

Semen Analysis and Sperm Function Tests as Diagnostic Tools for Male Animals' Infertility

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Abbreviations: AI: Artificial Insemination; BCF: Beat Cross Frequency; PI: Propidiumiodide; ZBA: Zona Binding Assay; DFI: DNA Fragmentation Index; HZA: Hemizona Assay; SCSA: Sperm Chromatin Structure Assay; AOT: Acridine Orange Test; SCD: Sperm Chromatin Dispersion Test; ROS: Reactive Oxygen Species; VSL: Straight Line Velocity; LIN: linearity; VCL: Curvilinear Velocity; CTC: Fluorescent Chlortetracycline

Introduction

Artificial insemination (AI) is a reproductive management tool that assisted a lot the increase of livestock productivity. Therefore, the impact of the male in the genetic progress, as well as, in the reproductive efficiency of the herds is more crucial. In commercial farms, a routine examination of semen is performed aiming to predict the male's fertility. Taking into consideration the aforementioned, it becomes more important to identify the sub-fertile or infertile male animals prior to entering the breeding herd and during their reproductive activity. It's well known that the evaluation of classical seminal parameters under commercial conditions, allows the identification of ejaculates with poor fertility potential, but does not have high predictable efficiency of field fertility. For this reason semen laboratory analysis has been developed and specified, including more and more examined parameters. Furthermore, a combination of modern tests, which can evaluate different steps of fertility process, is usually proposed to achieve reliable results about male's fertilizing ability. The current state of the field includes a variety of diagnostic tools, which can be classified as following:

Computer-assisted semen analysis (CASA)

Spermatozoa must run across the female genital tract to approach the oviduct and fertile the oocytes. Thus, motility is a sperm characteristic of high predictable value for the fertilizing capacity of the male. However, routine-manual semen analysis lacks the ability to measure the kinematics of spermatozoa. It is also subjective, depending on the experience of the person who performs the analysis. CASA semen analysis provided a solution for this problem, because motility parameters can be measured in an objective manner, enhancing the accuracy of semen motility assessment. CASA is also beneficial because of its capacity to analyze individual motion characteristics of spermatozoa, some of which have been shown to be related to fertility outcome [1]. Specifically, straight line velocity (VSL) and linearity (LIN) are associated with larger litter sizes in pigs [2], while curvilinear velocity (VCL) and average path velocity (VAP) are kinematic parameters with significant positive correlation with the ability to migrate in sheep cervical mucus [3]. Concerning bull, Kathiravan et al. [4] reported that the parameters VCL, VSL and VAP can be used to predict *in vivo* fertility, while according to Oliveira et al. [5] beat cross frequency (BCF), was also found to be predictor of bovine field fertility.

Sperm Viability - Membrane Integrity - Specific Sperm Function Tests

Sperm plasma membrane integrity is required to keep spermatozoa live and support their normal function. Recent advances in staining technology provide new means for assessing the functionality of spermatozoa. Different fluoresce dyes have been included in sperm viability evaluation instead of eosin-nigrosin traditional double stain. Usually SYBR-14, in combination with

propidiumiodide (PI), is used to estimate live/dead spermatozoa rate. SYBR-14, as well as, calcein AM and 6-carboxy fluoresce in diacetate (CFDA), stain live cells, while PI enters to the damaged cells, binds and stains the nuclear DNA, indicating dead cells. In modern spermatology more fluorescent dyes are used to collect information for specific functions of spermatozoa. Fluorescent chlortetracycline (CTC) and fluorescent lectin - conjugated agglutinin derived from *Pisum sativum* (FITC-PSA) are used to identify the capacitation and acrosomal status of spermatozoa. Moreover, mitochondrial membrane potential can be measured by JC-1 and Rhodamine-123 dyes.

Sperm-oocyte interaction in vitro tests (zona pellucida binding test, hemizona assay)

The evaluation of gametes' interaction is very crucial for the diagnosis of subfertility, because it reflects multiple sperm functions that are required for the fertilization process. Sperm-oocyte interaction *in vitro* test sperm it different endpoints in the early embryonic development, to be monitored. The currently most widely performed sperm-zona pellucida binding tests are zona binding assay (ZBA) and hemizona assay (HZA). Different *in vitro* protocols with heterologous or homologous, hemizona or intact zona binding assay have been performed (Clulow et al. 2010; Zhang et al. 1998) [6,7]. ZBA has been developed as diagnostic test for several species. [7] reported a significant correlation between ZBA with bull frozen-thawed semen and non-return to estrus rate. Positive results have been also found in buffalos, where the number of bounded spermatozoa to the zona pellucida had significant correlation with sperm plasma membrane integrity and mitochondrial membrane potential [8]. Moreover, HZA has been demonstrated as a method to estimate boar semen fertilizing ability, after its exposure to toxic factors.

Sperm chromatin integrity - sperm DNA damage

Sperm chromatin integrity is an independent quality parameter that may have a better diagnostic value than the standard semen analysis [9]. It has been confirmed that sperm DNA damage-fragmentation is strongly associated with impaired fertilization, slow early embryonic development, early embryonic death and sub-fertility. Although, it appears that there is a threshold of sperm DNA damage, which can be repaired by oocyte Derijck et al. [10], Evenson & Wixon [11] quantified that a DNA fragmentation index (DFI) in the range of about 8% and 10–20% is related with sub fertility in boars and

bulls, respectively. Additionally, Rybar et al. [12] found that bulls with a 43.3% pregnancy rate had significantly higher DFI than those with a 60.0% pregnancy rate. Depending on the required laboratory equipment, a variety of tests have been performed to evaluate sperm DNA fragmentation, such as acridine orange test (AOT), TUNEL assay, comet test, sperm chromatin structure assay (SCSA) and sperm chromatin dispersion test (SCD).

Assessment of Reactive Oxygen Species (ROS)

ROS are formed as a by-product of oxygen metabolism. The production of ROS is a normal physiological process, essential for the adequate spermatozoa's functionality [13]. ROS are involved in sperm capacitation, acrosome reaction, maintenance of fertilizing ability and embryo development at late stages [14]. However, an imbalance between ROS generation and scavenging activity is harmful for spermatozoa and it is associated with male sub- or infertility [15]. The excessively ROS production is mainly attributed to leukocytes and immature spermatozoa, induces damage of sperm DNA and has a clinical importance [16]. Moreover, the membranes of the mammalian spermatozoa are rich in polyunsaturated fatty acids (PUFAs), so they are sensitive to extended ROS effect and lipid per oxidation. In swine, ROS degrade boar sperm quality, because they may stimulate the acrosome reaction [17]. Taking into consideration the above mentioned the modern spermatology measures ROS levels as an additional marker of male infertility [18].

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