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Artificial Intelligence in Gamete Cell Selection and Microbiologic Analysis

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Abstract

Infertility is one of the most common problems worldwide. Half of the causes of infertility are due to women and half to men. For this reason, studies to overcome this problem are concentrated on the way of ensuring the continuation of the generation by using high technology. For this purpose, applications such as (in vitro fertilization) IVF and (intra cytoplasmic sperm injection) ICSI are widely applied. This process is done by experts and produces non-objective results. As a result, artificial intelligence (AI) technology has begun to be used. Devices such as computer-assisted sperm analysis (CASA), flow cytometry provide objective evaluation in the evaluation of semen. Artificial intelligence has also been used for oocyte and embryo. Similarly, artificial intelligence is used in microbiological analysis. There is a relationship between the presence of microbiota in semen and sperm quality. Thanks to artificial intelligence, rapid and reliable microbiological analysis, diagnosis, and antimicrobial resistance are measured. In this way, microbiological analyzes in semen are also measured with artificial intelligence. Therefore, it is one of the most important goals that the analyzes made in these processes give objective and reliable results and do not harm the cell to be used. This review also highlights the current computer-based software systems used in sperm, oocyte and embryo evaluation.

Keywords: Sperm; oocyte; embryo evaluation; artificial intelligence; CASA

Abbreviations: IVF: İn Vitro Fertilization, ICSI: İntra Cytoplasmic Sperm İnjection, AI: Artificial İntelligence, CASA: Computer-Assisted Sperm Analysis, ANA: Antinuclear Antibody, ML: Machine Learning, MALDI-TOF: Matrix-Assisted Laser Desorption/İonization-Time-of-Flight, VCL: Curvilinear Velocity, VAP: Average Path Velocity, VSL: Straight Line Velocity, ALH: Lateral Head Displacement, LIN: Linearity of The Curvilinear Path, STR: Straightness of The Average Path, BCF: Beat- Cross Frequency, Indo-1 AM: Indo-1 Acetoxymethylester, IBFC: İmage-Based Flow Cytometry, HSV: High-Security Pipettes, PDMS: Polydimethylsiloxane, QS: Quorum Sensing

Introduction

The use of artificial intelligence (AI) is increasing in our daily life and in the laboratory **[1]**. It can be used in application of antinuclear antibody (ANA) patterns and performing white blood cell differentials and performing white blood cell differentials **[2]**. A subset of AI algorithms is called "machine learning" (ML) **[3]**. It is used in the clinical microbiology laboratory of artificial intelligence, where the human mind cannot analyze all the variables at the same time. Such a wealth of visual information among, for example, data (i.e., images of microscopic slides and plate bacteria), matrix assisted laser desorption/ionization-time-of-flight (MALDITOF) mass spectra, and nucleic acid sequence data; without AI, analyzing such data is manual, tedious and requires experience **[4]**.

Infertility creates huge economic losses in the animal industry. 50% of this is associated with male infertility **[5]**. There are many artificial intelligence-based studies to contribute to infertility treatment such as live birth rate estimation **[6,7]**, semen analysis **[8]**, embryo selection **[9]**, uterus analysis **[10]**, embryo-based treatment outcome estimation **[11]**.

In embryo selection and semen analysis, which embryo will be transferred and which fertilization method will be preferred depends on this subject and the personnel who are experts in the field. The training of the personnel is also very important in terms of decision making at this stage. One of the biggest challenges in the subjective assessments of embryos is the high intra- and inter-operator variability in the assessment of morphology and morphokinetics **[12-14]**. In this context, embryos can be monitored continuously and the entire embryo development process can be evaluated more precisely with technologies accelerated by artificial intelligence studies **[15]**. Semen analysis (sperm count, motility, viability and other morphological analyzes) are the most common method used in the diagnosis of male infertility **[16]**. Traditionally, semen motility is assessed by a specialist who visually scores points through a microscope. This practice leads to subjective interpretations among experts **[17]**.

Computer aided sperm analysis (CASA) systems are used for many types of sperm analysis. Subjective motility estimation methods have been replaced by CASA **[18,19]**. The movement of each sperm is recorded as changes in center position in successive frames, and the calculations provide output measurements that describe movement. With CASA software, motility, kinematics and velocity parameters of semen in many species such as bovine **[20]**, horse **[21]**, cat **[22]**, dog **[23]**, ram **[24]**, human **[25]**, rabbit **[26]**, fish **[27]**, rat **[28]**, can be analyzed. In this analysis, semen is used quickly in fresh and frozen form, while a very small amount of total semen is used for analysis, while the remaining parts can be used for fertilization. Curvilinear velocity (VCL), average path velocity (VAP), straight line velocity (VSL), amplitude of lateral head displacement (ALH), linearity of the curvilinear path (LIN), straightness of the average path (STR), and beat- Cross frequency (BCF) are the parameters measured by CASA **[29]**.

Flow cytometry is one of the image-based methods in which morphological analyzes can be made in sperm. Since the end of the 1970s, the method mentioned with sperm analysis was published by Van Dilla et al **[30]**. Dead-viable sperm ratio is among the parameters determined by flow cytometry. SYBR-14 and PI are among the commonly used dyes **[31,32]**. Indo-1 acetoxymethylester (Indo-1 AM) has been used for measuring intracellular Ca^{2+} in spermatozoa by flow cytometry **[33]**. Mitochondrial membrane potential and rate of acrosomal damage can be determined by flow cytometry. JC-1 is widely used for mitochondrial membrane potential and PNA/PI is widely used for acrosomal damage **[34]**. The most popular method of sex determination and commercial use is Beltsville Sperm Sex Determination Technology **[35]**. Flow cytometry is the method used to determine sexed semen according to DNA weight of spermatozoa. The flow cytometer measures the DNA content based on the DNA-binding fluorescent probe Hoechst 33342 **[36]**. There is image-based flow cytometry (IBFC) that captures images of each cell at high speeds (500 to 2000 sperm/second) **[37]**. This technology IBFC in animal andrology may eventually lead to the development of new approaches to semen analysis **[36]**. Images from the IBFC are based only on bright field data, without the need for fluorescence detection, distinguishing motile and non-motile sperm in an ejaculate **[39]**.

There are many methods for the cryopreservation of human and animal sperm. In recent years, various technologies have been added to the sperm freezing process **[40]**. Slow freezing, fast freezing and ultra fast freezing are methods used in sperm freezing. Slow freezing is a method in which sperm cells are gradually cooled over a 2–4-hour period using a programmable machine **[41]**. In the fast freezing technique, a cryoprotectant is added to the spermatozoa and the suspension is drawn into a cryo-straw or cryovial and exposed to a liquid nitrogen vapor phase. There are methods in which fewer spermatozoa are used in the sperm freezing process. Empty zona pellucida **[42]**. Cryo-loops, microdroplets, cut pipettes, mini pipettes, open-drawn pipettes, alginate beads, agarose gel microspheres, cryotope, plastic capillaries, and high-security pipettes (HSV) **[43,44]**. Another ultrafast cooling approach to cryopreservation of sperm without cryoprotectant uses polydimethylsiloxane (PDMS) chips in microfluidics **[45]**. In the cryopreservation process for spermatozoa, it is necessary to use a controlled cooling system in which temperature ranges are determined **[46]**. In general, semen cryopreservation uses protocols of freezing curves ranging from 10 to 100 °C min-1. However, studies are underway to optimize cryopreservation protocols **[47]**. Cryopreservation of semen in an automatic freezing machine was first used by Almquist & Wiggins **[48]**. Freezing sperm in straws is an expensive, but accurate, effective method. After the semen samples are reconstituted and cooled, the sperm are drawn into 0.25 or 0.5 ml straws, placed on a rack and frozen in liquid nitrogen vapor with a styrofoam box or programmable device **[49]**. The advantage of the programmable freezer is the customization of the freezing curve **[50]**. In a study in horses, the programmable freezer provided a more consistent and reliable freezing rate than liquid nitrogen vapor. It has been noted that since the level of liquid nitrogen in the can is subjectively estimated and subject to evaporation and it is difficult to standardize it for each freezing process, it may have provided a more variable freezing rate than the programmable freezer **[51]**.

Embryo selection in In vitro fertilization (IVF) is one of the important steps for determining the quality of fertilized oocytes, and for subsequent transfer or cryopreservation **[52]**. In recent years, innovation and research in the field of artificial intelligence (AI) has greatly influenced in vitro fertilization (IVF) procedures **[53]**. Automated embryo selection using machine learning or computer vision based on embryo images is a new area of research **[54,55]**. Traditionally, the main purpose of embryo evaluation was to sort the embryos according to their implantation rate **[56]**. Experts select oocytes/embryos by simple observational examination focusing on the morphology of their development. The examination is often subjective and varies between experts **[57]**. In order to reduce the subjectivity of these observations, methods such as accelerated monitoring systems of embryo development **[58]**, the use of morphokinetics **[59]** have been tried.

Studies have shown that there is a relationship between inflammation in the male reproductive system and infertility **[60]**. Microorganisms have negative effects on semen quality **[61]**. Speed in microbiological diagnosis is among the most important problems in reducing the development of antimicrobial resistance. When microorganisms settle in the organs and tissues they have affinity for, they reproduce rapidly and increase the number of colonies. In this way, as the mass of microbes increases, the response of the immune system becomes long and difficult. In addition, the increased mass protects itself from antimicrobials thanks to virulence factors such as quorum sensing (QS) biofilm. In addition, mutant strains develop after gene exchange with each other in this colonization, making a big difference in competition with treatment protocols. Although artificial intelligence uses complex algorithms, AI is basically the way a computer is used. Artificial intelligence has the potential to make clinical microbiology applications more efficient, more accurate **[62]**. Thanks to artificial intelligence, rapid microbiological diagnosis and antimicrobial resistance monitoring, analysis and control of influencing virulence factors will make great contributions to humanity today and in the future **[63,64]**. Microbiological analyzes will become easier with genomic analyses, digital storage and imaging, slide scanning, bacteriological library. In the identification processes made with conventional methods, it is also necessary to distinguish between the colony morphologies of microorganisms, potentially disease-causing pathogens and microorganisms belonging to the flora. Artificial intelligence (AI) is becoming

indispensable in clinical fields. Examples of these applications range from image-based applications to deep learning algorithms and in silico clinical trials **[65]**.

Artificial insemination in livestock has been practiced for decades. In this way, the development of animal breeding, especially in cattle and pigs, has accelerated. The use of artificial intelligence in obtaining the best spermatozoa, sperm sex determination technologies and long-term storage methods of semen is increasing **[66]**. On the other hand, Artificial intelligence applications have started to be used in clinical microbiology laboratories to provide some convenience in all these situations **[67]**. In this way, by using genome analysis-based algorithms that determine the resistance genes of resistant microorganisms, increasing antimicrobial resistance and the economic loss of multidrug microorganisms, which are called red alert pathogens in the world, will be prevented in the health sector. In addition, it will be of great hope that it will contribute to the decrease in the mortality rate. However, after obtaining microbiome/proteome analyzes at the human/environment and animal/environmental level, with artificial intelligence applications, significant contributions can be made to the health of new generations in accordance with ethics **[68]**.

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