# MICROBIOTA AND REPRODUCTION IN AGRICULTURAL MAMMALS
(review)

## D.V. POPOV✉

*Afanas'ev Research Institute of Fur-Bearing Animal Breeding and Rabbit Breeding*, 6, ul. Trudovaya, pos. Rodniki, Ramenskii Region, Moscow Province, 140143 Russia, e-mail popov.bio@gmail.com (✉ corresponding author)
ORCID:
Popov D.V. orcid.org/0000-0001-7422-5470
The author declares no conflict of interests

A b s t r a c t

The use of specialized animal breeds of agricultural species is often accompanied by a decrease in reproductive success. In dairy cattle breeding, the number of service-period days, artificial insemination procedures per pregnancy, and the frequency of pregnancy losses are increasing (S.V. Guskova et al., 2014). Accumulated data on obtaining embryos by *in vivo* and *in vitro* methods and their transplantation indicate a significant level (30-60 %) of embryo losses (P.J. Hansen, 2020). The reasons for low rates in reproductive technologies are diverse and associated with both biotic and abiotic factors, and one of the key factors of embryo losses may be the imbalance of microbial communities in the reproductive system sections of both female donors and recipients. The study of the microbiota composition of various departments and systems of the multicellular organism has recently become an increasingly dominant topic in the scientific literature. Modern methods of microbial identification, e.g., metagenomic sequencing, reveals great microbial diversity in various anatomical departments of macroorganisms. The accumulated data show the microbial composition, dynamics in the organs of the reproductive system, and its relationship with the reproduction of mammals, reproductive success, the course of pregnancy, the prognosis of the possibilities of pathological processes. The review focuses on the impact of microbiota on the success of reproductive technologies, e.g., in vitro fertilization, embryo transplantation, and artificial insemination. For example, F. Marco-Jimĭnez et al. (2020) discuss the effect of symbiotic bacteria on fertility and semen quality. The understudied nature of this area for mammals and the extreme need for additional research on the microbiota of the reproductive tract of farm animals, the results of which will provide insight and insight into the unsuccessful and positive outcomes of reproduction, are noted. At the same time, the practical application of this information will increase the chances of success in reproductive biotechnology, reduce the costs associated with reproduction and therapeutic interventions in the treatment of pathological processes of the reproductive system, and open up the possibility of developing and implementing new methods such as microbial therapy. Thus, it can be concluded that the microbiota of mammalian reproductive system and organs influence the physiological processes of reproduction (R. Koedooder et al., 2019). It is clear that by being able to manage microbial communities, humans can increase the chances of reproductive success in the reproduction of highly specialized breeds of farm animals (P.J. Hansen, 2020; R.W. Hyman et. al., 2012; D.E. Moore et. al., 2000).

Keywords: endometrium, microbiota, microbiome, reproductive system, sperm, uterus, reproductive technology

Livestock reproduction at any livestock is the main technological stage in ensuring the success of anima husbandry. The development of progressive technologies, market economy, and competition provide the transition of most modern agricultural enterprises from extensive to intensive development. For this purpose, herds are created, consisting of specialized highly productive breeds, and in almost every branch of animal husbandry, reproductive biotechnologies, e.g. artificial insemination, obtaining embryos by in vivo and in vitro methods, embryo transplantation, etc., are used to reproduce livestock. An increase in the specialization of animals in the direction of productivity inevitably leads to a decrease in the potential of their biological characteristics, such as adaptive qualities, reproductive longevity, and reproductive success [1, 2]. Recent works aimed at studying the

microbial communities of organs and systems of a multicellular organism indicate that an imbalance in the composition of the microbiota can lead to negative phenomena and manifest itself in the form of acute pathological processes or a functional disorder in one or more physiological systems of the body.

All tissues and organs of a multicellular organism are colonized by a coexisting microbial community, which includes bacteria, viruses, fungi, yeasts, archaea and protozoa [3]. The diversity of microorganisms within a particular physiological system of a macroorganism is defined as microbiota, i.e. the species composition of the microbial community [4]. The prevailing microorganisms is called dominant, and each organ or system in a macroorganism has its own characteristic composition of microbial associations. Diversity of microorganisms is referred to as alpha and beta diversity. Alpha diversity characterizes the average species diversity in a sample of interest, while beta diversity reflects the diversity between different samples [5]. Components of the microbiota affect both the macroorganism and each other. The relationship between them can be mutualistic (mutually beneficial), commensal and parasitic. The totality of the genomes of these communities is defined as the microbiome [3, 4]. With the advent of methods for sequencing the conserved bacterial 16S rRNA gene (6, 7), next-generation sequencing (NGS) [8-10], whole genome sequencing (WGS) [11, 12], quantitative PCR (qPCR) [13, 14], a large amount of data on new genes, genome organization, and bacterial community structures has been obtained. Bioinformatic resources such as mothur [15] and Quantitative Insights Into Microbial Ecology (QIIME) [16] are available for processing such data. The main function of these information systems is to combine the obtained DNA sequences into operational taxonomic units (OTU) by various methods [17, 18] using external reference databases - Greengenes [19], SILVA [20], Ribosomal Database Project [21]. However, the shortcomings associated with the methodological features of research, analysis and interpretation of the obtained data can adversely affect the objectivity and quality of the results [22-25].

At present, the microbiome of various human systems and organs has been most studied [5], while the microbial communities of the organs of the reproductive system of agricultural mammals have not been studied enough.

The purpose of our review is to analyze current publications on the microbiota of the reproductive tract, describing the composition of microbial communities in various anatomical regions (vagina, cervix, endometrium and placenta) of mammals, and to consider the proposed mechanisms of the relationship between the abnormal composition of the microbiota of the reproductive organs and reproductive success in mammalian agricultural species. .

Microbiota and multicellular organism. The accumulated information about the microbiota of various species and breeds helps to expand our understanding of the processes of evolution and domestication. Thus, data have been obtained indicating that the most universal indicators of the domestication syndrome. i.e. a change in behavioral characteristics (decrease in aggressiveness, increase in socialization) [26, 27] and a significant expansion of phenotypic and population genetic variability [28] can manifest themselves and be closely related to each other. with a friend due to changes in the microbiota of animals formed in the same ecological niche together with humans [26]. The microbiota of the mammalian gastrointestinal tract has been called the "forgotten organ" [29], and its study has become the basis of the theory developed in recent years about the role of the microbiota in evolutionary processes [30]. Each mammalian species contains an intestinal microbiota, the variability of which is associated with the processes of adaptation and diversification of animals, contributing to the possibilities of changing the type of nutrition, phenotypic plasticity, and the work of

innate and adaptive immunity. The gut microbiota serves as an important target for environmental factors and as a selective agent shaping the adaptive evolution of the mammalian diet, phenotypic plasticity, gastrointestinal tract morphology, and immunity [30]. The concept of the role of interactions between a multicellular organism and microbiota in the process of evolution has been called the hologenomic theory of evolution, in which the relationship between them is considered as the main target of genomic transformations under the influence of environmental factors [31]. Comparative analysis of the microbiota of wild and closely related domesticated species provides insight into how domestication may have affected the composition of microbial associations in farm animals. For example, a comparative study of the microbiota of domestic pigs and wild boars showed, in particular, that some representatives of *Enterobacteriaceae*, which are considered the dominant bacterial groups in the intestinal microbiota of pigs, do not occur in wild boars. Interestingly, in recently domesticated wild boars, a corresponding shift in the species representation of *Enterobacteriaceae* was found. Taken together, this suggests that the composition and structure of the gut microbiota of domestic pigs may reflect the management practices of this livestock sector. It has also been shown that in cattle, inoculation with bison rumen content increases protein digestibility and nitrogen retention, but not fiber digestibility, which suggests the ability of microbial communities in the gastrointestinal tract of farm animals' ancestors to use nitrogen from plant feed mass for amino acid biosynthesis [32].

Ongoing microbiome research aims to reveal missing details in pathophysiological processes and to explain seemingly random variations in disease severity and phenotypic manifestations due to, for example, environmental-geographical and forage factors. Thanks to advances in the study of microbial communities, important information has been obtained that bacterial dysbiosis can lead, in particular, to disturbances in the functioning of the nervous system [33, 34]. There is evidence suggesting a role for the microbiota in many complex disorders such as obesity, cancer and inflammatory bowel disease [35]. It is now known that the gut microbiota significantly influences overall host metabolism and immune responses [36]. External factors (antibiotics, diet, and geographic location) can have a critical impact on the composition of the gut microbiota [37].

A similar trend is observed in the microbiota of the reproductive system under both physiological and pathological conditions [38]. The reproductive system of multicellular organisms is the main structure that determines the reproduction of a biological object. When studying the microbial communities of the reproductive organs, in particular in humans, it was shown that the differences between biosamples within the same physiological system (beta diversity) were significantly greater than the differences between samples obtained from the same organ (alpha diversity). The vaginal microbiota in mammals was characterized by the least alpha diversity with relatively low beta diversity at the genus level, but very high diversity among taxonomic units studied due to the predominance of lactobacilli.

It has been established that a symbiotic relationship between the host and microorganisms is necessary and disruption of this relationship can lead to a dysbiotic state [39, 40]. For example, bacterial vaginosis is characterized by a shift from a healthy, low pH values in a lactobacillus-dominated community to an elevated pH and a more diverse microbial community [41]. However, shifts between symbiosis and dysbiosis and vice versa are still not well understood.

In farm animals, these issues are very important because they are related to reproduction and therefore have significant economic importance, directly affecting the efficiency of livestock production [42].

**Microbial communities of the reproductive system in female mammals.** In mammals, the reproductive organs of both females and males are systems separated by anatomical or physiological barriers. In females, the reproductive tract consists of the following sections: vagina, cervix and uterine cavity, uterine horns, oviducts and ovaries. More and more evidence is accumulating, indicating that certain bacterial communities have an unequal impact on reproductive health and reproductive success. Thus, a specific microbial composition has been found in humans, which differs in the parts of the reproductive system [3, 43]. It has been found that the number of bacteria localized in the endometrium is significantly lower compared to their number in the vagina, suggesting that the cervix acts as a protective barrier to the ascending microbiota [44].

The vaginal microbiota can be divided into five (I-V) community state types (CSTs), four of which are dominated by lactobacilli. Group I is dominated by Lactobacillus crispatus (26.2%), group II by L. gasseri (6.3%), group III by L. iners (34.1%), and group V by L. jensenii (5.3%) [45, 46]. In group IV, there is no dominance of lactobacilli, but there are many more severe anaerobes [47]. CST IV-A is characterized by the presence of some species of *Lactobacillus* spp. and a variety of strictly anaerobic bacteria, the IV-B community combines representatives of the genera *Atopium*, *Prevotella*, *Sneathia*, and *Gardnerella* [48]. A.Y.K. Albert et al. [49] expanded our understanding of the range of bacterial communities. The authors, by changing the methodological approach, found that *Gardnerella* subgroups (CST IV-C and IV-D) predominate in the communities [49].

It was found that the vaginal microbiota is dynamic, as the species composition of communities undergoes modifications over time. It is known that CST IV-B often changes to CST III, but rarely to CST I, CST I often changes to CST III or CST IV-A, CST III changes 2 times more often to CST IV-B compared to CST IV- A, CST II rarely changes, with no change from CST I to CST II observed, and CST II is relatively stable compared to CST IV-A.

Differences in microbial composition are also reflected in vaginal pH. CST I appears to have the lowest median pH (4.0±0.3), while CST IV has the highest pH (5.3±0.6). The difference in pH between different CSTs is most likely due to the specific dominance of lactobacilli and the ability of each lactobacillus to produce lactic acid [50].

The vaginal microbiota of non-pregnant healthy women may change depending on a number of characteristics: the periods of the sexual cycle (estrus, ovulation, etc.), ethnic origin, ecological and geographical factors x47, 48, 51-53].

Hormonal status has a significant impact on the composition of the microbiota; for example, changes in microbial composition during pregnancy have been shown to be a response to increased estrogen levels [54].

During pregnancy, the abundance and biodiversity of the vaginal microbiota decreases, while closer to childbirth, it returns to a state characteristic of non-pregnant women [54, 55]. The predominance of *Lactobacillus* spp. during pregnancy [54, 56] reduces the risk of preterm birth [57] and protects against bacterial vaginosis [47]. Without the *Lactobacillus* spp. dominance, the opportunistic microbiota such as *Gardnerella* or *Ureaplasma* become abundant, which may increase the risk of preterm birth [58].

An important property of lactobacilli, which is associated with their ability to inhibit the growth of other bacteria, is the production of bacteriocins [57]. As already noted, lactobacilli synthesize both D- and L-isomers of lactic acid, while the macroorganism itself is capable of producing only the L-isomer [48, 50, 59]. The main beneficial effect of D-lactic acid is to reduce the activity of matrix metalloproteinase (MMP)-8, which allows the cervical plug to maintain integrity

and thereby limits the vertical transmission of vaginal bacteria to the uterus. Dactobacteria act as a mechanical barrier, binding to the surface of epithelial cells to prevent other bacteria from attaching [60].

Microbiota and reproductive health. The accumulated data suggest that the species and quantitative composition of microbial communities affects reproductive health in mammals. For example, infertility problems are often associated with a decrease in the abundance of lactobacilli in the cervix [61]. The presence of certain bacteria (particularly *Atopobium vaginae*, *Ureaplasma vaginae*, *U. parvum*, *U. urealyticum*, and gardnerella) and the reduced frequency of *Mycoplasmateceae* species compared with the microbiota in healthy individuals have been shown to result in a high prevalence of asymptomatic bacterial vaginosis [62, 63].

In infertility due to infection [61], a decrease in the number of lactobacilli and a higher diversity of microorganisms in the cervix were found, with a significant increase in the number of detections of *Gardnerella vaginalis*, *Prevotella* spp., *Leptotrichia*, *Sneathia* compared with controls in normal fertility [61, 64]. It has been established that bacterial vaginitis is the most common disease of the vagina of microbial etiology, described as polybacterial dysbiosis [65], affecting 30% of women of reproductive age [66]. Anaerobes, in particular *Gardnerella vaginalis*, *Atopobium vaginae*, *Mobiluncus*, *Mollicutes*, *Dialister invisus*, *Sneathia*, *Prevotella* spp., are considered as possible pathogens in bacterial vaginitis [67]. It is noted that in this pathology, the bacterial composition of the vaginal microbiota is more diverse [68]. It is important to note that bacterial vaginitis is associated with adverse reproductive outcomes such as infertility, miscarriage [69], recurrent pregnancy loss [70] and preterm birth [67].

Microbiota of the reproductive system of farm animals on the example of cattle. The study of the microbiota of the reproductive system of farm animals is important for understanding the role of microorganisms in pathological processes associated with reproduction. For example, in a study of cows with purulent uterine discharge, a significant positive correlation was found between the presence of *Trueperella pyogenes* and clinical endometritis, and between *Escherichia coli*, *Fusobacterium necrophorum*, *Prevotella melaninogenica*, *Bacteroides* spp. and metritis. In healthy cows, *Streptococcus* spp., *Staphylococcus* spp. and *Bacillus* spp. are commonly detected. A study of the bovine uterine microbiota has shown that the *Porphyromonadaceae*, *Ruminococcaceae* and *Lachnospiraceae* families are the most abundant, and the cow can carry a pregnancy despite the presence of potentially pathogenic bacteria in the uterus [71].

A study of the effect of *Trueperella pyogenes* on the reproductive function of cows showed that with endometritis caused by this pathogen, the frequency of successful pregnancy is 47% lower, and the average time for its onset is 57 days longer than in healthy cows [72]. Using PCR analysis, it was found that in the microbiota of the reproductive organs of cows with metritis and clinical endometritis, *Escherichia coli* acts as a precursor pathogen that predisposes cows to infection with *F. necrophorum* associated with metritis and *T. pyogenes* associated with clinical endometritis.

Metagenomic sequencing has expanded the knowledge of the bovine uterine microbiota. It has been established that in cows, bacteria are present in the uterus even before calving. In animals with developing metritis and healthy individuals, the structure of microbial associations is identical until the 2nd day of the postpartum period, after which the microbial community of the uterus of individuals with metritis changes towards a larger relative abundance of representatives of *Bacteroidetes* and *Fusobacteria* and a smaller one of *Proteobacteria* and *Tenericutes.* A potential route of infection by uterine pathogens has been found to be

hematogenous and that metritis is associated with a dysbiosis of the uterine microbiota characterized by reduced diversity and increased abundance of *Bacteroidetes* and *Fusobacteria*, especially *Bacteroides*, *Porphyromonas* and *Fusobacterium* [73-75]. In addition, the study of bovine endometritis revealed a significant effect of microbiota structure variability on immunity and general resistance of animals to adverse biotic and abiotic environmental factors. In endometritis, the inflammatory reaction caused by Gram-negative *E. coli* has been found to affect the expression of microRNA (miRNA) involved in the regulation of innate immunity [76]. It is interesting to note that the dominance of lactobacilli in the composition of the vaginal microbiota is unique for humans as a biological species, while in other mammals (including primates) the vaginal microbiota is rarely characterized by the dominance of lactobacilli, while the pH of the vagina in women is always lower than in females of other mammals [77]. J.D. Swartz et al. [78] emphasized that lactobacilli were common and found in vaginal samples in 80% of cows (16 individuals in a sample of $n = 20$) and 90% of sheep (18 individuals in a sample of $n = 20$), while lactobacilli always had low relative abundance ($0.36\pm0.66$ and $0.53\pm0.65\%$ of the population, respectively, as assessed by the 16S rRNA gene) and pH was almost always neutral [78].

Microbiota and reproductive technologies. The study of microbial communities of the reproductive system is important for the successful application of reproductive biotechnologies, e.g., in vitro fertilization (IVF), embryo transplantation, etc. Thus, the dominance of lactobacilli (*L. crispatus*, *L. iners*, *L. jensenii*, *L. gasseri*, or other lactobacilli species) in the vaginal microbiota in the pre-transplant cycle is associated with a positive outcome of the procedure [79, 80]. However, some vaginal microbial communities adversely affect pregnancy [79, 81]. An increase in the number of opportunistic microflora in the genital tract always correlates with a decrease in the frequency of lactobacilli species, which decreases the success of reproductive biotechnology methods [82, 83].

As a reason for an unfavorable result in the IVF procedure, the possibility of colonization of the follicular fluid by microorganisms during egg retrieval is considered. Negative pregnancy outcomes were noted in the presence of *Actinomyces* spp., *Bifidobacterium* spp., *Propionibacterium* spp. in the ovaries. and *Streptococcus* spp. and, conversely, positive outcomes occurred when *Lactobacillus* spp. are detected in the ovaries [80]. It has been previously demonstrated that the presence of *Streptococcus viridans* on embryo transfer instruments is associated with adverse outcomes of the procedure [80].

The microbiota of the upper reproductive tract is associated with the likelihood of conception both in vivo and with the use of reproductive technologies. Problems with conception may be due to changes in the structure of the microbiota due to the penetration of pathogens from the vagina into the upper reproductive system, which leads to an imbalance in the intrauterine environment (82). Lactobacilli contribute to the creation of favorable conditions for embryo implantation and pregnancy due to their protective and supportive properties [48, 50, 59, 85]. Recent studies have associated reproductive success with a predominance (>90%) of lactobacilli in the microbial profile of the endometrium [86]. The dominance of the genera *Gardnerella* (family *Bifidobacteriaceae*) and streptococci (family *Streptococcaceae*) in the endometrium is associated with a significant decrease in the likelihood of implantation and favorable delivery [86].

Endometrial microbiome profiles have been described that may be associated with chronic endometritis [87, 88] associated with a predisposition to infertility in endometriosis [87] and determine repeated implantation failures [87, 88]. There is evidence of a limited role for the microbial landscape of the cervical canal

and endometrium during embryo transfer and the absence of a significant microbial effect on the likelihood of pregnancy [85, 87]. Subsequent pregnancy rates have not been reported to be affected by prophylactic antibiotic use [92]. A number of studies [93] also found no statistically significant difference in reproductive success rates between those treated with antibiotics prior to embryo transfer and those who did not receive antibiotics.

Microbiota and pregnancy. The microbiota of the reproductive tract continues to play a role after pregnancy setting [94]. Dysbacteriosis in the vagina, endometrium or placenta can lead to an unfavorable pregnancy outcome.

At the end of the 1st trimester, the vaginal microbiota is mainly composed of lactobacilli — *L. crispatus*, *L. iners*, *L. gasseri* or *L. jensenii* [95]. Premature birth (before 34 weeks) is highly likely to be associated with the dominance of *L. iners* at the 16th week of pregnancy, while the predominance of *L. crispatus* presumably serves as an indicator of a successful pregnancy outcome.

Abnormal colonization of the vagina by *Klebsiella pneumonia* in the 2nd trimester increases the risk of preterm birth (before 28 weeks), and colonization of *Streptococcus agalactiae* in the 2nd trimester leads to an increased likelihood of late miscarriages [96, 97].

Embryonic development and growth are largely dependent on the function of the placenta. Once thought to be sterile, the placenta has been found to have its own unique microbiota. In humans, a significant presence of non-pathogenic commensal microbiota of the *Firmicutes*, *Tenericutes*, *Proteobacteria*, *Bacteroidetes*, and *Fusobacteria* phyla has been found in the uterus and placenta [98].

Manipulations that alter the structure of the uterine microbiota can help modulate the local immune system in preparation for embryo implantation and placenta formation [99], which can directly influence the development of preeclampsia [96].

Sperm microbiota and reproductive health. In males, the reproductive system is represented by the external part (penis and scrotum) and the internal part (testes, accessory glands, vas deferens and urethra).

Recent analyzes have shown that the seminal microbiota is most likely formed by the association of microbial communities from all parts of the male reproductive tract. The NGS method showed that the bacterial communities of the seed are divided into three groups, in which either lactobacilli, or Pseudomonas aeruginosa, or Prevotella predominate. It is important to note that 80% of the quality semen samples belonged to the group dominated by lactobacilli [100]. It has been established that low concentration and abnormal morphology of spermatozoa are associated with the presence of *Mycoplasma* spp. [101, 102]. The incidence of *Mycoplasma hominis* is significantly higher in infertile men than in fertile men, and antibiotic therapy has also been shown to improve sperm quality in infertile men [103].

Similar to the female reproductive tract, in male reproductive diseases, the abundance of lactobacilli will decrease with a higher species diversity of the microbial community [104]. An increase in abundance of *Neisseria*, *Klebsiella* and *Pseudomonas aeruginosa* and a decrease in the number of lactobacilli has been associated with increased seminal fluid viscosity and oligoasthenoteratozoospermia [105], therefore, sexually transmitted diseases reduce not only female but also male fertility. When studying the effect of the seed microbiota on reproductive success in rabbits, it was found that *Lysinibacillus* and *Flavobacterium* can act as markers of potential fertility [106].

Clearly, the microbiota of both sexes influence each other and appear to interact. Comparison of seminal and vaginal microbiota in surveyed couples revealed a large number of common DNA markers for microbiota components

[107]. Among the common microbiota components, the most common genera were *Lactobacillus*, *Veillonella*, *Streptococcus*, *Porphyromonas*, *Atopobium vagine*. Although the microbial communities of the semen were more diverse, the overall concentration of bacteria in the semen was lower than in the vaginal communities. The sperm microbiota significantly, albeit temporarily, influences the vaginal microbiota [108]. Earlier studies showed no effect on vaginal lactobacilli and pH 8-12 h post-coitus, with significantly more *E. coli* found in the vagina [109]. It is hypothesized that the physiological postcoital transient state of the vaginal microbiome, in which vaginal lactobacilli are replaced by *Gardnerella vaginalis* under the influence of ejaculate, leads to a change in pH [110]. Extensive rodent studies have shown that exposure to seminal fluid induces a spectrum of cytokines in the female reproductive tract, altering endometrial receptivity and pre-implantation developmental dynamics of the embryo [111]. Unfortunately, the interaction and influence on each other between the microbiota of the reproductive systems of male and female mammals is still poorly understood. When analyzing the influence of microbiota on reproductive success, one of the directions may be to study the temporary combined microbial community of the female and male reproductive systems, which is formed in the postcoital period and, possibly, even persists in the preimplantation period, which can contribute to successful conception and pregnancy.

So, it becomes obvious that the microbiota serves as a factor that unites all the physiological systems of the body. Any changes in microbial communities at the level of a system or even an organ lead to the emergence of pathological processes. At present, the accumulation of data on the microbiomes of agricultural animals is of both theoretical and practical importance. This scientific direction remains relevant and promising, since the productivity and adaptive potential of valuable agricultural species and animal breeds can be improved by purposeful changes in the qualitative and quantitative composition of their microbial communities. The study of the microbial communities of the reproductive system organs in farm animals will provide new data on the physiology of reproduction, increase the likelihood of reproductive success in the application of methods of reproductive biotechnology, as well as reduce the associated costs and apply new methods of treatment, such as microbial therapy. Today, the accumulated materials indicate the need to strengthen the biological control of the microbiota in seed production, the use of in vitro and in vivo technologies to obtain embryos of agricultural animals. In addition, important scientific areas include the study of the composition of microbial communities of the reproductive organs of producers and the possibility of reducing the predicted loss of embryos during preliminary colonization of certain types of bacteria in the parts of the reproductive system of female donors and female recipients.

REFERENCES

1. Gus'kova S.V., Turbina I.S., Eskin G.V., Kombarova N.A. *Myasnoe i molochnoe skotovodstvo*, 2014, 3: 10-13 (in Russ.).
2. Hansen P.J. The incompletely fulfilled promise of embryo transfer in cattle-why aren't pregnancy rates greater and what can we do about it? *J. Anim. Sci.*, 2020, 98(11): skaa288 (doi: 10.1093/jas/skaa288).
3. Koedooder R., Mackens S., Budding A., Fares D., Blockeel C., Laven J., Schoenmakers S. Identification and evaluation of the microbiome in the female and male reproductive tracts. *Human Reproduction Update*, 2019, 25(3): 298-325 (doi: 10.1093/humupd/dmy048).
4. Marchesi J.R., Ravel J. The vocabulary of microbiome research: a proposal. *Microbiome*, 2015, 3: 31 (doi: 10.1186/s40168-015-0094-5).
5. Stoma I.O., Karpov I.A. *Mikrobiom cheloveka* [Human microbiome]. Minsk, 2018 (in Russ.).
6. Lane D.J., Pace B., Olsen G.J., Stahl D.A., Sogin M.L., Pace N.R. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc. Natl. Acad. Sci USA*, 1985, 82(20):

6955-6959 (doi: 10.1073/pnas.82.20.6955).

7. Gray M.W., Sankoff D., Cedergren R.J. On the evolutionary descent of organisms and organelles: a global phylogeny based on a highly conserved structural core in small subunit ribosomal RNA. *Nucleic Acids Research*, 1984, 12(14): 5837-5852 (doi: 10.1093/nar/12.14.5837).

8. Metzker M.L. Emerging technologies in DNA sequencing. *Genome Res.*, 2005, 15: 1767-1776 (doi: 10.1101/gr.3770505).

9. Margulies M., Egholm M., Altman W.E., Attiya S., Bader J.S., Bemben L.A., Berka J., Braverman M.S., Chen Y.-J., Chen Z. Genome sequencing in microfabricated highdensity picolitre reactors. *Nature,* 2005, 437(7057): 376-380 (doi: 10.1038/nature03959).

10. Loman N.J., Misra R.V., Dallman T.J., Constantinidou C., Gharbia S.E., Wain J., Pallen M.J. Performance comparison of benchtop high-throughput sequencing platforms. *Nat. Biotechnol.*, 2012, 30(5): 434-439 (doi: 10.1038/nbt.2198).

11. Ranjan R., Rani A., Metwally A., McGee H.S., Perkins D.L. Analysis of the microbiome: advantages of whole genome shotgun versus 16S amplicon sequencing. *Biochem. Biophys. Res. Commun.*, 2016, 469(4): 967-977 (doi: 10.1016/j.bbrc.2015.12.083).

12. Roumpeka D.D., Wallace R.J., Escalettes F., Fotheringham I., Watson M. A review of bioinformatics tools for bio-prospecting from metagenomic sequence data. *Frontiers in Genetics*, 2017, 8: 23 (doi: 10.3389/fgene.2017.00023).

13. Ott S.J., Musfeldt M., Ullmann U., Hampe J., Schreiber S. Quantification of intestinal bacterial populations by real-time PCR with a universal primer set and minor groove binder probes: a global approach to the enteric flora. *Journal of Clinical Microbiology*, 2004, 42(6): 2566-2572 (doi: 10.1128/JCM.42.6.2566-2572.2004).

14. Malinen E., Kassinen A., Rinttila T., Palva A. Comparison of real-time PCR with SYBR Green I or 5´-nuclease assays and dot-blot hybridization with rDNA-targeted oligonucleotide probes in quantification of selected faecal bacteria. *Microbiology*, 2003, 149: 269-277 (doi: 10.1099/mic.0.25975-0).

15. Schloss P.D., Westcott S.L., Ryabin T., Hall J.R., Hartmann M., Hollister E.B., Lesniewski R.A., Oakley B.B., Parks D.H., Robinson C.J. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, 2009, 75(23): 7537-7541 (doi: 10.1128/AEM.01541-09).

16. Caporaso J.G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello E.K., Fierer N., Pena A.G., Goodrich J.K., Gordon J.I. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods*, 2010, 7: 335 (doi: 10.1038/nmeth.f.303).

17. Edgar R.C. Updating the 97% identity threshold for 16S ribosomal RNA OTUs. *Bioinformatics*, 2018, 34: 2371-2375 (doi: 10.1093/bioinformatics/bty113).

18. Westcott S.L., Schloss P.D. De novo clustering methods outperform reference based methods for assigning 16S rRNA gene sequences to operational taxonomic units. *PeerJ*, 2015, 3: e1487 (doi: 10.7717/peerj.1487).

19. McDonald D., Price M.N., Goodrich J., Nawrocki E.P., DeSantis T.Z., Probst A., Andersen G.L., Knight R., Hugenholtz P. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J.*, 2012, 6: 610-618 (doi: 10.1038/ismej.2011.139).

20. Pruesse E., Quast C., Knittel K., Fuchs B.M., Ludwig W., Peplies J., Glöckner F.O. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research*, 2007, 35(21): 7188-7196 (doi: 10.1093/nar/gkm864).

21. Cole J.R., Wang Q., Cardenas E., Fish J., Chai B., Farris R.J., Kulam-Syed-Mohideen A.S., McGarrell D.M., Marsh T., Garrity G.M., Tiedje J.M. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Research*, 2009, 37(suppl_1): D141-D145 (doi: 10.1093/nar/gkn879).

22. Chen W., Zhang C.K., Cheng Y., Zhang S., Zhao H. A comparison of methods for clustering 16S rRNA sequences into OTUs. *PLoS ONE*, 2013, 8: e70837 (doi: 10.1371/journal.pone.0070837).

23. Nguyen N.P., Warnow T., Pop M., White B. A perspective on 16 S rRNA operational taxonomic unit clustering using sequence similarity. *NPJ Biofilms Microbiomes*, 2016, 2: 16004 (doi: 10.1038/npjbiofilms.2016.4).

24. Haas B.J., Gevers D., Earl A.M., Feldgarden M., Ward D.V., Giannoukos G., Ciulla D., Tabbaa D., Highlander S.K., Sodergren E., Methé B., DeSantis T.Z., Human Microbiome Consortium, Petrosino J.F., Knight R., Birren B.W. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Res.*, 2011, 21(3): 494-504 (doi: 10.1101/gr.112730.110).

25. D'Amore R., Ijaz U.Z., Schirmer M., Kenny J.G., Gregory R., Darby A.C., Shakya M., Podar M., Quince C., Hall N. A comprehensive benchmarking study of protocols and sequencing platforms for 16 S rRNA community profiling. *BMC Genomics*, 2016, 17: 55 (doi: 10.1186/s12864-015-2194-9).

26. Glazko V.I., Zybaylov B.L., Kosovsky G.Yu., Glazko G.V., Glazko T.T. Domestication and microbiome *The Holocene*, 2021, 31(10): 1635-1645 (doi: 10.1177/09596836211025975).

27. Wilkins A.S. A striking example of developmental bias in an evolutionary process: The "domestication syndrome". *Evolution & Development*, 2020, 22(1-2): 143-153 (doi: 10.1111/ede.12319).

28. Glazko V., Zybailov B., Glazko T. Asking the right question about the genetic basis of domestication: what is the source of genetic diversity of domesticated species? *Adv. Genet. Eng.*, 2015, 4(2): 1000125 (doi: 10.4172/2169-0111.1000125).

29. O'Hara A.M., Shanahan F. The gut flora as a forgotten organ. *EMBO Rep.*, 2006, 7(7): 688-693 (doi: 10.1038/sj.embor.7400731).

30. Kolodny O., Callahan B.J., Douglas A.E. The role of the microbiome in host evolution. *Phil. Trans. R. Soc. B*, 2020, 375(1808): 20190588 (doi: 10.1098/rstb.2019.0588).

31. Zilber-Rosenberg I., Rosenberg E. Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol. Rev.,* 2008, 32(5): 723-735 (doi: 10.1111/j.1574-6976.2008.00123).

32. Ikeda-Ohtsubo W., Brugman S., Warden C.H., Rebel J.M.J., Folkerts G., Pieterse C.M.J. How can we define "optimal microbiota?": a comparative review of structure and functions of microbiota of animals, fish, and plants in agriculture. *Front. Nutr.,* 2018, 5: 90 ( doi: 10.3389/fnut.2018.00090).

33. Douglas-Escobar M., Elliott E., Neu J. Effect of intestinal microbial ecology on the developing brain. *JAMA Pediatr.*, 2013, 167(4): 374-379 (doi: 10.1001/jamapediatrics.2013.497).

34. Bercik P., Denou E., Collins J., The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology*, 2011, 141(2): 599-609 (doi: 10.1053/j.gastro.2011.04.052).

35. Knight R., Callewaert C., Marotz C., Hyde E.R., Debelius J.W., McDonald D., Sogin M.L. The microbiome and human biology. *Annual Review of Genomics and Human Genetics*, 2017, 18: 65-86 (doi: 10.1146/annurev-genom-083115-022438).

36. Li J.V., Swann J., Marchesi J.R. Biology of the microbiome 2: metabolic role. *Gastroenterol. Clin. North Am.*, 2017, 46(1): 37-47 (doi: 10.1016/j.gtc.2016.09.006).

37. Doré J., Blottière H. The influence of diet on the gut microbiota and its consequences for health. *Curr. Opin. Biotechnol.*, 2015, 32: 195-199 (doi: 10.1016/j.copbio.2015.01.002).

38. NIH Human Microbiome Portfolio Analysis Team. A review of 10 years of human microbiome research activities at the US national institutes of health, fiscal years 2007-2016. *Microbiome*, 2019, 7: 31 (doi: 10.1186/s40168-019-0620)

39. Peterson S.N., Snesrud E., Liu J., Ong A.C., Kilian M., Schork N.J., Bretz W. The dental plaque microbiome in health and disease. *PLoS ONE*, 2013, 8: e58487 (doi: 10.1371/journal.pone.0058487).

40. Yang F., Zeng X., Ning K., Liu K.L., Lo C.C., Wang W., Chen J., Wang D., Huang R., Chang X. Saliva microbiomes distinguish caries-active from healthy human populations. *ISME J.*, 2012, 6: 1-10 (doi: 10.1038/ismej.2011.71).

41. Fredricks D.N., Fiedler T.L., Marrazzo J.M. Molecular identification of bacteria associated with bacterial vaginosis. *N. Engl. J. Med.*, 2005, 353(18):1899-1911 (doi: 10.1056/NEJMoa043802).

42. Cotozzolo E., Cremonesi P., Curone G., Characterization of bacterial microbiota composition along the gastrointestinal tract in rabbits. *Animals (Basel)*, 2020, 11(1): 31 (doi: 10.3390/ani11010031).

43. Chen C., Song X., Wei W., Zhong H., Dai J., Lan Z., Li F., Yu X., Feng Q., Wang Z. The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. *Nat. Commun.*, 2017, 8: 875 (doi: 10.1038/s41467-017-00901-0).

44. Mitchell C.M., Haick A., Nkwopara E., Garcia R., Rendi M., Agnew K., Fredricks D.N., Eschenbach D. Colonization of the upper genital tract by vaginal bacterial species in nonpregnant women. *Am. J. Obstet. Gynecol.*, 2015, 212(5): 611.e1-611.e9 (doi: 10.1016/j.ajog.2014.11.043).

45. Hickey R.J., Zhou X., Pierson J.D., Ravel J., Forney L.J. Understanding vaginal microbiome complexity from an ecological perspective. *Transl. Res.*, 2012, 160(4): 267-282 (doi: 10.1016/j.trsl.2012.02.0080).

46. Noyes N., Cho K.-C., Ravel J., Forney L.J., Abdo Z. Associations between sexual habits, menstrual hygiene practices, demographics and the vaginal microbiome as revealed by Bayesian network analysis. *PLoS ONE*, 2018, 13: e0191625 (doi: 10.1371/journal.pone.0191625).

47. Ravel J., Gajer P., Abdo Z., Schneider G.M., Koenig S.K., McCulle S.L., Karlebach S., Gorle R., Russell J., Tacket C.O., Brotman R.M., Davis C.C., Ault K., Peralta L., Forney L.J. Vaginal microbiome of reproductive-age women. *Proc. Natl. Acad. Sci.*, 2011, 108(supplement_1): 4680- 4687 (doi: 10.1073/pnas.1002611107).

48. Gajer P., Brotman R.M., Bai G., Sakamoto J., Schütte U.M.E., Zhong X., Koenig S.S.K., Fu L., Ma Z.S., Zhou X., Abdo Z., Forney L.J., Ravel J. Temporal dynamics of the human vaginal microbiota. *Sci. Transl. Med.*, 2012, 4(132): 132ra152 (doi: 10.1126/scitranslmed.3003605).

49. Albert A.Y.K., Chaban B., Wagner E.C., Schellenberg J.J., Links M.G., Van Schalkwyk J., Reid G., Hemmingsen S.M., Hill J.E., Money D. A study of the vaginal microbiome in healthy Canadian women utilizing cpn60-based molecular profiling reveals distinct *Gardnerella* subgroup community state types. *PLoS ONE,* 2015, 10: e0135620 (doi: 10.1371/journal.pone.0135620).

50. O'Hanlon D.E., Moench T.R., Cone R.A. Vaginal pH and microbicidal lactic acid when *Lactobacilli* dominate the microbiota. *PLoS ONE*, 2013, 8(11): e80074 (doi: 10.1371/journal.pone.0080074).

51. Anahtar M.N., Byrne E.H., Doherty K.E., Bowman B.A., Yamamoto H.S., Soumillon M., Padavattan N., Ismail N., Moodley A., Sabatini M.E., Ghebremichael M.S., Nusbaum C., Huttenhower C., Virgin H.W., Ndung'u T., Dong K.L., Walker B.D., Fichorova R.N., Kwon D.S. Cervicovaginal bacteria are a major modulator of host inflammatory responses in the female genital tract. *Immunity*, 2015, 42(5): 965-976 (doi: 10.1016/j.immuni.2015.04.019).

52. Anukam K.C., Osazuwa E.O., Ahonkhai I., Reid G. Lactobacillus vaginal microbiota of women attending a reproductive health care service in Benin city, Nigeria. *Sexually Transmitted Diseases*, 2006, 33(1): 59-62 (doi: 10.1097/01.olq.0000175367.15559).

53. Pendharkar S., Magopane T., Larsson P.-G., de Bruyn G., Gray G.E., Hammarstrum L., Marcotte H. Identification and characterisation of vaginal *Lactobacilli* from South African women. *BMC Infect. Dis.*, 2013, 13: 43 (doi: 10.1186/1471-2334-13-43).

54. MacIntyre D.A., Chandiramani M., Lee Y.S., Kindinger L., Smith A., Angelopoulos N., Lehne B., Arulkumaran S., Brown R., Teoh T.G., Holmes E., Nicoholson J.K., Marchesi J.R., Bennett P.R. The vaginal microbiome during pregnancy and the postpartum period in a European population. *Sci. Rep.,* 2015, 5: 8988 (doi: 10.1038/srep08988).

55. Aagaard K., Riehle K., Ma J., Segata N., Mistretta T.A., Coarfa C., Raza S., Rosenbaum S., Van den Veyver I., Milosavljevic A. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. *PLoS ONE,* 2012, 7: e36466 (doi: 10.1371/journal.pone.0036466).

56. Romero R., Hassan S.S., Gajer P., Tarca A.L., Fadrosh D.W., Nikita L., Galuppi M., Lamont R.F., Chaemsaithong P., Miranda J., Chaiworapongsa T., Ravel J. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of nonpregnant women. *Microbiome*, 2014, 2: 4 (doi: 10.1186/2049-2618-2-4).

57. Hyman R.W., Fukushima M., Jiang H., Fung E., Rand L., Johnson B., Vo K.C., Caughey A.B., Hilton J.F., Davis R.W., Giudice L.C. Diversity of the vaginal microbiome correlates with preterm birth. *Reproductive Sciences*, 2014, 21(1): 32-40 (doi: 10.1177/1933719113488838).

58. Stout M.J., Zhou Y., Wylie K.M., Tarr P.I., Macones G.A., Tuuli M.G. Early pregnancy vaginal microbiome trends and preterm birth. *Am. J. Obstet. Gynecol.*, 2017, 217(3): 356.e1-356.e18 (doi: 10.1016/j.ajog.2017.05.030).

59. Mendes-Soares H., Suzuki H., Hickey R.J., Forney L.J. Comparative functional genomics of *Lactobacillus* spp. reveals possible mechanisms for specialization of vaginal Lactobacilli to their environment. *Journal of Bacteriology*, 2014, 196(7): 1458-1470 (doi: 10.1128/JB.01439-13).

60. Ojala T., Kankainen M., Castro J., Cerca N., Edelman S., Westerlund-Wikstrum B., Paulin L., Holm L., Auvinen P. Comparative genomics of *Lactobacillus crispatus* suggests novel mechanisms for the competitive exclusion of *Gardnerella vaginalis*. *BMC Genomics*, 2014, 15: 1070 (doi: 10.1186/1471-2164-15-1070).

61. Graspeuntner S., Bohlmann M.K., Gillmann K., Speer R., Kuenzel S., Mark H., Hoellen F., Lettau R., Griesinger G., König I.R. Microbiota-based analysis reveals specific bacterial traits and a novel strategy for the diagnosis of infectious infertility. *PLoS ONE*, 2018, 13: e0191047 (doi: 10.1371/journal.pone.0191047)

62. Babu G., Singaravelu B.G., Srikumar R., Reddy S.V. Comparative study on the vaginal flora and incidence of asymptomatic vaginosis among healthy women and in women with infertility problems of reproductive age. *Journal of Clinical and Diagnostic Research*, 2017, 11(8): DC18-DC22 (doi: 10.7860/JCDR/2017/28296.10417).

63. Campisciano G., Florian F., D'Eustacchio A., Stankovi D., Ricci G., De Seta F., Comar M. Subclinical alteration of the cervical-vaginal microbiome in women with idiopathic infertility. *J. Cell. Physiol.,* 2017, 232(7): 1681-1688 (doi: 10.1002/jcp.25806).

64. Di M.P., Filardo S., Porpora M.G., Recine N., Latino M.A., Sessa R. HPV/Chlamydia trachomatis co-infection: metagenomic analysis of cervical microbiota in asymptomatic women. *New Microbiologica*, 2018, 41(1): 34-41.

65. Van de Wijgert J.H.H.M, Borgdorff H., Verhelst R., Crucitti T., Francis S., Verstraelen H., Jespers V. The vaginal microbiota: what have we learned after a decade of molecular characterization? *PLoS ONE*, 2014, 9: e105998 (doi: 10.1371/journal.pone.0105998).

66. Workowski K.A., Bolan G.A. Sexually transmitted diseases treatment guidelines, 2015. *Recommendations and Reports,* 2015, 64(RR3): 1-137.

67. Onderdonk A.B., Delaney M.L., Fichorova R.N. The human microbiome during bacterial vaginosis. *Clinical Microbiology Reviews*, 2016, 29(2): 223-238 (doi: 10.1128/CMR.00075-15).

68. Gottschick C., Deng Z.-L., Vital M., Masur C., Abels C., Pieper D.H., Wagner-Döbler I. The urinary microbiota of men and women and its changes in women during bacterial vaginosis and antibiotic treatment. *Microbiome*, 2017, 5: 99 (doi: 10.1186/s40168-017-0305-3).

69. Donders G.G., Van Calsteren K., Bellen G., Reybrouck R., Van den Bosch T., Riphagen I., Van Lierde S. Predictive value for preterm birth of abnormal vaginal flora, bacterial vaginosis and aerobic vaginitis during the first trimester of pregnancy. *BJOG: An International Journal of Obstetrics & Gynaecology*, 2009, 116(10): 1315-1324 (doi: 10.1111/j.1471-0528.2009.02237.x).

70. Işik G., Demirezen Ş., Dönmez H.G., Beksaç M.S. Bacterial vaginosis in association with spontaneous abortion and recurrent pregnancy losses. *Journal of Cytology*, 2016, 33(3): 135-140 (doi: 10.4103/0970-9371.188050).

71. Karstrup C.C., Klitgaard K., Jensen T.K., Agerholm J.S., Pedersen H.G. Presence of bacteria in the endometrium and placentomes of pregnant cows. *Theriogenology*, 2017, 99: 41-47 (doi: 10.1016/j.theriogenology.2017.05.013).

72. Bicalho M.L., Lima F.S., Machado V.S., Meira E.B. Jr., Ganda E.K., Foditsch C., Bicalho R.C., Gilbert R.O. Associations among *Trueperella pyogenes*, endometritis diagnosis, and pregnancy outcomes in dairy cows. *Theriogenology*, 2016, 85(2): 267-274 (doi: 10.1016/j.theriogenology.2015.09.043).

73. Galvão K.N., Bicalho R.C., Jeon S.J. Symposium review: The uterine microbiome associated with the development of uterine disease in dairy cows. *Journal of Dairy Science*, 2019, 102(12): 11786-11797 (doi: 10.3168/jds.2019-17106).

74. Bicalho V.S., Machado C.H., Higgins F.S., Lima R.C. Genetic and functional analysis of the bovine uterine microbiota. Part I: Metritis versus healthy cows. *Journal of Dairy Science*, 2017, 100(5): 3850-3862 (doi: 10.3168/jds.2016-12058).

75. Bicalho M.L.S., Lima S., Higgins C.H., Machado V.S., Lima F.S., Bicalho R.C. Genetic and functional analysis of the bovine uterine microbiota. Part II: Purulent vaginal discharge versus healthy cows. *Journal of Dairy Science*, 2017, 100(5): 3863-3874 (doi: 10.3168/jds.2016-12061).

76. Umar T., Yin B., Umer S., Ma X., Jiang K., Umar Z., Akhtar M., Shaukat A., Deng G. MicroRNA: could it play a role in bovine endometritis? *Inflammation*, 2021, 44(5): 1683-1695 (doi: 10.1007/s10753-021-01458-3).

77. Miller E.A., Beasley D.E., Dunn R.R., Archie E.A. Lactobacilli dominance and vaginal pH: why is the human vaginal microbiome unique? *Front. Microbiol.*, 2016, 7: 1936 (doi: 10.3389/fmicb.2016.01936).

78. Swartz J.D., Lachman M., Westveer K., O'Neill T., Geary T., Kott R.W., Berardinelli J.G., Hatfield P.G., Thomson J.M., Roberts A., Yeoman C.J. Characterization of the vaginal microbiota of ewes and cows reveals a unique microbiota with low levels of Lactobacilli and Near-Neutral pH. *Frontiers in Veterinary Science*, 2014, 1: 19 (doi: 10.3389/fvets.2014.00019).

79. Hyman R.W., Herndon C.N., Jiang H., Palm C., Fukushima M., Bernstein D., Vo K.C., Zelenko Z., Davis R.W., Giudice L.C. The dynamics of the vaginal microbiome during infertility therapy with in vitro fertilization-embryo transfer. *Journal of Assisted Reproduction and Genetics*, 2012, 29(2): 105-115 (doi: 10.1007/s10815-011-9694-6).

80. Moore D.E., Soules M.R., Klein N.A., Fujimoto V.Y., Agnew K.J., Eschenbach D.A. Bacteria in the transfer catheter tip influence the live-birth rate after in vitro fertilization. *Fertility and Sterility*, 2000, 74(6): 1118-1124 (doi: 10.1016/s0015-0282(00)01624-1).

81. Hillier S.L., Nugent R.P., Eschenbach D.A., Krohn M.A., Gibbs R.S., Martin D.H., Cotch M.F., Edelman R., Pastorek J.G., Rao A.V., McNellis D., Regan J.A., et all., for the Vaginal Infections and Prematurity Study Group. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. *New England Journal of Medicine*, 1995, 333: 1737-1742 (doi: 10.1056/NEJM199512283332604).

82. Haahr T., Jensen J.S., Thomsen L., Duus L., Rygaard K., Humaidan P. Abnormal vaginal microbiota may be associated with poor reproductive outcomes: a prospective study in IVF patients. *Human Reproduction*, 2016, 31(4): 795-803 (doi: 10.1093/humrep/dew026).

83. Mangot-Bertrand J., Fenollar F., Bretelle F., Gamerre M., Raoult D., Courbiere B. Molecular diagnosis of bacterial vaginosis: impact on IVF outcome. *European Journal of Clinical Microbiology & Infectious Diseases*, 2013, 32: 535-541 (doi: 10.1007/s10096-012-1770-z).

84. Pelzer E.S., Allan J.A., Waterhouse M.A., Ross T., Beagley K.W., Knox C.L. Microorganisms within human follicular fluid: effects on IVF. *PLoS ONE*, 2013, 8: e59062 (doi: 10.1371/journal.pone.0059062).

85. Petrova M., Lievens E., Malik S., Imholz N., Lebeer S. *Lactobacillus* species as biomarkers and agents that can promote various aspects of vaginal health. *Frontiers in Physiology,* 2015, 6: 81 (doi: 10.3389/fphys.2015.00081).

86. Moreno I., Codocer F.M., Vilella F., Valbuena D., Martinez-Blanch J.F., Jimenez-Almazán J., Alonso R., Alamá P., Remohí J., Pellicer A. Evidence that the endometrial microbiota has an effect on implantation success or failure. *Am. J. Obstet. Gynecol.*, 2016, 215: 684-703 (doi: 10.1016/j.ajog.2016.09.075).

87. Cicinelli E., Matteo M., Tinelli R., Lepera A., Alfonso R., Indraccolo U., Marrocchella S., Greco P., Resta L. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. *Human Reproduction*, 2015, 30(2): 323-330 (doi: 10.1093/humrep/deu292).

88. Cicinelli E., Matteo M., Tinelli R., Pinto V., Marinaccio M., Indraccolo U., De Ziegler D., Resta L. Chronic endometritis due to common bacteria is prevalent in women with recurrent miscarriage as confirmed by improved pregnancy outcome after antibiotic treatment. *Reproductive Sciences*, 2014, 21(5): 640-647 (doi: 10.1177/1933719113508817).

89. Khan K.N., Fujishita A., Kitajima M., Hiraki K., Nakashima M., Masuzaki H. Intra-uterine microbial colonization and occurrence of endometritis in women with endometriosis. *Human Reproduction*, 2014, 29(11): 2446-2456 (doi: 10.1093/humrep/deu222).

90. Fotouh I.A., Al-Inany M.G. The levels of bacterial contamination of the embryo transfer catheter

relate negatively to the outcome of embryo transfer. *Middle East Fertility Society Journal*, 2008, 13(1): 39-43.

91. Franasiak J.M., Werner M.D., Juneau C.R., Tao X., Landis J., Zhan Y., Treff N.R., Scott R.T. Endometrial microbiome at the time of embryo transfer: next-generation sequencing of the 16S ribosomal subunit. *Journal of Assisted Reproduction and Genetics*, 2016, 33(1): 129-136 (doi: 10.1007/s10815-015-0614-z).

92. Kroon B., Hart R.J., Wong B., Ford E., Yazdani A. Antibiotics prior to embryo transfer in ART. *Cochrane Database of Systematic Reviews*, 2012, 3: CD008995 (doi: 10.1002/14651858.CD008995.pub2).

93. Brook N., Khalaf Y., Coomarasamy A., Edgeworth J., Braude P. A randomized controlled trial of prophylactic antibiotics (co-amoxiclav) prior to embryo transfer. *Human Reproduction*, 2006, 21(11): 2911-2915 (doi: 10.1093/humrep/del263).

94. Schoenmakers S., Steegers-Theunissen R., Faas M. The matter of the reproductive microbiome. *Obstetric Medicine*, 2019, 12(3): 107-115 (doi: 10.1177/1753495X18775899).

95. Kim J.H., Yoo S.M., Sohn Y.H., Jin C.H., Yang Y.S., Hwang I.T., Oh K.Y. Predominant *Lactobacillus* species types of vaginal microbiota in pregnant Korean women: quantification of the five *Lactobacillus* species and two anaerobes. *Journal of Maternal-Fetal & Neonatal Medicine*, 2017, 30(19): 2329-2333 (doi: 10.1080/14767058.2016.1247799).

96. Beckers K.F., Sones J.L. Maternal microbiome and the hypertensive disorder of pregnancy, preeclampsia. *American Journal of Physiology-Heart and Circulatory Physiology*, 2020, 318(1): H1-H10 (doi: 10.1152/ajpheart.00469.2019).

97. McElrath T.F., Hecht J.L., Dammann O., Boggess K., Onderdonk A., Markenson G., Harper M., Delpapa E., Allred E.N., Leviton A., ELGAN Study Investigators. Pregnancy disorders that lead to delivery before the 28th week of gestation: an epidemiologic approach to classification. A*merican Journal of Epidemiology*, 2008, 168(9): 980-989 (doi: 10.1093/aje/kwn202).

98. Aagaard K., Ma J., Antony K.M., Ganu R., Petrosino J., Versalovic J. The placenta harbors a unique microbiome. *Sci. Transl. Med.*, 2014, 6(237): 237ra65 (doi: 10.1126/scitranslmed.3008599).

99. Benner M., Ferwerda G., Joosten I., Van der Molen R.G. How uterine microbiota might be responsible for a receptive, fertile endometrium. *Human Reproduction*, 2018, 24(4): 393-415 (doi: 10.1093/humupd/dmy012).

100. Weng S.-L., Chiu C.-M., Lin F.-M., Huang W.-C., Liang C., Yang T., Yang T.-L., Liu C.-Y., Wu W.-Y., Chang Y.-A., Chang T.-H., Huang H.-D. Bacterial communities in semen from men of infertile couples: metagenomic sequencing reveals relationships of seminal microbiota to semen quality. *PLoS ONE*, 2014, 9(10): e110152 (doi: 10.1371/journal.pone.0110152).

101. Gdoura R., Kchaou W., Chaari C., Znazen A., Keskes L., Rebai T., Hammami A. *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis* and *Mycoplasma genitalium* infections and semen quality of infertile men. *BMC Infect. Dis.*, 2007, 7: 129 (doi: 10.1186/1471-2334-7-129).

102. Zinzendorf N.Y., Kouassi-Agbessi B.T., Lathro J.S., Don C., Kouadio L., Loukou Y.G. *Ureaplasma urealyticum* or *Mycoplasma hominis* infections and semen quality of infertile men in Abidjan. *Journal of Reproduction and Contraception*, 2008, 19(2): 65-72 (doi: 10.1016/S1001-7844(08)60008-5).

103. Ahmadi M.H., Mirsalehian A., Gilani M.A.S., Bahador A., Talebi M. Asymptomatic infection with *Mycoplasma hominis* negatively affects semen parameters and leads to male infertility as confirmed by improved semen parameters after antibiotic treatment. *Urology*, 2017, 100: 97-102 (doi: 10.1016/j.urology.2016.11.018).

104. Mändar R., Punab M., Korrovits P., Türk S, Ausmees K., Lapp E., Preem J.K., Oopkaup K., Salumets A., Truu J. Seminal microbiome in men with and without prostatitis. *Int. J. Urol.*, 2017, 24(3): 211-216 (doi: 10.1111/iju.13286).

105. Monteiro C., Marques P.I., Cavadas B., Damiro I., Almeida V., Barros N., Barros A., Carvalho F., Gomes S., Seixas S. Characterization of microbiota in male infertility cases uncovers differences in seminal hyperviscosity and oligoasthenoteratozoospermia possibly correlated with increased prevalence of infectious bacteria. *Am. J. Reprod. Immunol.*, 2018, 79(6): e12838 (doi: 10.1111/aji.12838).

106. Marco-Jiménez F., Borrás S., Garcia-Dominguez X., D'Auria G., Vicente J.S., Marin C. Roles of host genetics and sperm microbiota in reproductive success in healthy rabbit *Theriogenology*, 2020, 158: 416-423 (doi: 10.1016/j.theriogenology.2020.09.028).

107. Mändar R., Punab M., Borovkova N., Lapp E., Kiiker R., Korrovits P., Metspalu A., Krjutškov K., Nõlvak H., Preem J.K., Oopkaup K., Salumets A., Truu J. Complementary seminovaginal microbiome in couples. *Research in Microbiology,* 2015, 166(5): 440-447 (doi: 10.1016/j.resmic.2015.03.009).

108. Borovkova N., Korrovits P., Ausmees K., Turk S., Joers K., Punab M., Mändar R. Influence of sexual intercourse on genital tract microbiota in infertile couples. *Anaerobe*, 2011, 17(6): 414-418 (doi: 10.1016/j.anaerobe.2011.04.015).

109. Eschenbach D.A., Patton D.L., Hooton T.M., Meier A.S., Stapleton A., Aura J., Agnew K. Effects of vaginal intercourse with and without a condom on vaginal flora and vaginal epithelium. *The Journal of Infectious Diseases*, 2001, 183(6): 913-918 (doi: 10.1086/319251).

110. Leppaluoto P.A. Bacterial vaginosis: what is physiological in vaginal bacteriology? An update and opinion. *Acta Obstetricia et Gynecologica Scandinavica*, 2011, 90(12): 1302-1306 (doi: 10.1111/j.1600-0412.2011.01279.x).
111. Robertson S.A., Sharkey D.J. Seminal fluid and fertility in women. *Fertility and Sterility*, 2016, 106(3): 511-519 (doi: 10.1016/j.fertnstert.2016.07.1101).