Journal of Clinical Veterinary Research

(ISSN: 2771-313X) *Open Access*

Review Article Volume 2 – Issue 1

Relationship of Microbiota with Male Reproductive Potential

Ali Dogan Omur[1,6,7,*], Serkan Ali Akarsu[2], Buşra Ayyildiz[3], Demet Celebi[4,*], Mehmet Akif Aydin[5]

[1]Atatürk University Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Erzurum, Turkey

[2]Elbistan Vocational School, Kahramanmaras İstiklal University, Kahramanmaras, TR

[3]Department of Medical Microbiology, Faculty of Medicine, Ataturk University, Erzurum, TR

[4]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ataturk University, Erzurum, TR

[5]Food and Livestock Application and Research Center, Ataturk University, Erzurum, TR

[6]Atatürk University Faculty of Veterinary Medicine, Department of Biochemistry, Erzurum, Turkey

[7]Ataturk University, Faculty of Science, Department of Molecular Biology and Genetics, Erzurum, Turkey

[*]Corresponding author: [1]Ali Dogan Omur, Ataturk University Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Erzurum, Turkey

[2]Demet Celebi, Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ataturk University, Erzurum, TR

Received date: 06 May, 2022 | **Accepted date:** 17 May, 2022 | **Published date:** 20 May, 2022

Citation: Omur AD, Akarsu SA, Ayyildiz B, Celebi D, Aydin MA. (2022) Relationship of Microbiota with Male Reproductive Potential. J Clin Vet Res 2(1): doi https://doi.org/10.54289/JCVR2200105

Abstract

Microbiota is a cluster of physiological and pathogenic microorganisms found in many tissues and organs of living things, including such as bacteria, viruses, fungi. Microbiota has important roles on many system functions such as immune system, urinary system, digestive system. However, these microorganisms can cause obstruction in the genital tract, epididymitis and orchitis, and thus may lead to infertility. In addition, pathogenic and apathogenic microorganisms such as bacteria, viruses and fungi have negative effects on spermatology. Decreased sperm motility, disruption of membrane integrity, and acrosome damage are the most common of these negativities. Approximately 15% of male infertility in the world is associated with infection in the genital tract. The most common microorganisms found in the male genital tract and semen are Enterobacter, Lactobacillus and Staphylococci. In addition, microorganisms in semen cause infections in the female genital tract, birth of offspring with anomalies and embryonic deaths. In this study, the current literature on the microorganisms found in the male genital tract and semen in different species has been compiled.

Keywords: Infertility; Microbiota; Semen

Abbreviations: GU: Genitourinary, STD: Sexually Transmitted Diseases, ART: Assisted Reproductive Technologies, CFU: Colony Forming Units, LAB: Lactic Acid Bacteria, IPV: Infectious Pustular Vulvovaginitis, IBR: Infectious Bovine Rhinotracheitis, IPB: Irifexiosis Pustular Balanopostilis, FMD: Foot and Mouth Disease, BT: Bluetongue, BVD: Bovine Viral Diarrhea, BL: Bovine Leukemia, EF: Ephemeral Fever, LSD: Lumpy Skin Disease

Introduction

The terms abiogenesis and biogenesis were explained by Thomas Henry Huxley (18251895). He proposed that the term abiogenesis be used to mean the spontaneous process of formation, and the term biogenesis to be used to refer to the process of life arising from similar life. Microorganisms were

discovered in the late 17th century by Robert Hooke and Antoni van Leeuwenhoek **[1].**

Microbiota; while it contributes to the development of the immune system, has important roles on skin, urinary system, respiratory system, digestion and absorption of nutrients, production of vitamins, development and functions of the gastrointestinal immune system, the living body also provides a rich nutrient environment for the survival of microorganisms. Although there have been studies explaining the role of microbiota in the organism in recent years, it is important to create optimum health conditions of the body by understanding the molecular mechanisms in more detail with the point reached today.

Previous studies have defined that microbiota in the organism is related to homeostasis and health **[2,3]** including male and female genital tracts **[4].** Microbiomes play an important role in disease and some etiologies. The skin, intestines, oral cavity, vagina, and urethra are among the sites that host microbial communities. The composition of the microbiomes can alter its effect on metabolism **[5-7].**

Microbiota in Semen

Sexually transmitted diseases (STDs) can affect individual morbidity, mortality and fertility **[8].** Semen is not sterile and may contain microorganisms even after processing for Assisted reproductive technologies (ART) **[9].** Infections in the genitourinary (GU) canal account for up to 15% of the causes of male infertility **[10].** In the sequencing studies performed in the genitourinary system of men and women, genes of pathogenic factors were found, and it was stated that these factors have negative effects on the health of the reproductive system **[11].** Studies have showed that a large number of infectious bacteria, viral, fungal and protozoan species can enter the normal genital-urinary tract through sexual transmission, intracanalicular spread of infected urine **[12,13].** The bacterial microbiome is referred to as the extracellular microenvironmental component **[14].** Also, bacteria can have a direct negative impact on spermatozoa physiology, reducing viability or motility and decrease mitochondrial activity **[15-18]** One of the most important causes affecting spermatogenesis is infections that directly affect the testicles **[19,20].** These infections prevent the continuity of spermatogenesis. It can affect spermatozoa as well as cause defects in the formed spermatozoa **[21].** Some infections, inflammation in the tissues, enlargement of the genital tract, epididymitis and orchitis may be related to male infertility **[22].** Microorganism load in semen increases the number of macrophages and polymorphonuclear granulocytes, forming the primary line of defense. This defense mechanism leads to the production of reactive oxygen species (ROS) in the semen, and thus, with a large increase in the number of dead spermatozoa, ROS production also increases and the semen begin to lose functions **[23-25].**

Genital Mycoplasma (Mycoplasma genitalium and Mycoplasma hominis) colonizes the genitourinary tract of both males and females **[26].** M.genitalium caused sperm agglutination and decreased motility in sperm in vitro. It is thought that this situation may also affect fertilization **[27].**

Various microorganisms cause problems such as balanitis, postitis, seminal vesiculitis, prostatitis, urethral inflammations, testicular degenerations, orchitis, epididymitis, ampullitis, resulting in male infertility **[28].** Microorganisms in semen can be viruses, bacteria, chlamydia, rickettsia or fungi and are generally classified as pathogenic, potentially pathogenic, and nonpathogenic **[29].** Semen is an important vector for the spread of viral infections. Some viruses can be found in semen cells (leukocytes, macrophages, sperm) and free semen **[30].** Hepatitis B, C, HIV, Papillomavirus, Herpesvirus, Cytomegalovirus can spread through semen in humans **[27].** In addition, in humans, Trichomonas vaginalis is a protozoan and causes infection around the urethra, especially since it prefers squamous cells **[31].**

Various microorganisms, including viruses, can be found in semen due to infections. These agents can be transmitted to susceptible animals by artificial and natural insemination. This causes the infection to be transmitted to animals inseminated with semen and even to encounter various consequences affecting the embryo **[32].** The bacterial load in fresh semen is between 10^4 and 10^6 colony forming units (CFU) /mL **[33].** Bacterial load in semen often leads to loss of sperm motility and sperm agglution and sperm plasma membrane damage, resulting in infertility and economic damage **[34].**

In the figure below **(Figure 1)**, the ratio information about bacterial microorganisms in (A) normal semen and (B) case samples is given.

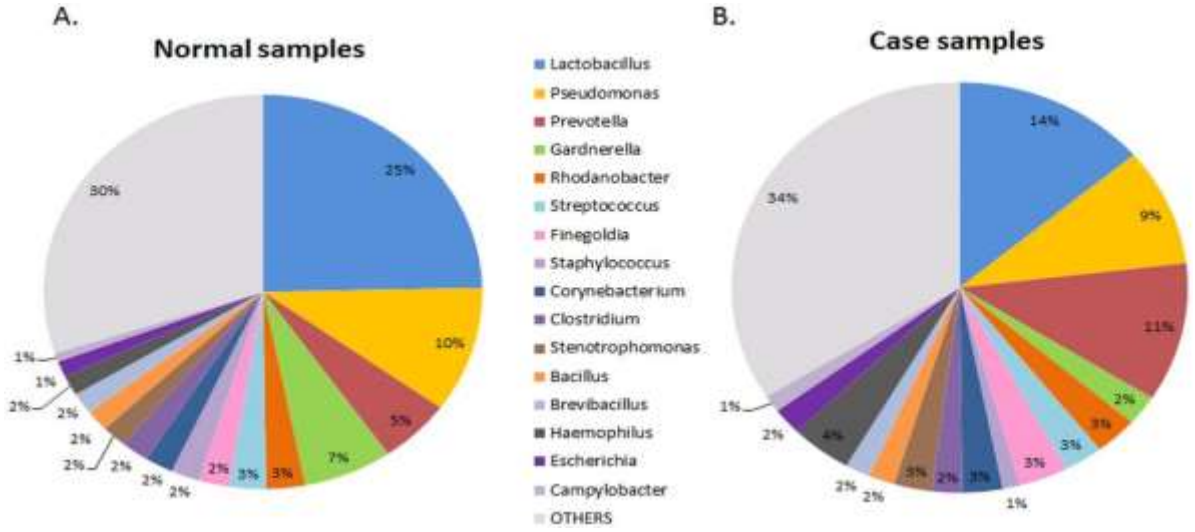

Figure 1. Proportion of bacterial microorganisms in (A) normal semen and (B) case samples **[11].**

Probiotics are defined as live microorganisms that confer a health benefit on the host **[35].** The most commonly used probiotics are lactobacilli and bifidobacteria, which produce lactic acid as the primary metabolite of sugar metabolism. Lactobacilli and bifidobacteria strains have also been reported to produce antioxidants **[36].** Lactobacillus, which contains about 180 species, is not known to have any negative effects on the living thing. Lactic acid bacteria (LAB) such as Lactobacillus, Lactococcus, Pediococcus, Streptococcus and Enterococcus are in this class **[37,38].** Studies have shown that lactic acid bacteria (LAB) in the genus Lactobacillus spp. prevent pathogenic microorganisms that may occur, and that they can do this by consuming resources, stimulating the host immune system, decreasing the pH, and producing acetic and lactic acid with hydrogen peroxide **[39,40].** Depending on the species, environmental factors can also contaminate sperm in semen examination and analysis in the laboratory **[41].**

The presence of bacteria in pig semen has been demonstrated by studies **[42].**

Brucellosis, Chlamydophilosis, Leptospirosis and Enterobacteriaceae are among the subjects studied in pig semen **[43,44].** A study has shown that Lactobacillus can be found in pig semen and its prolonged incubation will have a negative effect on spermatozoa **[45].**

Artificial insemination is one of the most frequently used methods in dogs **[46].** Semen is not a sterile secretion as it contains physiological microbial flora from the dog's urogenital tract [47; Osborne and Lees, 1995). Fractions present in canine ejaculate stimulate bacterial growth. Escherichia coli, Staphylococcus aureus, Klebsiella spp., Mycoplasma canis, Pseudomonas aeruginosa and Streptococcus spp. bacteria are more common in the first fraction of dog's ejeculate **[47,48].** Bacterial growth of less than 10^5 colony forming units (CFU)/mL in ejaculate from dogs is considered physiological, while high values are a cause of pathological risk **[49,50].**

Various exogenous and endogenous factors affect semen quality in rams. Bacteria found in ram ejaculates can originate from different sources such as wool, skin, urine, feces. However, contaminated feed, water, and poor hygiene conditions in the production area may increase bacterial contamination of semen **[51].** Aeromonas veronii, Bacillus subtilis, Enterobacter bugandens, Escherichia coli, Escherichia hermannii, Klebsiella variicol, Lactobacillus curvatus, Mannheimia haemolytica, Providencia rettgeri, Pseudomonas lutea, Staphylococcus delphini, Staphylococcus capitis, Staphylococcus chromogenes, Staphylococcus sciura, Staphylococcus simulans and Staphylococcus vitulinus are bacteria found in the ram semen **[52].** In a study performed before mating in rams, swap samples were taken from the glans penis and bacteria such as

Staphylococcus aureus, Streptococcus pyogenes, Proteus mirabilis and Brucella abortus were isolated **[53].** In horses, Staphylococcus spp, Micrococcus spp, Pseudomonas spp were isolated in semen **[54].** Taylorella equigenitalis is located in the distal part of the genital tract in horses and can remain there for a long time **[55].**

In poultry, pathogenic microorganisms that can potentially contaminate semen are transmitted through the cloaca **[56].** Salmonella spp. Campylobacter spp., Staphylococcus spp., Coliform spp., Streptococci spp. and Bacillus spp. are among the bacteria found in the ejaculate of poultry **[57].** Similarly in a study conducted in turkeys, only Enterobacter spp. was isolated from semen **[58].**

In fish semen, bacteria of the genus Pseudomonas spp are expressed as part of the microbiota of the semen **[59, 60].** In a study, Argyrosomus regius, Flavobacterium spp., Aeromonas spp. and Corynebacterium spp., Pseudomonas spp. and Vibrio sp. are isolated **[61].**

Microbiological control of ejaculates and spermatozoon count in straws aim to eliminate the prevalence of bacteriospermia from 7% to 99% of ejaculates **[62-66].** While bacteria such as Bacterioidetes, Actinobacteria, Proteobacteria, Firmicutes, Fusobacteria or Cyanobacteria are present in the semen of bulls, sometimes opportunistic pathogenic bacteria such as Staphylococcus, Streptococcus, Mycoplasma, Pseudomonas, Corynebacterium or Bacillus can also be found **[67,68].** Among the viruses isolated from bovine semen; Infectious Bovine Rhinotracheitis (IBR), Infectious Pustular Vulvovaginitis (IPV) Irifexiosis Pustular Balanopostilis (IPB), Foot and Mouth Disease (FMD), Bluetongue (BT), Bovine Viral Diarrhea (BVD), Bovine Leukemia (BL), Ephemeral Fever (EF), and Lumpy Skin Disease (LSD) viruses can be counted **[69-76].**

It was determined that Chlamydia, Campylobacter, Chlamydophila were found in preputial swabs of goats **[77].** In a study conducted in goats, it was determined that the bacterial load increased in goats over 5 years old, the rainy season increased the number of bacteria, and Jamunapari goats had a higher bacterial load **[78].**

In a study of Australian saltwater crocodile, an unidentified Pseudomonas species Citrobacter braakii, Enterobacter agglomerans, Klebsiella ornitholytica, Klebsiella pneumonia, Proteus Aeromonas veronii bio sobria, Bacillus cereus, Chryseosepticia bacterium avium, Providenceosepticia cocciantobacterium, Providetobacterium Enterosepticia cocciantuca mirabilis, Pseudomonas aeruginosa, Salmonella arizonae were detected in penile shaft, sulcus and semen **[79].** In koalas, Corynebacterium species were found both in the foreskin and semen **[80].** On the other hand, Staphylococcus spp. and Bacillus spp. were reported to be among the main isolated micro-organisms in camel **[81].** In a study conducted in Pecari tajacu, Corynebacterium spp, Staphylococcus spp, Bacillus sp, Rhodococcus spp, Dermabacter spp, Microbcterium spp species were found in semen and foreskin **[82].**

Another work conducted in rabbits, Proteobacteria, Firmicutes, Fusobacteria and Bacteroidetes were found in the sperm microbiota. It has been determined that host genetics influence the bacterial community composition in the sperm microbiota. In addition, in their discriminant analysis, they stated that Lysinibacillus and Flavobacterium are biomarkers for fertility **[83].**

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding: The authors received no financial support for the research, authorship, and/or publication of this article.

ORCID ID: Ali Doğan ÖMÜR https://orcidorg/0000-0002-2976-4368

ORCID ID: Serkan Ali AKARSU https://orcidorg/0000-0003-4450-6540

ORCID ID: Büşra AYYILDIZ https://orcidorg/0000-0001-5116-1400

ORCID ID: Demet ÇELEBİ https://orcidorg/0000-0002-2355-0561

ORCID ID: Mehmet Akif AYDIN https://orcidorg/0000-0002-6238-018X

References:

1. Gest H. (2004) The discovery of microorganisms by Robert Hooke and Antoni Van Leeuwenhoek, fellows of the Royal Society. Notes and records of the Royal Society of London. 58(2): 187-201.

2. Mahboubi MA, Carmody LA, Foster BK, Kalikin LM, VanDevanter DR, et al. (2016) Culture-based and culture-independent bacteriologic analysis of cystic fibrosis respiratory specimens. Journal of Clinical Microbiology. 54(3): 613-619.
3. Moreno I, Codoñer FM, Vilella F, Valbuena D, Martinez-Blanch JF, et al. (2016). Evidence that the endometrial microbiota has an effect on implantation success or failure. American Journal of Obstetrics and Gynecology. 215(6): 684-703.
4. Altmäe S, Franasiak JM, Mändar R. (2019) The seminal microbiome in health and disease. Nat Rev Urol. 16: 703-721.
5. Stumpf RM, Wilson BA, Rivera A, Yildirim S, Yeoman CJ, et al. (2013) The primate vaginal microbiome: comparative context and implications for human health and disease. American Journal of Physical Anthropology. 152: 119-134.
6. Madupu R, Szpakowski S, Nelson KE. (2013) Microbiome in Human Health and Disease. Science Progress.96(2): 153-170.
7. Cox MJ, Cookson WO, Moffatt MF. (2013) Sequencing the human microbiome in health and disease. Human Molecular Genetics. 22(1). 88-94.
8. Mccormack W. (1994) Pelvic inflammatory disease. New England Journal of Medicine. 330:115-119.
9. Mändar R, Punab M, Korrovits P, Türk S, Ausmees K, et al. (2017). Seminal microbiome in men with and without prostatitis. International Journal of Urology.24(3): 211-216.
10. Diemer T, Huwe P, Ludwig M, Hauck EW, Weidner W. (2003) Urogenital infection and sperm motility. Andrologia. 35(5): 283-287.
11. Weng SL, Chiu CM, Lin FM, Huang WC, Liang C, et al. (2014). Bacterial communities in semen from men of infertile couples: metagenomic sequencing reveals relationships of seminal microbiota to semen quality. PloS one. 9(10): e110152.
12. Ochsendorf FR. (2008) Sexually transmitted infections: impact on male fertility. Andrologia. 40:72-5.
13. Keck C, Gerber-Schäfer C, Clad A, Wilhelm C, Breckwoldt M. (1998) Seminal tract infections: impact on male fertility and treatment options. Human Reproduction Update. 4(6): 891-903.
14. Blaser M, Bork P, Fraser C, Knight R, Wang J. (2013) The microbiome explored: recent insights and future challenges. Nature Reviews Microbiology. 11(3): 213-217.
15. Reichart M, Kahane I, Bartoov B. (2000) In vivo and in vitro impairment of human and ram sperm nuclear chromatin integrity by sexually transmitted Ureaplasma urealyticum infection. Biology of Reproduction. 63(4): 1041-1048.
16. Hosseinzadeh S, Brewis IA, Eley A, Pacey, AA. (2001) Co-incubation of human spermatozoa with Chlamydia trachomatis serovar E causes premature sperm death. Human Reproduction. 16(2): 293-299.
17. Baud D, Vulliemoz N, Ammerdorffer A, Gyger J, Greub G, et al. (2018) Waddlia chondrophila, a Chlamydia-related bacterium, has a negative impact on human spermatozoa. Human Reproduction 33(1): 3-10.
18. La Vignera S, Vicari E, Condorelli RA, d'Agata R, Calogero AE. (2011) Male accessory gland infection and sperm parameters. International Journal of Andrology. 34(5pt2): e330-e347.
19. Hales DB, Diemer T, Hales KH. (1999) Role of cytokines in testicular function. Endocrine. 10(3): 201-217.
20. Diemer T, Ludwig M, Huwe P, Hales DB, Weidner W. (2000) Influence of urogenital infection on sperm function. Current Opinion in Urology. 10(1): 39-44.
21. Gülyüz A, Uslu BA. (2016) Escherichia coli ile Kontamine Edilen Boğa Spermalarında Bazı Spermatolojik Parametrelerinin Değerlendirilmesi. Van Veterinary Journal. 27(2): 91-96.
22. Gimenes F, Souza RP, Bento JC, Teixeira JJ, Maria-Engler SS, et al. (2014) Male infertility: a public health issue caused by sexually transmitted pathogens. NatureReviews Urology. 11(12): 672-687.
23. Ochsendrof FR. (1988) Infection and reactive oxygen species. Andrologia. 30(1):81-6.
24. Aitken RJ. (1995) Free radicals, lipid peroxidation and sperm function. Reproduction, Fertility and Development. 7(4): 659-668.

25. Griveau J F, Dumont E, Renard P, Callegari JP, Le Lannou D. (1995) Reactive oxygen species, lipid peroxidation and enzymatic defence systems in human spermatozoa. Reproduction. 103(1): 17-26.
26. Manhart LE, Broad JM, Golden MR. (2011) Mycoplasma genitalium: should we treat and how? Clinical Infectious Diseases. 53(3): 129-142.
27. Hatipoğlu H, Altındiş M. (2016) Association of sexually transmitted diseases with male infertility Andrology Bulletin. 18(67): 269-74.
28. Selçuk M, Nizam MY, Selçuk Z. (2019) Infertility in buffaloes. The Problem of Infertility in Male Animals and Current Approaches to the Solution. 1st Edition. Ankara: Turkey Clinics. 32-39.
29. Gangadhar KS, Rao AR, Krishnaswamy S, Rao SU. (1986) Bacterıal and fungal types and theır load in the frozen-semen of buffalo bulls. Indian Veterinary Journal. 63(1): 48-53.
30. Lorusso F, Palmisano, M, Chironna M, Vacca M, Masciandaro P, et al. (2010) Impact of chronic viral diseases on semen parameters. Andrologia. 42(2): 121-126.
31. Topçu AW, Söyletir G, Doğanay M, et al. (2008) Infectious Diseases and Microbiology. 3rd Edition Istanbul: Nobel Medicine Bookstore, 1549-1580.
32. Yavru S, Öztürk, F, Şimşek A, Yapkıç O, Yıldız C. (1998) Virus Isolation from Semen of Bulls that using Artificial and Natural Insemination. Eurasian Journal of Veterinary Sciences. 14(2): 39-46.
33. Althouse GC, Lu KG. (2005) Bacteriospermia in extended porcine semen. Theriogenology. 63(2): 573-584.
34. Althouse GC, Kuster CE, Clark SG, Weisiger RM. (2000) Field investigations of bacterial contaminants and their effects on extended porcine semen. Theriogenology. 53(5): 1167-1176.
35. Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) (2002) Guidelines for the evaluation of probiotics in food. Report of a Joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food.
36. Amaretti A, Di Nunzio M, Pompei A, Raimondi S, Rossi M, Bordoni A. (2013) Antioxidant properties of potentially probiotic bacteria: in vitro and in vivo activities. Applied microbiology and biotechnology. 97(2): 809-817.
37. Reid G, Burton J. (2002) Use of Lactobacillus to prevent infection by pathogenic bacteria. Microbes and Infection. 4(3): 319-324.
38. Gärtner MA, Bondzio A, Braun N, Jung M, Einspanier R, et al. (2015) Detection and characterisation of Lactobacillus spp. in the bovine uterus and their influence on bovine endometrial epithelial cells in vitro. PLoS One. 10(3): e0119793.
39. Charteris WP, Kelly PM, Morelli L, Collins JK. (2001) Antibacterial activity associated with Lactobacillus gasseri ATCC 9857from the human female genitourinary tract. World Journal of Microbiology and Biotechnology. 17(6): 615-625.
40. Eschenbach DA, Davick PR, Williams BL, Klebanoff SJ, Young-Smith K, et al. (1989). Prevalence of hydrogen peroxide-producing Lactobacillus species in normal women and women with bacterial vaginosis. Journal of Clinical Microbiology. 27(2): 251-256.
41. Schulze M, Ammon C, Ru¨diger K, Jung M, Grobbel M. (2015). Analysis of hygienic critical control points in boar semen production. Theriogenology. 83(3): 430-437.
42. Althouse GC, Rossow K. (2011) The potential risk of infectious disease dissemination via artificial insemination in swine. Reproduction in domestic animals. 46: 64-67.
43. Maes D, Nauwynck H, Rijsselaere T, Mateusen B, Vyt P, et al. (2008) Diseases in swine transmitted by artificial insemination: an overview. Theriogenology. 70(8): 1337-1345.
44. Bussalleu E, Yeste M, Sepúlveda L, Torner E, Pinart E, et al. (2011) Effects of different concentrations of enterotoxigenic and verotoxigenic E. coli on boar sperm quality. Animal Reproduction Science. 127(3-4): 176-182.
45. Schulze M, Schäfer J, Simmet C, Jung M, Gabler C. (2018) Detection and characterization of Lactobacillus

spp. in the porcine seminal plasma and their influence on boar semen quality. PLoS One. 13(9): e0202699.

46. Linde-Forsberg C. (2005) Recent advances in small animal reproduction. Ithaca, NY: International Veterinary.
47. Bjurström L, Linde-Forsberg C. (1992) Long-term study of aerobic bacteria of the genital tract in breeding bitches. American journal of veterinary research. 53(5): 665-669.
48. Osborne CA, Lees GE. (1995) Bacterial infections of the canine and feline urinary tract. Canine and Feline Nephrology and Urology. 759-797.
49. Johnston SD. (1991) Performing a complete canine semen evaluation in a small animal hospital. Veterinary Clinics of North America: Small Animal Practice. 21(3): 545-551.
50. Goericke-Pesch S, Weiss R, Wehrend A. (2011). Bacteriological findings in different fractions of canine ejaculates showing normospermia, teratozoospermia or azoospermia. Australian Veterinary Journal. 89(8): 318-322.
51. Otter A. (2008) Bacterial isolates from the semen of rams with suspected infertility. The Veterinary Record. 162(19): 623.
52. Tvrdá E, Kačániová M, Baláži A, Vašíček J, Vozaf J, et al. (2021) The Impact of Bacteriocenoses on Sperm Vitality, Immunological and Oxidative Characteristics of Ram Ejaculates: Does the Breed Play a Role? Animals. 12(1): 54.
53. Zaid NW, Al-Zubaidy IA. (2009) The effect of natural mating on the bacterial pollution in the endogenous ram. Al-Anbar J. Vet. Sci. 2(1): 31-35.
54. Al-Kass Z, Eriksson E, Bagge E, Wallgren M, Morrell J M. (2020) Microbiota of semen from stallions in Sweden identified by MALDI-TOF. Veterinary and Animal Science. 10: 100143.
55. Schlueter H, Kuller HJ, Friedrich U, Selbitz HJ, Marwitz T, et al. (1991) Epizootiology and therapy of contagious equine metritis (CEM) with special regard to treatment of infected stallions. Praktische Tierarzt (Germany, FR). 72(6): 503-511.
56. Vohra A, Syal P, Madan A. (2016) Probiotic yeasts in livestock sector. Animal Feed Science and Technology. 219: 31-47.
57. Gale C, Brown KI. (1961) The identification of bacteria contaminating collected semen and the use of antibiotics in their control. Poultry Science. 40(1): 50-55.
58. Ngu GT, Etchu KA, Butswat ISR, Woogeng IN. (2014). Semen and microbial characteristics of two breeds of turkeys in an arid tropical environment of Bauchi State, Nigeria. African Journal of Microbiology Research 8(21): 2174-2182.
59. Boonthai T, Khaopong W, Sangsong J, Sooksawat T, Nimrat S, et al. (2016) Semen collection methods affect the bacterial composition of post-thawed semen of silver barb (Barbodes gonionotus). Animal reproduction science. 166: 90-98.
60. Jenkins JA, Tiersch TR. (1997) A preliminary bacteriological study of refrigerated channel catfish sperm. Journal of the World Aquaculture Society. 28(3): 282-288.
61. Santos M, Soares F, Moreira M, Beirão J. (2018) Evaluation of different extenders for the cold storage of meagre (Argyrosomus regius) semen. Aquaculture Research. 49(8): 2723-2731.
62. Yániz JL, Marco-Aguado MA, Mateos JA, Santolaria P. (2010) Bacterial contamination of ram semen, antibiotic sensitivities, and effects on sperm quality during storage at 15 C. Animal Reproduction Science. 122(1-2): 142-149.
63. Bresciani C, Cabassi CS, Morini G, Taddei S, Bettini R, et al. (2013). Boar semen bacterial contamination in Italy and antibiotic efficacy in a modified extender. Italian Journal of Animal Science. 13(1): 3082.
64. Abro SH, Abro R, Tunio M, Rind R, Bughio S. (2015) Evidence of bacterial contamination in the frozen bovine semen. Pakistan Journal of Agriculture, Agricultural Engineering and Veterinary Sciences. 31(1): 102-108.
65. Gaczarzewicz D, Udala J, Piasecka M, Blaszczyk B, Stankiewicz T. (2016) Bacterial contamination of boar semen and its relationship to sperm quality preserved in commercial extender containing gentamicin sulfate. Polish Journal of Veterinary Sciences, 19(3).

66. Mitra J, Chowdhury S, Panda S, Chakraborty M, Singha A. (2016) Microbiological evaluation of bovine frozen semen samples in West Bengal, India. Explor. Anim. Med. Res. 6(2): 185-191.
67. González-Marín C, Roy R, López-Fernández C, Diez B, Carabaño MJ, et al. (2011) Bacteria in bovine semen can increase sperm DNA fragmentation rates: A kinetic experimental approach. Animal Reproduction Science. 123(3-4): 139-148.
68. Wierzbowski S, Nowakowski W, Wayda E, Kuzniak S. (1984) Antibiotic level and bacterial contamination of frozen bull's semen (streptomycin, penicillin). Medycyny Weterynaryjna (Poland). 40(5): 284-287.
69. Breckon RD, Luedke AJ, Walton TE. (1980) Bluetongue virus in bovine semen: viral isolation. American Journal of Veterinary Research. 41(3): 439-442.
70. Cottral GE, Gailiunas P, Cox BF. (1968). Foot-and-mouth disease virus in semen of bulls and its transmission by artificial insemination. Archiv für die gesamte Virusforschung. 23(4): 362-377.
71. Loewen KG, Darcel CLQ. (1985) A comparison of two methods for the isolation of bovine herpesvirus 1 (BHV-1) from extended bovine semen. Theriogenology. 23(6): 935-943.
72. Parsonson IM, Thompson LH, Walton TE. (1994) Experimentally induced infection with bluetongue virus serotype 11 in cows. American Journal of Veterinary Research. 55(11): 1529-1534.
73. Paton DJ, Brockman S, Wood L. (1990) Insemination of susceptible and preimmunized cattle with bovine viral diarrhoea virus infected semen. British Veterinary Journal. 146(2): 171-174.
74. Van Engelenburg FAC, Maes RK, Van Oırschot JT, et al. (1993) Development of a rapid and sensitive poIymerase chain reaction assay for detection of bovine herpesvirus type 1 in bovine semen. J.eILMic. 31(12): 3129-3135.
75. Weldon SL, Blue JL, Wooley RE, Lukert PD. (1979) Isolation of picornavirus from feces and semen from an infertile bull. Journal of the American Veterinary Medical Association. 174(2): 168-169.
76. Wentink GH, van Oirschot JT, Pelgrim W, Wensing T, Gruys E. (1993) Experimental transmission of bovine leukosis virus by rectal palpation. Veterinary Record: Journal of the British Veterinary Association.
77. Gangwar C, Kumaresan G, Mishra AK, Kumar A, Pachoori A, et al. (2020) Molecular detection of important abortion-causing microorganisms in preputial swab of breeding bucks using PCR-based assays. Reproduction in Domestic Animals. 55(11): 1520-1525.
78. Gangwar C, Mishra AK, Gururaj K, Kumar A, Kharche SD, et al. (2021) Semen quality and total microbial load: An association study in important Indian Goat breeds during different seasons. Andrologia. 53(4): e13995.
79. Johnston SD, Lever J, McLeod R, Oishi M, Qualischefski E, et al. (2014) Semen collection and seminal characteristics of the Australian saltwater crocodile (Crocodylus porosus). Aquaculture. 422: 25-35.
80. Johnston SD, OBoyle D, Frost AJ, McGowan MR, Tribe A, et al. (1998) Antibiotics for the preservation of koala (Phascolarctos cinereus) semen. Australian Veterinary Journal. 76(5): 335-338.
81. Ghoneim IM, Waheed MM, Al-Hofofi AN, Fayez MM, Al-Eknah MM, et al. (2014). Evaluation of the microbial quality of fresh ejaculates of camel (Camelus dromedarius) semen. Animal Reproduction Science. 149(3-4): 218223.
82. Santos CS, Silva AM, Maia KM, Rodrigues GSO, Feijó FMC, et al. (2020) Composition of semen and foreskin mucosa aerobic microbiota and its impact on sperm parameters of captive collared peccaries (Pecari tajacu). Journal of Applied Microbiology. 129(3): 521-531.
83. Marco-Jiménez F, Borrás S, Garcia-Dominguez X, D'Auria G, Vicente JS, et al. (2020) Roles of host genetics and sperm microbiota in reproductive success in healthy rabbit. Theriogenology. 158: 416-423.