extracted organs (*in vitro*) or (*in vivo*) using ultrasound scanning methods or palpation. This fact prevent the scientists from making the conclusions regarding the quantitative and structural patterns of the lateral (left-right) differentiation of the generative and secretory functions of paired gonads. All current guidance and methodological publications and recommendations are based on generalized data on the functional activity of the ovaries (Pener, 1993; Geodakyan, 2005; Davydova, 2006; Buhrov, 2009; Bugrov, 2013). Quantitative indicators of lateral dimorphism in different types of laboratory, productive and wild animals differ significantly. The data is often contradictive and sometimes asymmetry of paired gonads in animals and people is denied (Pener, 1993; Dominguez, 2003; Davydova, 2006; Buhrov, 2009).

The most scientists consider that according to size, morphology and activity the right ovary is dominant in mammals (Pener, 1993; Davydova, 2006; Buhrov, 2009; Bugrov, 2013). Yet there is now consensus regarding absolute or relative domination, the topography pattern of physiological ovulation in single or multiple females. All these anatomical and physiological patterns directly influence the reproduction of farm animals. Furthermore, lateral dimorphism may influence on ovarian pathologies, which is crucial in reproduction of farm animals as well as rare endangered species, the preservation of each individual of which is crucial for the diversity of the fauna. There are a number of theoretical concepts regarding the origin of functional asymmetry of paired organs (genetic, cytogenetic, pathological, ecological, chemical, biochemical, neurohumoral, etc.). They do not explain the biological significance deviations from symmetry in the bilateral structure of different animal species.

The goal of our research was to study the patterns of functional asymmetry of paired gonads of cows in different climatic zones, different technology of maintenance, breeding, growing and exploitation of dairy cows and heifers in Ukraine. The common goal included methodologically developed stages of research on the collection of statistical data and was divided into the following tasks:

- Development of a methodological research model (Sidashova & Sahlo, 2014);
- Selection of cows and mature heifers into groups according to the "small herd" using the principle of research periods in accordance with the scientific and industrial tasks (lactating cows, heifers, embryo donor cows, cows and embryo recipients) (Sidashova & Sahlo, 2014; Sidashova, 2017);
- Carrying out methodological monitoring of the morphofunctional state of the females ovaries by palpation according to the cyclical-lateral principle, with data being fixed in the established format (Sidashova & Sahlo, 2014; Sidashova, 2014);
- Determining the structure of the lateral profiles of the functional asymmetry of different herds (micropopulations) of cattle in the dynamics of the follicular and luteal phases of the sexual cycle;
- Study of the effect of induction of female sexual activity by hormonal preparations on changes in the lateral dominance of paired gonads;
- Fixation and compilation of data obtained at different stages of the study, followed by statistical processing and analysis.

Materials and Methods

Research and production research was carried out during 2009-2017 on a large number of cows and heifers (n = 1177) of five dairy breeds. Dairy enterprises were located in six different regions of Ukraine, which varied considerably in there climatic and environmental characteristics. All enterprises were focused on industrial technologies of milk production, but the organization of technological processes of raising calves, reproduction of breeding stock, milking and feeding of livestock had significant differences determined by economic features and the type of grazing land nature of the region. The presented numbering of enterprises is used in the text and the tables: No. 1 – JSC "Agro-Soyuz" of Dnipropetrovsk region; No. 2 – JSC "Poltavaplemservice" of the Poltava region; № 3 – PZ "Stepnoy" Zaporozhye region; № 4 – JLLC "RVD – Agro" of Cherkasy region; №. 5 – DP DG them. Decembrists of the Poltava region; № 6 – "Bogoyavlenskiy" Donetsk region; No. 7 – AF "Petrodolinskoe" of Odessa region.

The livestock of all enterprises was provided with a stable feed ration in accordance with the zootechnical standards, the zoohygienic conditions of maintenance animals of different age groups met the current requirements. All animals were provided with anti-epidemic measures and vaccinations against infectious diseases in accordance with current veterinary instructions.

All biotechnological research and production procedures were carried out when animals were fixed with the help of special machines; no animal was harmed.

In accordance with the goals and tasks of research, in each herd, females in the sexual arousal stage were identified (by a clinical-visual-reflexological method (Bugrov, 2013; Sidashova, 2017)), differential diagnosis of the clinical, morphometric and morphofunctional state of paired gonads of cows and heifers in stadially fixed periods of the sexual cycle has been studied. Palpation diagnostics was performed by the same operator to eliminate the ambiguity of the interpretation of morphological data obtained *in vivo* in real time. A modified rectal differentiated cyclic diagnostic technique based on lateral dimorphism of the gonads and data visualization is presented in Figures 1 and 2 and in our previous publications (Sidashova et al., 2016; Sidashova, 2017).

In the enterprises No. 1, 3, 5 and 7, in parallel, work was carried out to stimulate polioovulation in donor cows, followed by non-surgical leaching of preimplantation embryos (*in vivo*), their assessment (*in vitro*) and transcervical transplantation to recipients (*in vivo*), the results of which are presented in our previous publications (Dovgopol et al., 1994; Sidashova, 2014; Sidashova & Sahlo, 2014; Sidashova et al., 2016; Sidashova, 2017).

We used the investigations of Russian scientists (Geodakjan, 1993; Wall, 1996) in order to theorize about the proposed methodology of studying the functional asymmetry of the paired organs of animals. The abovementioned authors considered the evolutionary hypothesis of asynchronous asymmetry based on the crucial regulatory values of interhemispheric asymmetry of the brain. The formation of asymmetry of the organism and population under the changeable environmental factors is presented as the basic biological principle of the evolutionary progress of all species on Earth.

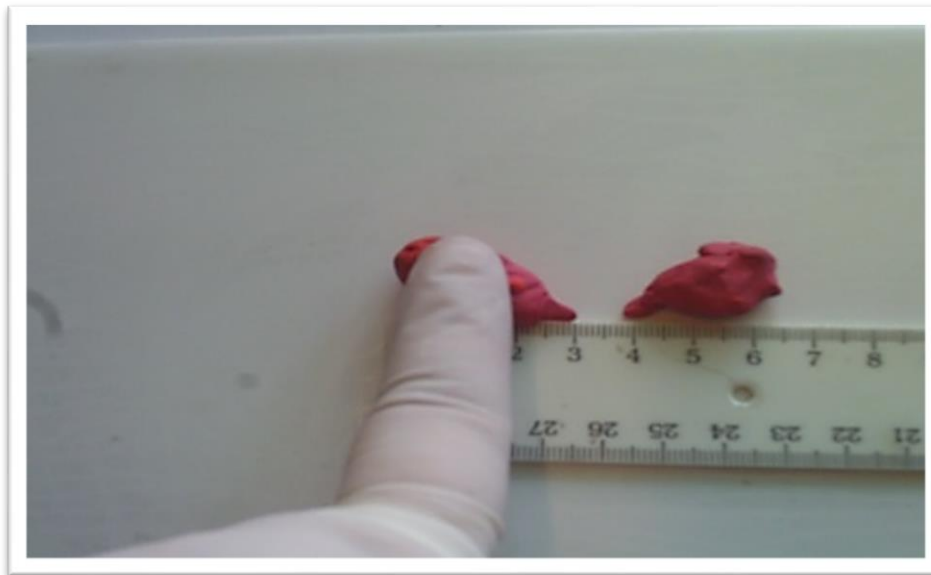


Figure 1. Method of lateral control of ovulation of the dominant follicle and palpation morphometry using volumetric models of a cow's ovaries made in real time after a rectal *in vivo* study.



Figure 2. An example of photographs of volumetric models of a cow's ovaries made according to palpatory diagnosis during fixed terms of the luteal or follicular phase of the estrous cycle (from left to right): lysis of the corpus luteum, preovulatory follicle, ovulation fossa, functional corpus luteum of the 7th day of the cycle. Author's elaboration: the technique is intended for use during practical training of biotechnologists (Sidashova, 2014; Sidashova et al., 2016; Sidashova, 2017).

The methodology of step-by-step and stage-by-stage research (in the chronological aspect) and structural-comparative analysis (in the spatial aspect) were used as an innovative methodological approach and allowed to confirm experimentally and develop the conclusions of the concept of asynchronous-asymmetric dominance evolution using the example of paired gonads of cows. The organizational model in Figure 3 presents the general scheme of research. The considered evolutionary concept was based on the hypothesis of deepening the asymmetry of living systems in the process of evolutionary development. Isomorphism of the laws and mechanisms of two fundamental differentiations- sexual and lateral were the basis of this process. They ensure conservative-operative specializations in the structure of bilateral organisms by an economical form of informational contact with the environment. The female gender and left organs with the regulating role of the right hemisphere of the brain are introduced into the conservative subsystem, and the operative group includes the male gender and the right organs with the regulating left hemisphere. Such an organization of conservative-operative specialization of organs allows testing various evolutionary advances in the population, without the risk of unsuccessful fixation, which is achieved by asynchronous evolution of subsystems (operational develop earlier than conservative ones).

In constantly changing environment, the structure of the lateral (based on the feedback mechanism of the neural junction in the hemispheres of the brain) brain connections with the body allows to maintain an equilibrium of lateral dimorphism. The manifestation of the functional asymmetry of the paired organs of organisms in accordance with the concept of asynchronous asymmetry is considered as an adaptive mechanism for the preservation of proven adaptive features and the acquisition of innovative development directions. The data were statistically processed by Statistics - 2011 v. 20 software.

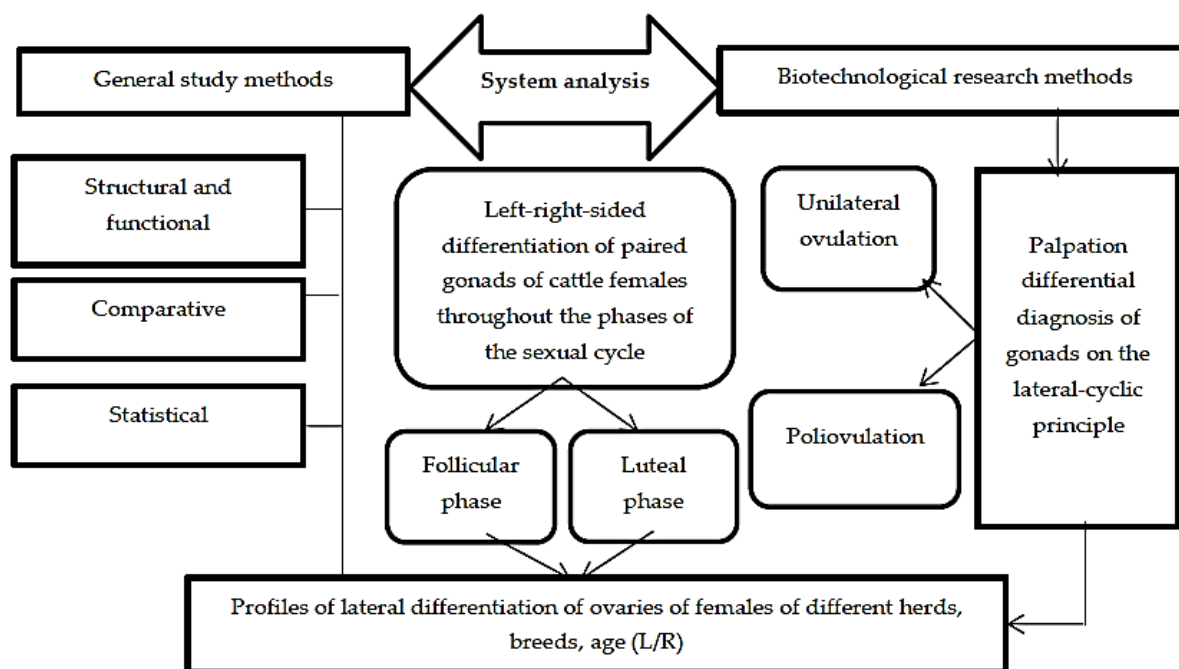


Figure 3. Organizational research model of functional asymmetry of paired gonads of cows in various dairy enterprises of Ukraine

Results and Discussion

The analysis of research results obtained at different stages (n=714 cycles), presented in Table 1. For the first time it was confirmed experimentally that the ratio of the activity of the right and left ovaries of cows of different dairy herds (minipopulations) was characterized by the structured morphogenesis in the left-right ovarian morphofunctional formations throughout the different phases of the sexual cycle. Our data confirmed the dominance of the function of the right ovary of cows. For the first time the proportionality of the asymmetry of the dominance of the right gonad in relation to the left one had been showed. The depth of asymmetry is different during the follicular and luteal phases of the sexual cycle. During the period of maturation and ovulation (stage of sexual arousal), the activity of the right ovary in cows of different herds varied between 75-56%, and in the stage of sexual rest (the luteal phase of the cycle), when corpus luteum were formed and functioned in the ovaries, 53% of all cycles examined.

Table 1. The results of structural and functional studies of dairy cows ovaries of different herds, M ± m (differential palpation diagnosis *in vivo*, 2009-2012).

№ of farm	Cattle breed	Cycle phase *	n	Morphological lesions diagnosed on the left or right, respectively, the phase of the cycle M ± m **: ± m				
				L ^c		R ^d		± m
				cycles	%	cycles	%	
1	Holstein black and motley	F	151	67	44.37 ^a	84	55.63 ^b	0.13
		CL	159	59	37.11 ^a	100	62.89 ^b	0.53
2	Holstein black and motley + Ukrainian red motley dairy	F	11	3	27.27	9	72.73	2.31
		CL	15	6	40.00	6	60.00	1.50
3	Ukrainian black and white milk	F	8	2	25.00	6	75.00	3.00
		CL	17	8	47.06	9	52.94	1.13
4	Ukrainian red-and-white dairy + Ukrainian black-and-white dairy	F	21	7	33.33	14	66.67	2.00
		CL	26	8	30.37	18	69.23	2.25
5	Ayrshire	F	21	7	33.33	14	66.67	1.63
		CL	74	32	43.24	42	56.76	0.56
6	Ukrainian red-and-white milk	F	65	50	34.25 ^a	96	65.75 ^b	1.92
		CL	146	114	41.15 ^a	163	58.85 ^b	1.43
Together in 6 herds:		F	277	114	41.15	274	58.85	1.43
		CL	437	163	37.30	437	62.70	1.68
On average throughout the cycle in all herds:		F+CL	714	277	38.80	437	61.20	1.58

Note: * - F - follicular phase, CL - luteal phase of the sexual cycle; ** - L - activity of the left ovary, R - activity of the right ovary (hereinafter - for all tables and charts); c-d (p < 0.001) except a-b (p > 0.05).

The average level of lateral dimorphism of cycling ovaries in the examined cows of different herds appeared in the form of a proportional ratio that is close to harmonic, namely: L:P as a ratio of 1,000:1.612 or 38.80%: 61.20%. No direct influence on the

level of fluctuation of left-right asymmetry of paired gonad of dairy cows has been established, but indirect effects of paratypical factors have been revealed (Lavrus, 2000; Sidashova, 2014; Sidashova et al., 2016; Sidashova, 2017), this requires more detailed study. Investigating the correlative relationships of the structural connection of the activity of the right and left ovaries of cows in different phases of the sexual cycle, we found that they are significant, positive and vary slightly: $r = + 0.667 - +0.997$.

According to the dynamics of the profiles of the lateral dimorphism of the ovaries of lactating cows we established for the first time the enantiomorphic (mirror) nature of the relationship between the paired gonad morphogenesis at the population level (Orlov et al., 2002). The dynamics of the left or right ovaries activity indicators during the sexual cycle from the folliculogenesis stage to the luteogenesis stage (Figure 4) have clear signs of enantiomorphism. However the conceptual and terminological meaning of this direction of studying the structural organization of complex biological objects is still insufficiently studied and needs to be improved (Lavrus, 2000; Sidashova, 2017).

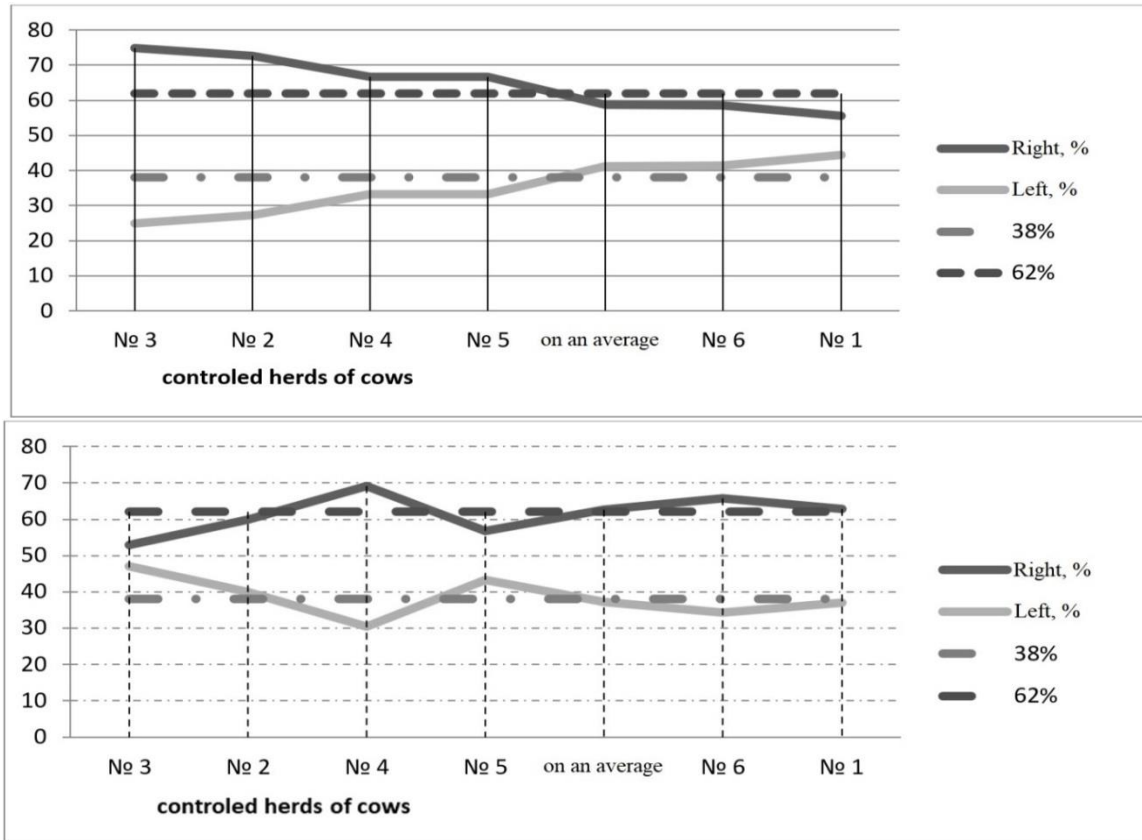


Figure 4. Dynamics of profiles of functional asymmetry of the dairy cows ovaries in different herds, % of the left-right localization of the active ovary during different phases of the sexual cycle (at the top is the follicular phase, at the bottom is the luteal phase) Research carried out by a similar method for the population ($n = 463$ cows) mature heifers aged 13–19 months, which were grown in two very noticeably different regions of Ukraine in terms of environmental and climatic parameters. Studies had confirmed the previously established harmonic and proportional ratio of right dominance in the functioning of paired gonads (Figure 5) in cases of enantiomorphism occur under the influence of exogenous factors (farm number 5) in the process of formation of functional structures in ovaries at different stages of the sexual cycle.

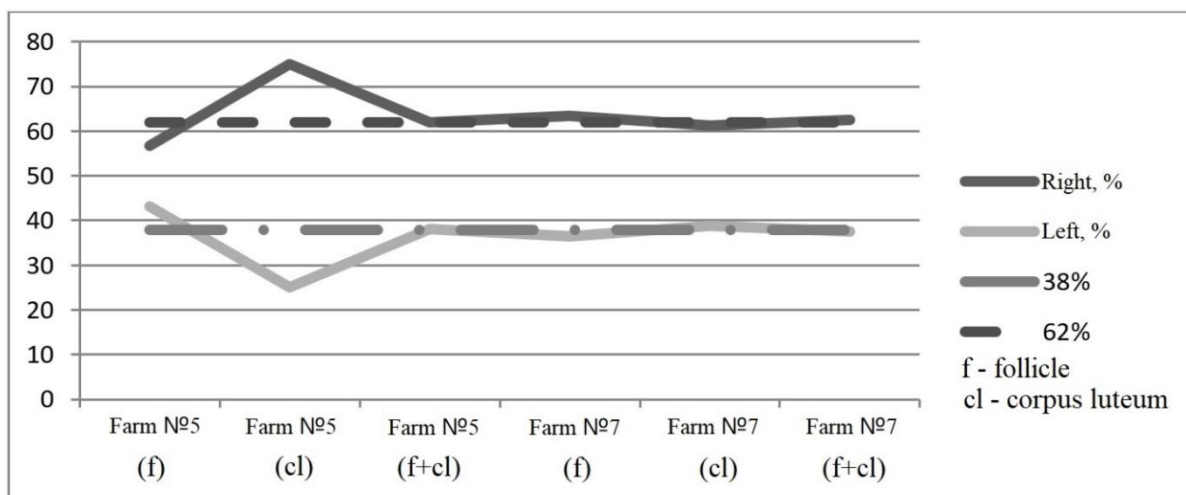


Figure 5. Dynamics of the lateral profiles of two herds of heifers in different dairy breeds, ecological and climatic regions of Ukraine (differential palpation diagnostics *in vivo* 2015-2017).

In heifers of herd No. 5 (Ayrshire breed), there was a significant increasing in dominance of the right ovary at the stage of corpus luteum formation (75.00% of functional yellow bodies on the 7th-12th day of the cycle), which is 13.74% more than in the herd of heifers of the Ukrainian red dairy breed ($p < 0.05$). However, the average statistical activity ratios of the right and left ovaries during the sexual cycle approach the already established harmonic ratio of 1:1.622 or 37.80%: 62.20% ($p < 0.05$). The results of similar experiments had showed in detail the morphometric features of the dominance of the right ovary in cows and heifers in a spontaneous cycle, and confirmed the presence of hormonal maintenance of a more specialized and developed right organ (Davydova, 2006; Buhrov, 2009; Bugrov, 2013).

The method of studying the functional asymmetry of ovaries in cows allowed us to obtain comparable data on unilateral ovulation in dairy cows and mature heifers and multiple ovulation in embryo-donor cows after hormonal stimulation of polioovulation (in accordance with the requirements of existing methods of cattle embryo transfer (Donaldson, 1984; Dovgopol et al., 1994; Sidashova et al., 2016)). The number of corpus luteum on the 7th day of the luteal phase on the left ovary by 5.88% compared with the right cows ($p < 0.05$) had been observed, in cows donor dairy complex number 2 ($n = 67$). Figure 6 shows the experimentally established phenomenon of the inversion of the dominance of the right ovary under the influence of the injection of high doses of gonadotropic drugs to cows (FSH - follicle-stimulating hormone). Consequently, the right-sided dominance of paired gonads of cows, and possibly of all mammals, is not absolute, but relative, with the ability to change lateral localization under the influence of exogenous factors.

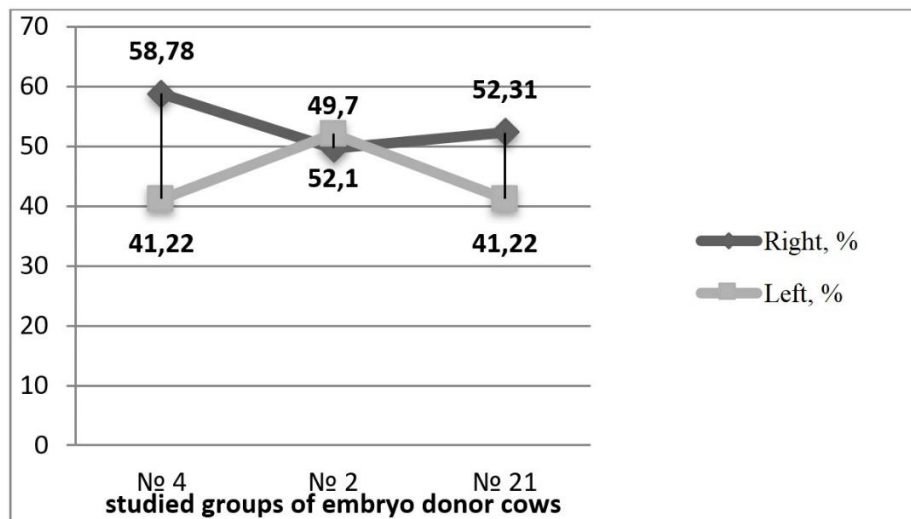


Figure 6. Inversion of lateral ovarian dominance in cows - embryo donors under the influence of hormonal stimulation of polioovulation with gonadotropic drugs ($n = 84$ donor cows).

In general, under the influence of high doses of follicle-stimulating hormones to induce multiple folliculogenesis of the ovaries to the generation of multiple eggs, a significant change in the structure of functional asymmetry of paired gonadal cows had been observed. The structure of ovaries was restoring to the initial population level during the next spontaneous cycle of donor groups (Dovgopol et al., 1994; Sidashova et al., 2016). The phenomenon of dominance inversion discovered by us was used to improve the selection of effective potential donors of embryos capable of stable high levels of folliculogenesis and the formation of high-quality (transferable) embryos (Sidashova et al., 2016).

The boundaries of the phenotypic variability of the lateral differentiation of paired gonads of cattle females require further clarification, since our studies have established harmonic proportions of lateral activity. This proportion may preliminarily indicate the desire of living systems for an optimal structural balance in the development of the population. Our previous studies have shown the level of pregnancy in cows and heifers depended on the lateral localization of a matured and ovulated egg during the uni- or bilateral cycle (Sidashova, 2014). These findings are of great practical importance for single pregnancy animals with a long period of gestation and are confirmed by clinical data in the number of sources (Orlov et al., 2002; Selk, 2014).

The data obtained in recent years in biology, clinical medicine, and animal reproductive biotechnology have made it possible to revise substantially the systemic organization of the reproduction processes of females of various mammalian species. Normally, with each ovulation, the reproductive system is fully prepared for pregnancy, as evidenced by the synchronous movement of the fertilized egg cell through the oviduct and the physiological state of the endometrium for implantation of the embryo. It was established experimentally that not the entire surface of the endometrium was homogeneous, which would be energetically uneconomical, but only a specific zone that the early embryo - blastocyst finds with precision, the mechanism of which still needs to be studied (Orlov et al., 2002; Geodakyan, 2005).

This zone is located ipsilateral relative to the dominant follicle. Clinical studies of several authors (Orlov et al., 2002) showed that in such a laterally localized area of the endometrium in comparison with the contralateral, there is an increase in blood flow and mitotic processes, moreover, long before fertilization, that is, at the stage of maturation of the follicle. The authors consider the established mother - placenta - fetus connections to be genetically determined, which is not supported by other authors (Wall, 1996; Orlov et al., 2002). The results of our research are based on the hypothesis of the structural organization of functional asymmetry of bilateral organisms. Our data confirm the conclusion regarding the structural principle of the spatio-temporal adaptation of the reproductive system of females and harmonization of the follicular and luteal phases of ovarian activity at both the population and species levels, and at the organism level (Geodakjan, 1993).

The results of our research had shown that the change of localization of spontaneous ovulations in singleton females is not a chaotic process, but a structurally regulated in time and space, which seeks to harmonize the functional asymmetry between the right and left ovaries. Consequently, it had been hypothesized that the structure of functional asymmetry of paired organs for all cattle species and, probably, for all mammal species is isomorphic. This conclusion so remains controversial and requires further research.

A step-by-step study of different herds, minipopulations, farms, breeds, and different age of females and conditions of their production exploitation allowed us to compare the data of lateral ovarian specialization of cows as a structurally organized integrated bilateral system adapted for performing secretory and generative functions. Studies of Ukrainian scientists on the functional asymmetry of the breeding organs of pigs carried out the previous years (Martynenko, 1956) showed promising directions for the further development of scientific research, which is confirmed by our investigation of pigs of different breeds (Sidashova et al., 2015).

Experimentally established laws of the enantiomorphic principle of organization of functional asymmetry of cow ovaries confirm the conclusions of the evolutionary concept of asynchronous asymmetry of the paired organs of animals as a regular process that ensures the adaptation of the species to the changing environment. The structural laws regulation the left-right differentiation of the paired gonad females clarified the general biological mechanism of self-regulation of complex living systems in accordance with the universal principle of saving energy resources.

Experienced lateral patterns of functional asymmetry had not only a fundamental aspect, but also an applied value, the results of which were described in our previous publications (Sidashova, 2014; Sidashova, 2017). The theses of investigation are presented below:

- The selection of optimal methods for managing the reproduction of the population of cows and heifers, considering the dynamics of the functional asymmetry indicators of the ovaries in various industrial groups (lactating cows, embryo donor cows, recipients, heifers);
- Assessment, selection and control of the reproductive potential of the ovaries of embryo-donor cows by the criterion of lateral dimorphism;
- Selection and correction of the reproductive state of effective recipients of pre-implantation embryos by taking into account ipsi- and contralateral ovulations in synchronized sexual cycles;
- Assessment of pre-implantation transferable embryos by lateral localization of the formation in donor gonads, followed by labeling of L (left) or R (right) and laterally separate storage of cryopreserved genetic resources;
- improving the effectiveness of transfers (*in vivo*) of deconserved or fresh embryos due to the additional structural ipsilateral compliance factor at all stages of embryo transplantation procedures: *in vivo* (donor) - *in vitro* (laboratory and cryobank) - *in vivo* (recipient).

Conclusion

We experimentally established the population patterns of lateral specialization of the right (dominant) ovary of cows, which had a dynamic volatile nature during the sexual cycle with asymmetry deepening into the follicular phase and approaching the universal harmonic proportion (L:R as 1:1.612-1.622) in the stage of sexual rest. We also proved that the dynamics of the lateral profiles of the functional asymmetry of the ovaries of cows has a structured proportional nature, organized according to the enantiomorphic dominant principle. We discovered the phenomenon of dominance inversion in the function of paired gonads after injection of hormonal gonadotropic drugs stimulating folliculogenesis.

We revealed the patterns of lateral dimorphism of cattle ovaries that confirmed the adaptive functional asymmetry in paired organs as an universal biological mechanism for adapting the species and population to changing environment. We established the population structure of paired gonads functional asymmetry in cows of different herds and breeds, which makes it possible to hypothesize regarding the species nature of proportions of the dominant and subordinate ovaries for all cattle species and, possibly, for other mammal species, taking into account anatomical features of different species.

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