Understanding motility dynamics of crossbred bull spermatozoa when analyzed by Computer Assisted Semen Analyzer (CASA)

Saroj Rai*, S. Tyagi†, M. Kumar‡, M. Karunakaran, M. Mondal, A. Mandal and R. Behera

ICAR- National Dairy Research Institute ERS-Kalyani, Kalyani- 741 235, West Bengal, India

Received: 04-11-2016 Accepted: 24-01-2017

ABSTRACT

The study was conducted to understand sperm kinetics of Frieswal bull spermatozoa using Computer Assisted Semen Analyzer (CASA). Fifty bull ejaculates were collected from ten healthy bulls that were in routine semen collection. The semen samples were diluted in Tris buffer at a concentration of 25x10⁶ spermatozoa/ ml for analysis. Rapidly moving spermatozoa represented the one with better velocity and progressiveness while spermatozoa with medium motility had low velocity with short distance travelled in spite of its ability to move in a straight line (straightness, STR >70 %). The slow moving cells had good head and flagella movement but they followed a circular path with straight line velocity (VSL, μm/sec), linearity (LIN %) and straightness (STR %) of 10.91, 9.00 and 22.54, respectively. Results indicated that individual sperm cells tracked by CASA as rapid, medium and slow motile were highly variable (p<0.001). However, the sperm motility between bulls varied (p<0.05) only in lateral head displacement (ALH, μm) and beat cross frequency (BCF, Hz).

Key words: Computer Assisted Semen Analyzer (CASA), Frieswal bull, Sperm motility.

INTRODUCTION

In artificial insemination the main concern is to use the semen of good quality although the sperm survival is thought to be influenced by numerous factors during cryopreservation. Sperm assessment using computer assisted semen analysis (CASA) is objective, detailed, accurate and highly repeatable in human as well as animal species (Farrell et al., 1996). Studies carried out using CASA system in mammalian ejaculates from species as gazellas, boars, stallions, dogs or bulls have identified the existence of sperm subpopulations defined by specific movement characteristics (Rivera et al., 2005 etc.). Forward progressive motility is thought to be required for the passage of spermatozoa through uterotubal junction for the colonization of the oviduct reservoir, whereas non-progressive, poorly motile or hyperactive spermatozoa are unable to migrate efficiently into the oviducts (Scott, 2000). The assessment of sperm quality in terms of motility, swimming pattern, sperm head behavior, etc. may help in better understanding of the possible sperm functions, semen quality and selection of semen and bulls for cryopreservation (Mortimer, 1997).

The purpose of the investigation is to understand motility dynamics of crossbred bull spermatozoa when analyzed by CASA system and to find out the kinematic differences between rapid, medium and slow motile cells.

*Corresponding author’s e-mail: dsaroj.rai@gmail.com,
†ICAR- Central Institute for Research on Cattle (CIRC), Meerut Cantt, Uttar Pradesh- 250001
help of playback facilities the video sequence allowed verification of sperm and non sperm particles along with its trajectories by the click of mouse and helped in removing non sperm particles. All progressively motile cells were shown by a cyan track and static cells with large red dots.

**STATISTICAL ANALYSIS**

All data were reported as Mean ± Standard Error (SE). Differences in the sperm kinetic values due to motility were tested by one way ANOVA. Difference were considered significant when \( p \leq 0.05 \).

**RESULTS AND DISCUSSION**

Visually a spermatozoon is usually considered to be motile if its flagellum is twitching even though it may not exhibit forward progression while Davis and Katz (1993) reported that a spermatozoon must achieve minimum VSL to be motile. Accordingly, three different sperm motility were tracked by CASA as rapid, medium and slow determined from eight kinetic parameters also confirmed by Muino et al. (2008). The following results are presented in Table 2 and Fig. 2. The overall motility MOT \%, PMOT \% , VAP \( \mu \)m/sec, VSL \( \mu \)m/sec, ALH \( \mu \)m, BCF Hz and STR \% of 50 ejaculates were 74.00±1.44, 47.88±2.05, 115.88 ± 2.44, 78.25 ± 2.33, 7.95 ± 0.10, 29.01 ± 0.37 and 71.06 ± 1.43, respectively while Hoflack et al., (2005) found 82.9±12.0, 68.9±14.4, 109.4±14.2, 4.5±0.8, 39±3.0 and 89.2±3.1, respectively in Holstein Friesian bulls. From the above results, it can be inferred that overall samples had good velocity and progression as VAP (mm/s) and STR (%) is more than 50 and 70, respectively. The three different types of motility tracked by CASA as rapid, medium and slow motile varied significantly \( (p<0.01) \) while sperm motility between bulls varied \( (p<0.05) \) only in lateral head displacement (ALH, mm) and beat cross frequency (BCF, Hz) which is also the kinetics for head movement. This variable as supported by Blasco (1984) may be due to many factors such as age, time between ejaculations, degree of sperm maturation, energy stores, presence of surface active agents in the cell membrane, viscosity, osmolarity, pH, temperature, ionic compositions in the seminal plasma, hormones, kinins and prostaglandins.

The rapid motile spermatozoa had higher values for velocity and progressiveness (VCL, VAP, VSL, ALH, STR and LIN) as similar patterns were observed by Hoflack et al. (2005) in Holstein Friesian bulls. The spermatozoa in medium motile subpopulations although having straight line progression had poor velocity (low VAP, VCL, ALH and BCF). This low velocity may probably be due to lower BCF which is also the propelling force that helps sperm cell to cover enough distance. Such theories have also been supported by Amann et al. (2000). The slow motile spermatozoa had better velocity (VAP and VCL) and good bobbing movement of the head and flagella (ALH and BCF) yet, it has failed to move in a straight path instead, followed an errant circular path (low VSL, STR and LIN). Similar motility type was observed by Boryshpolets et al. (2013) in rainbow trout and common carp. In human sperm, the motility which had straightness STR<30% and LIN<15% were considered to be hyperactive.

The study concludes that the rapid motile spermatozoa had forward progressive motility most while medium motile
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Average of 50 ejaculates</th>
<th>Sperm Sub-populations (485 individual sperm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOT%</td>
<td>74.00 ± 1.44</td>
<td>Rapid: 17.80±1.89a  Medium: 82.67±1.57a  Slow: 219.89±3.69a</td>
</tr>
<tr>
<td>PMOT%</td>
<td>47.88 ± 2.05</td>
<td>42.41±0.71a 31.28±1.73a 10.91±0.62a</td>
</tr>
<tr>
<td>VAP (mm/s)</td>
<td>115.88 ± 2.44</td>
<td>(2.99-203.30) (30.30-57.30) (16.20-186.40)</td>
</tr>
<tr>
<td>VSL (mm/s)</td>
<td>78.25 ± 2.33</td>
<td>(10.9-168.90) (15.10-119.20) (1.40-46.80)</td>
</tr>
<tr>
<td>VCL (mm/s)</td>
<td>209.40 ± 4.26</td>
<td>(83.90 - 149.90) (2.99-203.30) (30.30-57.30)</td>
</tr>
<tr>
<td>ALH (mm)</td>
<td>7.95 ± 0.10</td>
<td>8.90±0.14a  5.32±0.23b  8.94±0.41a</td>
</tr>
<tr>
<td>BCF (Hz)</td>
<td>29.01 ± 0.37</td>
<td>(3.50-15.80) (1.90-10.30) (1.50-20.80)</td>
</tr>
<tr>
<td>STR (%)</td>
<td>71.06 ± 1.43</td>
<td>72.10±1.03a 70.39±2.05a 22.54±1.89b</td>
</tr>
<tr>
<td>LIN (%)</td>
<td>54.04 ± 0.79</td>
<td>(43 – 88) (29.00-97.00) (1.00-68.00)</td>
</tr>
</tbody>
</table>

Means with different superscripts significantly (P<0.01) varied from each other

spermatozoa in spite of having the potential to move in straight line had poor velocity and head/ tail movement. The slow motile spermatozoa had hyperactive-like motility believed to be unable to migrate efficiently into the oviducts. Deeper investigation is required regarding different motility patterns and its fertilizing ability in vivo. Hence, the use of highly sophisticated instrument like CASA need to be further explored for assessment of sperm quality in terms of motility, swimming pattern, head behavior, etc. in understanding possible sperm functions, semen quality and for cryopreservation.

REFERENCES


