A Review on Animals Semen Characteristics: Fertility, reproduction and development

Farzad Moradpour[1]

1- Department of Animal Science, Faculty of Agriculture, Garmian University, Kalar, Al-Sulaimaniyah, KRG, Iraq

Corresponding author e-mail: Farzad-moradpour@gmail.com

ABSTRACT

In this research, the goal of review was summarizing the current knowledge of the methods available to assess in vitro quality of frozen-thawed bovine spermatozoa also, a review on animal's semen characteristics: fertility, reproduction and development after AI with that semen. Artificial insemination (AI) is the first generation reproductive biotechnology that has made a deep contribution to the genetics improvement in several animals. A fertile ejaculate must meet certain semen characteristics quality standards, such as: normal morphology, active energy metabolism, progressive motility, structural integrity and functionality of the membrane, penetration capacity and optimum transfer of genetic material. The percentage of total motile spermatozoa in normal canine ejaculates is between 70 to 90%. By the way, there are a lot of parameters that able to change on the composition and structure of various sperm plasma member domains, such as change temperature and sensitive to any theirs environments in vivo and vitro (tropical climates), season also nutrition. Computer-assisted semen analysis (CASA) is primarily used to obtain accurate and objective kinetic sperm measurements that gives extensive information about the kinetic property of the ejaculate based on measurements of the individual sperm cells.

Keyword: Fertility, Semen technical, Semen, Reproduction

I. INTRODUCTION

Artificial insemination (AI) is the first generation reproductive biotechnology that has made a deep contribution to the genetics improvement in several animals. This technology would not have been possible without successful freezing of bull semen. A fertile ejaculate must meet certain semen characteristics quality standards, such as: normal morphology, active energy metabolism, progressive motility, structural integrity and functionality of the membrane, penetration capacity and optimum transfer of genetic material[1] in other word, Semen characteristics are usually used as indicators of reproductive soundness of study.

By the way, there are a lot of parameters that able to change on the composition and structure of various sperm plasma member domains, such as change temperature and sensitive to any theirs environments in vivo and vitro (tropical climates), season also nutrition[2] Age, breed and season of semen collection are reported as some of the major factors affecting semen quality[3].

As it has been demonstrated the cryopreservation makes damage on sperm membranes, cytoskeleton, motile apparatus and nucleus, alter cell metabolism As a about result of fertility from the AI with frozen thawed semen is poorer than that obtained with fresh semen, which can be partially compensated by inseminating greater numbers of live spermatozoa.

Besides, one of the major issues on breeding in farm animals is fertility and reproduction, and approximately 30% of the problems are related to the males[4].

In this research, the goal of review was summarizing the current knowledge of the methods available to assess in vitro quality of frozen-thawed bovine spermatozoa also, a review on animal's semen characteristics: fertility, reproduction and development after AI with that semen.

II. EVALUATION OF SEMEN

A. Semen quality

The ultimate purpose of semen evaluation is to find one or a few parameters to predict the fertilizing ability of the animal's semen. Many different methods have been evaluated throughout the last decades, but only few methods have been adopted for practical work.

Recently, the studies have used light microscopic evaluations of classical sperm parameters, including sperm motivation, concentration, potentially fertile spermatozoa, sensitive and intolerance to hear stress, morphology and viability [5].

Also, Sperm morphology, motility, sperm concentration and volume per ejaculate are common criteria for evaluating semen quality at most AI stations.

Major factors that affect semen quality are also those related to the underdevelopment and complete purity of the testis. As well as, all factors that related to testicular degeneration, including hereditary and pathological conditions should be carefully considered as they may seriously affect semen quality via testicular development [6].

In last years, the reported by Hoogenboezem & Swanepoel (2000) that testicular degeneration might be due to exposure to heat stress, nutritional deficiencies and management-related factors such as fat deposition in the scrotum and poor body condition [3, 7].

Mathevon et al. (1998) reported that season significantly affected semen morphological characteristics in young bulls but did not significantly affect ejaculate volume and sperm motility in mature bulls[8].

B. - Motivation

Motility of semen in animal farm is a manifestation of structural and functional competence of spermatozoa; therefore, the percentage of by degree motile spermatozoa is usually positively correlated with that of plasma membrane integrity and normal morphology. By the way, after semen collection the spermatozoa motility should be evaluated immediately [9].

The percentage of total motile spermatozoa in normal canine ejaculates is between 70 to 90% [10]. In order to motility of semen studies show been that, quality of motility also may be assessed; a canine spermatozoon with normal motility should traverse the microscopic field of view in 2–3 sec [11]. Morrow (1980) defined the relationship between spermatozoa morphology and motivation and the reproduction potential and indicated that when more than 30% of the ejaculated spermatozoa have structural defects, reduced fertility may occur in domestic animals [12].

C. - Hyperactivated motility

Capacitated mammalian spermatozoa exhibit vigorous, non- progressive, non- linear motion called ‘hyperactivation’ shortly before or about the time of acrosome reaction as they progress through the female oviduct.

D. - Colour

In animal farm, a clear semen sample contains no spermatozoa. Cloudy or milky samples probably contain spermatozoa but always should be checked microscopically for confirmation, Yellow colour can indicate the urine contamination; Green colour denotes presence of pus, Red or brown colour indicates fresh or hemolysis blood in semen. The most common reasons for blood in the semen include prostatic disease or damage to blood vessels on the penis. Presence of blood in the semen has no effect on motility of spermatozoa until six hours of contact [13].

E. - Volume

The volume of semen is not an indicator of semen quality in animal farms. However, the volume should be measured as the part of the calculation of total number of spermatozoa in the sample. The volume of the first and third fraction especially the latter are variable and the volume is controlled by the person collecting the sample [11].

F. - Morphology

Head, tail and acrosomal defects may be better evaluated whit stained smears and reported that, abnormality is measured via counting about 100 to 200 spermatozoa in a stained semen slide under 100× (oil immersion). Normal semen samples should have <10% primary abnormalities and <20% secondary abnormalities. Total abnormalities should be <10-20% [14, 15]. Bull spermatozoa have an overall length of about 68-74/lm; the head is about 8-10 /lm, the neck, which connects the base of the head to the mid-piece and contains the proximal centriole, is about 0.3-1.5 /lm long. The mid-piece is 8-10 /lm and the tail is about 45-50 /lm long [16].

G. - PH

In several animals' seminal plasma has an average pH ranging from 7.0 to 7.4. Threlfall (2003) recommended that evaluation of pH be performed immediately after collection using accurate equipment (presumably a pH meter) and strongly discouraged use of a ''dipstick'' method [11].

H. - Concentration

Concentration of sperm has little value as an indicator of semen quality as well as, the concentration is inversely related to volume of semen collected. In last year's, the studies have showed been that, total number of spermatozoa is dependent on testicular size and it may has relationship with frequent semen collection[17].

The traditional technique for assessing spermatozoa concentration was done with the use of haemocytometer. The hemacytometer technique has been reported to be equally accurate or more accurate than CASA systems and it's considered the gold standard [17].

The concentration of sperm per ejaculate for bulls, rams and stallions was also reported by Bearden & Fuquay (1997) as being 1.2 billion/ml, 2.0 billion/ml and 150billion/ml respectively.

I. -Semen evaluation techniques

Most of the methods used so far focus on sperm viability and DNA integrity also, Semen Quality and gross Morphology estimated via light microscopy (Fig 1).

Computer assisted semen analysis (CASA, The CASA system commonly consists of a microscope attached to a video camera, a video frame grabber card and a computer) is objective method that gives extensive information about the kinetic property of the ejaculate based on measurements of the individual sperm cells(Tab 1)[18, 19].

The commonly reported CASA parameters include total motility, progressive motility, track speed (V CL), path velocity (V AP), progressive velocity (VSL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), straightness (STR) and linearity (LIN).

J. -Sperm morphology

The evaluation of sperm concentration and morphology is based on the direct relation between the incidence of abnormal spermatozoa and the type of certain morphological defects with the in vivo fertility of the bull [20].

Surely, there is a correlation between motility and fertility, also for morphology and fertility, as well as, there is a wide range of variation in the quality of the parameters assessed as well as fertility obtained with that semen. However, these correlations are reduced concomitantly with an increase in the lower limit set to accept an ejaculate for further processing.

Recently, reported that, computerized methods focus on evaluating the measurements that are able to quantify and classify sperm morphology correctly and offers repeatable and objective method of assessing bull sperm head morphometric within and between technicians.

It has been observed, however, that certain parameters regarding texture of the digitized image of the sperm head correlates with in vivo bulls' fertility, expressed as non-return ranks [21].

The relevance of the sperm morphology evaluation relates to its indicative role for presence of eventual deviations of the processes of spermatogenesis and of sperm maturation in the epididymis and the presence of pathologies in the accessory sexual glands or, even of procedures during semen collection and handling of semen thereof. The presence of a large number of sperm abnormalities in the semen is not only indicative of pathological genital processes [21, 22] , it is also associated with decreased fertility of the semen [23].

The results of sperm morphology evaluation are thus enough, if correctly interpreted, to allow for the culling of males intended for breeding and particularly if their semen is to be used for assisted reproductive technologies [24]

On the other hand, use of semen morphology as a measurement of semen fertility (or better, sire fertility) has been discussed for its value when semen within normal values is assessed (for a review, see [21]

K. -Sperm chromatin structure

Abnormal chromatin structure may lead to problems in packaging of sperm nuclear material possibly related to morphologically abnormal spermatozoa. As well as, morphological shapes of spermatozoa determined by visual examination of the sperm in light microscopy and visually classifying the cells as either normal or abnormal were low or inconsistent with abnormal sperm chromatin structure [23].

Abnormal sperm chromatin structure may be assessed by Sperm Chromatin Structure Assay (SCSA), which defines abnormal chromatin structure as susceptibility of DNA to denaturation in situ such defects may or may not be reflected in deviations of sperm head shape, albeit some are considered related, including pear-shaped sperm heads [25]. In the SCSA, whole spermatozoa, or sonication-released nuclei are either heated or treated with HCl to denature DNA in situ and then stained with the metachromatic dye acridine orange (AO) [23, 26]. Compared to visual estimation of sperm morphological abnormalities, SCSA is a very sensitive test.

Karabinus et al [27] showed that chromatin structural changes in spermatozoa after scrotal insulation of Holstein bulls could be detected 3 days after heat stress, whereas light microscopic observations did not detect abnormalities until 11 days.

The design of automated, computer-assisted sperm head morphometry analysis (ASMA) instruments [28] can surpass this problem and further analyze sperm head dimensions objectively. Most relevant, there is a relation-ship between sperm morphometry (bull semen) and the normal structure of the chromatin (the latter in direct relation to fertility) [26].

L. - Vital staining of spermatozoa

One of damaged in sperm in the several animal, spermatozoa are not able to reseal the compromised plasmalemma. Therefore, cannot maintain those ion and co-factor concentrations essential to sperm Survival. The development of staining technology using fluorophores (used for determining sperm membrane integrity) for nucleic acid, intra cytoplasmic enzymes has provided with new tools for assessing the functionality of frozen-thawed spermatozoa.

The has been reported that, the most commonly used classic nucleic acid stains are bisbenzimidazoles Hoechst 33258 (H258) and Hoechst 33342 (H342) and phenanthridines, such as ethidium bromide (EtBr), propidium iodide (PI) and ethidium homodimer (EthD-1). In additional, whereas promising results have been obtained in other studies

In species largely selected for sperm quality for AI (dairy cattle, pigs), a frame of “normality” is described when the frequency of abnormal sperm heads does not surpass 10% and when none of the other parameters (acrosomes, mid-piece, tails, proximal cytoplasmic droplets) do not surpass 5% each or totals 10% to 15%[29].

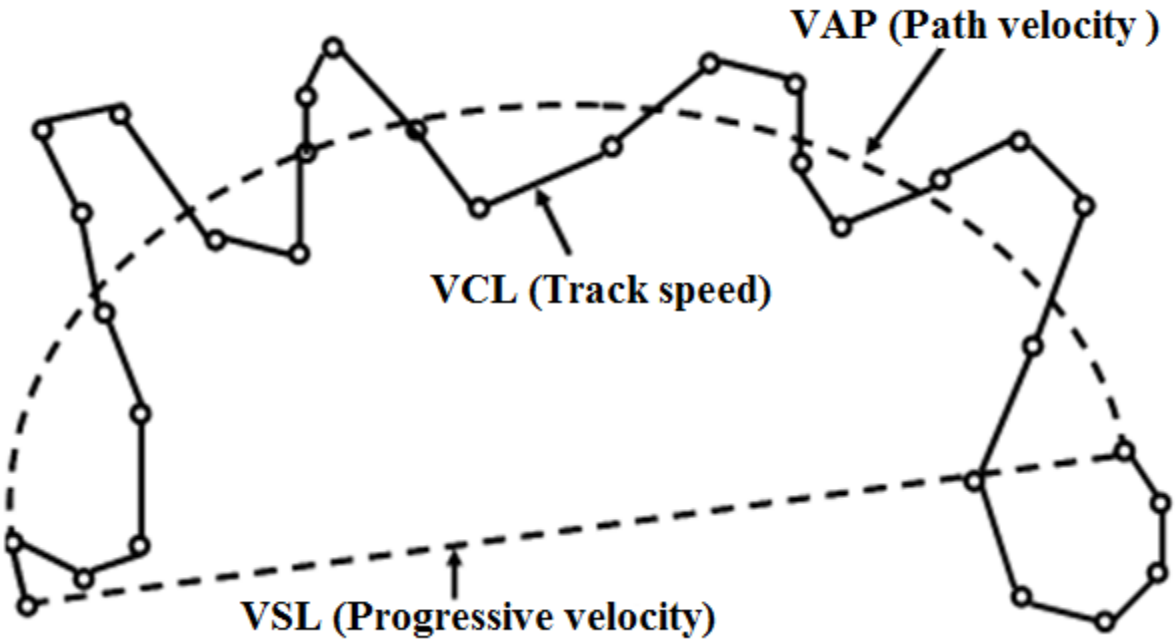

Fig. 1. Sperm motion track showing different motion characteristics assessed by CASA.

Table 1.Different settings of computer-assisted semen analyser for bull sperm analysis (Taylor et al., 2018)

Parameter\ rmdiurm used	TEYG	SCC	TALP	CUE	TALP	TEYG	Phy Saline
Frames acquired	-	-	30	30	30	30	30
Frame rate (Hz)	7	6	60	60	30	60	60
Minimum contrast	15	15	30	20	8	60	20
Minimum cell size(pixeis)	-	-	4	5	8	5	10
Threshold straightness%	-	-	80	80	60	64	70
Low VAP cut-off	-	-	25	75	20	15	30
Medium VAP cut- off	-	-	75	35	80	80	-
Head size, Non- motile (pixels)	-	-	5	7	13	7	5
Head Intensity, Non motile	-	-	55	30	21	80	20
Static head size (pixels)	0.6- 3	0.6- 3	0.7- 2.40	0.85- 2.40	0.40- 1.6	0.70- 1.95	-
Static head intensity	0.5- 3.8	0.5- 3.8	0.3- 2	0.40- 1.80	0.30- 1.6	0.41- 1.44	0.50- 3
Static elongation (pixels)	-	-	0- 65	0-60	-	16-84	0-85
Temperature	-	-	37	37	37	37	37

TEYG, tris- egg yolk- glycerol; SCC, sodium citrate-casein; TALP, Tyrodes albumin- lactate- pyruvate; CUE, Cornell University extender; Phy saline, physiological saline, VAP, average path velocity.

However, the presence of specific effects in spermatogenesis (generally hereditary) that result in typical morphological abnormalities (such as nuclear vacuoles [diadem defect], acrosomal plicae [knobbed defect], decapitated spermatozoa, short or mutilated tails [tail stump], coiled tails [Dag defect], or corkscrewed midpieces [corkscrew defect]); are very serious defects, because they interfere with fertilization and can lead to sterility and are of ten classified as major defects[30].

M. Sperm culture

Semen is not sterile. A wide variety of normal flora is present in semen. Before collection, clean the prepuce and tip of penis with sterile saline wet gauze. Culture of the distal urethra to compare flora may also be useful [31]. Greater than 10,000 CFU of aerobic bacteria per mL of semen indicates infection. Mycoplasma is a normal finding in the distal canine reproductive tract. However, it can cause the problems if present in excess amounts [32].

N. Analysis of multiple sperm attributes

It has been shown that combination of several post-thaw sperm quality attributes, as compared to any single sperm quality trait, can explain more variation in fertility between the bulls [4]. For this reason, in order to increase the predictive power of the test, simultaneous analysis of multiple sperm Attributes or outcomes of several laboratory assessments can be combined statistically to look for the overall effect of several Independent sperm parameters [1, 2].

III. LINKING GENETIC VARIANTS WITH SPERM PROTEIN PHENOTYPES MEASURABLE IN SEMEN

A deleterious non-synonymous substitution mutation in a gene controlling spermatogenesis and sperm function could render the carrier sire and some of its male on spring subfertile via a change in the quantity and function of the sperm protein encoded by this gene. By sequencing the genomes of subfertile bulls with sperm phenotypes characterized by cell imaging and proteomic analysis by using high throughput multiplex flow cytometry candidate variants can be identified that underlie abnormal phenotypes [33].

Recently, the reported that sperm biomarkers can be directly linked to sperm phenotypes using IBFC of spermatozoa labeled with fluorescently conjugated antibodies against target biomarkers [34].

In additional, Illustrating this approach, IBFC revealed the absence of fertilization-associated WBP2NL (syn. PAWP) protein from the spermatozoa of bulls with grossly malformed sperm heads and revealed a correlation with sperm-defect associated proteins such as ubiquitin, and with abnormal patterns of sperm labeling with lectins PNA and LCA [35].

In addition to using genome-based biomarker discovery approaches, nanopurification or other methods (sperm swim-up, gradient centrifugation, magnetic-activated cell sorting) can be used to enrich samples for defective spermatozoa, and proteomic analyses (mass spectrometry) can be used to identify proteins that are enriched in the defective sperm fractions. For example, seminal plasma-derived binder of sperm protein BSP5 [35, 36]was found to be enriched in the defective sperm fraction after nanopurification [33].

IV. CONCLUSIONS AND PERSPECTIVES

In the husbandry industry, although many efforts have been made, so far there are no currently available methods, or sperm assessment techniques that could accurately predict sperm fertilizing potential. Recently, Artificial insemination (AI) has proven to be the most effective tool in order to genetic improvement of animals, especially in the cattle, sheep and goat industry. Knowledge of reproduction, health and fertility in livestock's are an objective of great importance for the production of semen, which is achieved by good analysis and association of the semen. Result in major savings for AI enterprises, for the special bulls, sheep and other animal with lowly fertility could be selected and culled prior entering the fertility and progeny testing program.

Most promising tests available are sperm chromatin structure assay (SCSA) and sperm viability assessed by flow cytometry, although the present review and result of studies have shown that today we still do not have single in vitro sperm quality assessment method that can accurately predict sperm fertilizing potential. CASA has been demonstrated to be a useful tool to objectively analyses the sperm head movement and effect of different treatment son sperm kinematics. The ability of CASA to classify different sperm sub-populations provides an opportunity to establish criteria for different functional aspects of spermatozoa related to fertility, such as ability to penetrate cervical mucus, to undergo capacitation and acrosome reaction, penetration of cumulus, zona-binding. However, there has been a lack of uniformity among users and in defining universally accepted values for nor mal and abnormal sperm motion.

In order to enhance the predictive power of the test, assessment of several sperm attributes must be combined in a simultaneous analysis, of several sperm quality measurements and several laboratory assessments must be combined statistically to look for the overall effect of several independent sperm parameters. The high incidence of conception failure is due to poor breeding management and mistimed breeding.

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