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Computer assisted sperm analysis of Brahman crossbred breeding bull semen

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Abstract

The present study was conducted at the Central Cattle Breeding and Dairy Farm, Saver, Dhaka from November 2015 to February 2016. Sixty ejaculates were collected from eight matured Brahman crossbred breeding bulls with a view to assess the motility and velocity parameters of ejaculates sperm using Computer-Assisted Sperm Analyser (CASA) with Andro Vision software. CASA parameters included as total motility (TM) (%), forward progressive motility (FPM) (%), fast motility (FM) (%) and circular motility (CM) (%); velocity parameters such as curvilinear velocity (VCL) (μm/sec), straight line velocity (VSL) (μm/sec), average path velocity (VAP) (μm/sec), linearity (LIN) (%), straightness (STR) (%), wobble (WOB) (%), amplitude of lateral head displacement (ALH) (μm), radius (RD) (%), rotation (ROT) (%) and beat/cross-frequency (BCF) (Hz) were measured by CASA analyser. The result revealed that these parameters were favorable for breeding purposes and most of the sperm motility and velocity were desirable and positively correlated with each other. The scrotal circumference had positive correlations with semen volume and curvilinear velocity, and body weight also positively correlated with scrotal circumference. It was concluded that the CASA was effective for a quick and objective analysis of motility and velocity parameters in Brahman crossbred bull semen.

Key words: CASA, scrotal circumference, motility, velocity, phenotypic correlation

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Introduction

The economy of Bangladesh is based primarily on agriculture, and an essential component of the rural economy and the livelihood of the subsistence farmers. The agricultural sector comprising crops, livestock, fisheries and forestry have been playing important roles to mitigate this challenge (Islam, 1998). Livestock is the most important agricultural component which alone contributes about 1.78% of total GDP in 2013-14 (BER, 2015). Although the cattle population in Bangladesh is considerably high but the productivity is not satisfactory in terms of productive and reproductive performance as well as calf crop production, probably due to lack of technical knowledge and appropriate breeding program. The basic aim of the present cattle breeding program in Bangladesh is to improve the genetic potentiality of local cattle through the infusion of exotic blood to meet the increasing demand of meat because of the rapid increase in population, the spread of education and growing nutrition awareness.

AI is being used as an economical tool for the rapid exploitation of superior male germplasm. But the success of AI program mainly depends on the semen quality, skill of AI technician, heat detection and awareness of farmers. It is well-known that when only highly fertile bulls are used, better conception rates are achieved, which reduces costs of reproductive programs (Sudano *et al.,* 2011). Andersson *et al.* (2002) observed a high variability in fertility among bulls using different sperm concentrations per dose at AI. Safilho *et al.* (2009) reported a high variation in conception rates depending on the bull utilized in a Timed-AI program. The ability of a bull to produce an adequate quantity and quality of semen influences the number of females that can be impregnated, either artificially or naturally, and his genetic contribution to subsequent generations (Willett and Ohms, 1957; Chenoweth, 1980). A bull has a larger impact on herd productivity than a single female: a common statement is that "the bull is half of the herd".

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Not only the bull but also the process of semen evaluation has a larger impact on herd productivity. Subjective semen analysis is carried out by conventional method such as assessment of sperm concentration, motility and morphology by phase contrast microscope (Iguer-ouada and Verstegen, 2001). But very sophisticated CASA techniques yields accurate, objective assessment, repeatable and reliable results on different semen parameters such as total motility, forward progressive motility and different velocity parameters (Verstegen *et al*., 2002) based on the measurement of individual sperm cells. Recent reports suggested that CASA does not only measure the proportion of motile spermatozoa but also measures other sperm motion parameters derived from individual sperm cells and it has more predictive power on fertility potential of semen ejaculates (Mortimer, 1994). In bovine species, specific motion parameters have been positively correlated with fertility (Budworth *et al*., 1987; Farrell *et al*., 1998). In addition to the use of computerized techniques to predict semen fertility, CASA also provides a useful tool to study the effects of various in vitro procedures on sperm motility as well as the means to study the phenomenon of sperm hyperactivation. Spermatozoa forward progressive motility (FPM) along with certain velocity parameters is essential for the spermatozoa to achieve expected fertilization. Spermatozoa kinematic parameters such as FPM, curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP) and linearity (LIN) were positively correlated with bull fertility (Farrell *et al*., 1996; Perumal *et al*., 2011).

The per capita availability of meat in Bangladesh is 65.03 gm/head/day where FAO recommended per capita requirement is 120 gm/head/day (MoFL, 2013). So there is a huge gap between demand and availability of meat. But there is no recognized beef breed in Bangladesh. At this perspective crossbreeding of native cattle with Brahman breed for beef production has been advocated as a breeding policy across the country (GOB, 2007). Considering weather, agro-climatic condition, heat tolerance, disease and insect resistance, longevity, grazing ability, calving ease, mothering ability and management, Brahman breed is considered to be the most suitable and compatible beef breed in tropical and sub-tropical regions (Antonio *et al.,* 2006) that is why evaluation of productive performance of Brahman crossbred bull is necessary to disseminate Brahman breed in Bangladesh for the improvement of beef cattle by up-grading Local cattle.

Many experiments have already been done for the evaluation of common semen parameters manually in Bangladesh but in this present work computer based semen evaluation techniques was used for first time in Bangladesh to evaluate the motility and velocity parameters of bull semen which is vitally important to maintain optimum fertility level of cows.

Materials and methods

Experimental animals

Eight apparently healthy Brahman crossbred (50% Local × 50% Brahman) breeding bulls of approximately 4 to 7 years of age were selected from Central Cattle Breeding and Dairy Farm, Savar, Dhaka. The average body weight of these bulls was 658.6 kg (570 to 710 kg) and maintained under uniform feeding, housing, and lighting conditions. Each experimental animal was fed as per the farm feeding schedule.

Scrotal circumference measurements

Breeding bulls were restrained in a squeeze chute and the scrotal content was held in the ventral scrotum from the cranial side of the scrotum. Scrotal circumference was measured in centimeters using measuring tape.

Semen collection and evaluation

A total of 60 ejaculates were collected from eight bulls using artificial vagina (AV) during the period from November 2015 to February 2016. Immediately after collection, the samples were kept in a water bath at 37°C and evaluated for volume, colour, consistency, mass activity and pH. After the preliminary evaluations, ejaculates were subjected to the initial dilution with prewarmed (37°C) Sodium-citrate extender. The partially diluted samples were then brought to the laboratory in an insulated flask containing warm water (37°C) for further processing.

Computer-assisted semen analysis (CASA)

The motility and velocity parameters were evaluated by Andro Vision AXIO, latest version 6.0.1 (Minitub GmbH12500, Germany). Andro Vision is a highly precise CASA system for standardized, interactive semen analysis. The CASA provided two files with results for each of the bull, one was a summary file Data Base Summary (DBS) in which each of the bulls had overall means for each of the parameter and the second Data Base Tracked (DBT) was a file that had every live cell tracked and the parameters for each cell. The seminal parameters were similar across the two files and consist of VAP, VSL, VCL, amplitude of lateral head displacement (ALH), beat cross frequency (BCF), straightness (STR), linearity (LIN), elongation, SIZE, size in pixels, and intensity. The DBS file stores other variables such as total concentration, percent alive, percent motile and percent progressively motile as well.

Table 1. Definition for some standardized abbreviations of CASA kinematic parameters

Acronym	Meaning
VCL	Curvilinear velocity: the time-average velocity of the sperm head along its actual trajectory.
VSL	Straight-line velocity: the time-average velocity of the sperm head along a straight line from its first detected position to its last detected position.
VAP	Average-path velocity: the time-average velocity of the sperm head along its average trajectory. The average trajectory is computed by smoothing the actual trajectory.
LIN	Linearity: the linearity of the curvilinear trajectory (VSL/VCL).
STR	Straightness: the straightness of the average path (VSL/VAP).
ALH	Amplitude of lateral head displacement: the amplitude of variations of the actual sperm-head trajectory along its average trajectory.
BCF	Beat-cross frequency: the time-average rate at which the actual sperm trajectory crosses its average path trajectory.

This CASA system consists of a phase-contrast microscope, camera, mini thermal heating stage, image digitizer, and computer for saving and analyzing the data. The software settings adjusted with the reading: temperature of analysis 37ºC; frame rate (Frames/sec) 30; duration of data capture 15 sec.; minimum motile speed (microns/sec) 28; maximum burst speed (microns/sec) 600; Distance scale factor (microns/sec) 7.50; minimum cell size (pixels) 6; maximum cell size (pixels) 6; sperm count per field analysis >1000; minimum number of fields per sample 3. One small drop of diluted semen was placed on a clean prewarmed (37ºC) slide and examined under Computer Assisted Microscope with cover slip at 10x zoom. Analysis was performed at least three new fields of each of slide. The following CASA parameters, motility parameters such as total motility (TM) (%), forward progressive motility (FPM) (%), fast motility (%) and circular motility (%); velocity parameters such as curvilinear velocity (VCL) (μm/sec), straight line velocity (VSL) (μm/sec), average path velocity (VAP) (μm/sec), linearity (LIN) (%), straightness (STR) (%), wobble (WOB) (%), amplitude of lateral head displacement (ALH) (μm), radius (RD) (%), rotation (ROT) (%) and beat/cross-frequency (BCF) (Hz) were measured by CASA analyser.

Statistical analysis

The results were analyzed statistically and expressed as the mean±SE. The significance was tested by least-squares analyses of variance using the Generalized Linear Model (GLM) procedure of the Statistical Analysis System (SAS) (SAS institute Inc., 2009) computer package, version 9.1.3. Correlation between the motility and velocity parameters were established by means of the procedure CORR (SAS Institute Inc., 2009).

Results and Discussion

Scrotal Circumference and body weight

Table 2 shows that average left and right testes length, and scrotal circumference were 19.32±0.37 and 18.27±0.39, and 36.56±1.00 cm, respectively. This observation strongly supports with Ha *et al.* (2012) who reported that average scrotal circumference of Holstein Friesian and Brahman bulls were 33 and 32 cm, respectively. According to Jayawardhana 2006 who found that conception rates in cows mated to bulls with a scrotal circumference of less than 30 cm were low; but were satisfactory in cows mated to bulls with a scrotal circumference of 30 cm or greater. Ball *et al*. (1983) argued that the perimeter of the testicles in semen production in bulls of any breed should always reach over 31cm in 15-18 months, and 32 cm at over 18 months old. The testes length and scrotal circumferences vary due to breed, age and overall nutrition. So when famers will select their breeding bulls, the measurements of testes length and scrotal circumferences must be considered. The average body weight of Brahman crossbred bull was 658.62±15.75 kg. Sarder (2008) reported that highest proportion of total sperm abnormalities (17.07%) was recorded in bulls of > 650 kg body weight followed by 450- <550 kg, 550- < 650 kg and < 450 kg abnormalities of 16.44%, 16.34% and 15.81%, respectively. It seems to say that extremely low and higher body weight of breeding bulls should be avoided during selection of breeding bulls.

Table 2. Comparative measurements of left and right testes length, scrotal circumference and body weight of Brahman crossbred breeding bulls

Factor	Basic statistics			
	Number of records	Least squares means±SE	Maximum	Minimum
LTL (cm)	8	19.32±0.37	21.50	18.50
RTL cm)	8	18.27±0.39	20.40	17.30
SC (cm)	8	36.56±1.00	40.10	31.50
BWT kg)	16	658.62±15.75	710.00	570.00

LTL, Left Testes Length; RTL, Right Testes Length; SC, Scrotal Circumference; BWT, Body Weight

Motility and velocity parameters

The sperm motility and velocity parameters were evaluated by CASA and result is presented in Table 3. The mean ejaculate volume of semen was 6.24±0.15 ml/ejaculation (Table 2). This observation strongly supports with the findings of Rahman *et al.* (2014) and Akhter *et al.* (2013) who found the mean value as 7.19±0.19 ml for Holstein-Friesian x Zebu bulls and 6.92±0.2 ml for 1/4 Local x 3/4 Friesian bulls. Tania (2012) reported 2.66 to 4.16 ml of semen produced from Brahman crossbred bulls which was lower than the present study.

Table 3. Least square mean with standard error of motility and velocity parameters of bulls

Parameter	Number of records	Brahman crossbred bull (50%)
Volume (ml)	60	6.24±0.15
TM (%)	41	72.85±1.46
FPM (%)	41	64.80±1.51
FM (%)	41	45.91±2.09
CM (%)	41	1.92±0.48
VCL (µm/s)	52	166.05±13.00
VSL (µm/s)	52	80.93±7.03
VAP (µm/s)	52	112.01±7.51
LIN (VSL/VCL) (%)	52	48.73±0.86
STR (VSL/VAP) %)	52	72.25±0.03
RD (%)	52	5.75±2.55
ALH (µm)	52	7.36±3.54
ROT (%)	52	53.01±0.05
BCF (Hz)	52	28.58±0.97
WOB (%)	52	55.13±0.02

TM, Total Motility; FPM, Forward Progressive Motility, FM=fast motility, CM=Circular Motility, VCL=Curvilinear Velocity, VSL=Straight line Velocity, VAP=Average Path Velocity, LIN=linearity, STR=Straightness, WOB=Wobble, ALH=Amplitude of Lateral Head Displacement, RD=Radius, ROT=Rotation and BCF=Beat/Cross-Frequency

The least square mean value of total motility, forward progressive motility, fast motility and circular motility were 72.85±1.46, 64.80±1.51, 45.91±2.09 and 1.92±0.48%, respectively (Table 3). This observation strongly agrees with the studies of Vincent *et al.* (2012) and Contria *et al.* (2010) who obtained the mean value of total motility of semen as 76, 57.5±4.5, 60.5±0.5% for A, B and C categories of bulls and 75.6±6.5, 76.6±4.5, 68.0±4.9 and 67.1±6.3% for four types of setup under CASA in bovine semen, respectively. According to Galmessa *et al.* (2014) who reported the overall mean percent motility of fresh semen on the basis of scrotal circumferences (SC) were 78.49±17.27 for Sahiwal bulls which is slightly higher than the present study. Ahmed *et al.* (2014) who obtained forward progressive motility were 56.6±2.3 to 60.5±1.5% for local and Friesian bulls and this observation strongly supports with the result. However, the present observation differs from that of Vincent *et al.* (2012) who reported higher (70%) progressive motility of bovine semen. According to Contria *et al.* (2010) who obtained the mean value of progressive motility were 45.0±5.00 which is slightly lower than present study. There are no other findings in the literature for comparison to fast and circular motility. The sperm motility is very much correlated with herd fertility. In bovine artificial insemination industry, the minimum level of motility percentage required is 50 percent for achieving better conception rates. But in some developing countries, due to the limitation of such CASA techniques could not found the actual percent of motility sperm and that's why could not be given emphasize on bull selection by motility observation.

The mean value of velocity parameters such as curvilinear velocity (VCL), straight line velocity (VSL) and average pathway velocity (VAP) were 110.05±13.00, 52.93±7.03 and 64.01±7.51 µm/s, respectively. This observation is strongly supports with the result of Perumal *et al.* (2014) and Amanda (2011) who reported that average VCL, VSL and VAP were 188.83±4.65, 89.77±3.49 and 118.58±3.72 µm/s for Mithun semen and 193.93±71.50, 90.69±40.60 and 111.37±40.97 µm/s for Holstein bulls. However, the present observation differs from that of Sundararaman *et al*. (2012) who reported lower velocity in bull semen (152.1, 71.5 and 90.5 µm/s for VCL, VSL and VAP). A spermatozoon has significantly higher VCL and ALH, indicating that there is major bending of the mid piece and large amplitude of lateral head displacement. This signifies the hyperactivation of the spermatozoa. Hyperactivation in turm implies high energy state of the spermatozoa, which is essential for sperm penetration through cervical mucus, zona pellucida, fuse with the oocytes, and successful fertilization (Aitken *et al*., 1985). Linearity (LIN, VSL/VCL) and straightness (STR, VSL/VAP) in this study was 48.73 and 72.25% which was agreeable to the average of results found in previous studies (46.94 and 74.81% Perumal *et al.*, 2014, 48.24 and 80.74% Amanda, 2011) and comparable (39.34%; Farrell *et al.,* 1998, Budworth *et al*., 1988). The linearity or linear motility is higher indicating that spermatozoa have higher rate of fertilization potential in comparison to the total motility percentage (Cremades *et al*., 2005) and semen samples containing such spermatozoa have higher fertility rates and pregnancy rates after artificial insemination (Farrell *et al*., 1998). The results (7.36±3.54) of amplitude of lateral head displacement (ALH) (μm) from this study were approximately equal to studies of Perumal *et al.* 2014 and Amanda, 2011. Similarly the results (28.58±0.97 Hz) of beat/cross-frequency (BCF) (Hz) from this study were also approximately equal to studies of Perumal *et al.* 2014 and Amanda, 2011 and dissimilar (30.5 Hz) to Sundararaman *et al*. (2012). The percent value of wobble in this study was 55.13±0.02 which is similar to the results of Perumal *et al.* who found 62.85% in Mithun semen. The parameter LIN is a measure of linearity and the BCF motion parameter indicates the number of times the sperm track crosses the smoothed path, both of which indicate linear progression. The radius and rotation value were 5.75 and 53.01% (Table 3). There are no other findings in the literature for comparison. In bovine, sperm velocity is highly correlated with the 59-day non-return rate (Farrell *et al*., 1998). So when we select the breeding bulls, determining the sperm velocity is necessary but it is not possible for manual evaluation of semen parameters only possible when using CASA or related techniques.

Table 4. Phenotypic correlation between the motility and velocity parameters

	TM	FPM	FM	VCL	VSL	VAP	LIN	STR	ALH	BCF	WOB
TM	1.00	0.78**	0.59	0.65**	0.75**	0.55*	0.58	0.66	0.67	0.74*	-0.02
FPM		1.00	0.55**	0.71**	0.84**	0.65*	0.64	0.68	0.65	0.83**	-0.07
FM			1.00	0.54	0.75	0.60	0.52	0.46	0.52	0.46	0.21
VCL				1.00	0.84**	0.92**	0.26	0.32*	0.67**	0.41**	0.12
VSL					1.00	0.97**	0.62**	0.59**	0.36**	0.59**	0.45**
VAP						1.00	0.51**	0.46**	0.50**	0.56**	0.39**
LIN							1.00	0.83**	-0.07	0.59**	0.85**
STR								1.00	0.02	0.58**	0.47**
ALH									1.00	0.32	-0.08
BCF										1.00	0.46**
WOB											1.00

TM=Total Motility, FPM=Forward Progressive Motility, FM=fast motility, VCL=Curvilinear Velocity, VSL=Straight line Velocity, VAP=Average Path Velocity, LIN=linearity, STR=Straightness, WOB=Wobble, ALH=Amplitude of Lateral Head Displacement, RD=Radius, ROT=Rotation and BCF=Beat/Cross-Frequency; *Significant at 5% level ($p<0.05$); **Significant at 1% level ($p<0.01$).

Phenotypic correlation

The phenotypic correlation between the motility and velocity parameters of Brahman crossbred breeding bulls semen is presented in Table 4.

Semen samples with high TM and FPM had significantly ($p<0.05$) higher positive correlation with velocity parameters. The samples with high FPM had higher VAP, VCL and VSL (Table 4). This was similar to the findings of Anil Kumar *et al.* 2011 and Perumal *et al.* 2011 for path velocity. The average path velocity was significantly and positively correlated with VCL, VSL and ALH. The high positive correlation observed between VAP, VSL, VCL, and ALH, between VSL and VCL, and between ALH with VAP, VSL and VCL indicated that the velocity parameters were correlated and interrelated among them and with ALH. BCF was significantly and positively correlated with ALH. ALH was negatively correlated with WOB. The CASA variable such as velocity parameters is positively interrelated among them means that spermatozoa have higher rate of fertilization potentiality (Cremades *et al*., 2005) and semen samples containing such spermatozoa have higher fertility rates and pregnancy rates after artificial insemination (Farrell *et al*., 1996).

Relationship between scrotal circumferences with semen parameters

The relationship between scrotal circumferences with ejaculate volume and curvilinear velocity of Brahman crossbred breeding bulls is represented in Figure 1 and 2. The scrotal circumference had positive correlations with semen volume and curvilinear velocity of Brahman crossbred breeding bulls. It means that when the size of scrotal circumference will be more, the ejaculate volume should be increased. The determination coefficients were 0.37 for volume and 0.59 for curvilinear velocity in Brahman crossbred bulls, respectively (Figure 1 and 2). The results of this study are consistent with the research of Latif *et al.* (2009) and Ha *et al.* (2012) in crossbred Sahiwal and Brahman, Holstein Friesian bulls.

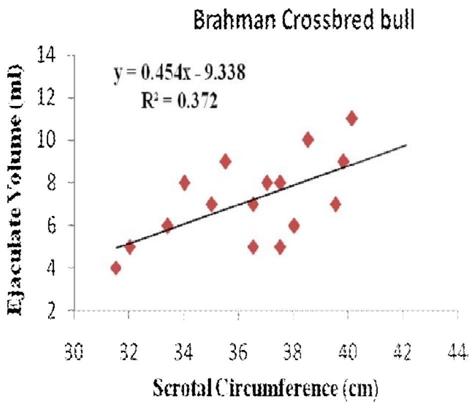

Figure 1. The relationship between scrotal circumference and ejaculate volume of Brahman crossbred breeding bulls.

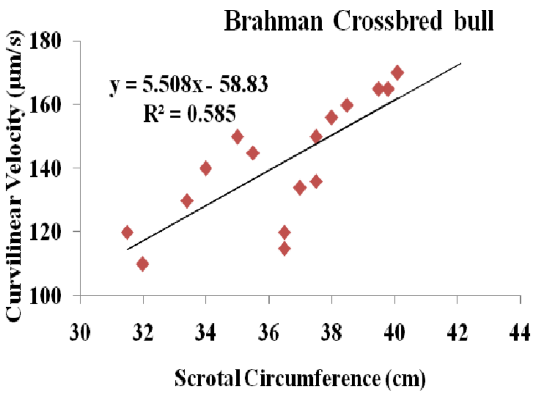

Figure 2. The relationship between scrotal circumference and curvilinear velocity of Brahman crossbred breeding bulls

Relationship between body weights with scrotal circumferences

The relationship between body weights with scrotal circumference of Brahman crossbred breeding bulls is presented in Figure 3. The body weight also had positive correlations with scrotal circumference and determinate coefficients were 0.03 (Figure 3). This observation supports with Mahmood *et al.* (2014) who reported that body weight was significant positive correlation with average testicular length ($r=0.920$, $P<0.01$), paired testicular volume ($r=0.819$, $P<0.05$) and scrotal circumference ($r=0.68$), respectively.

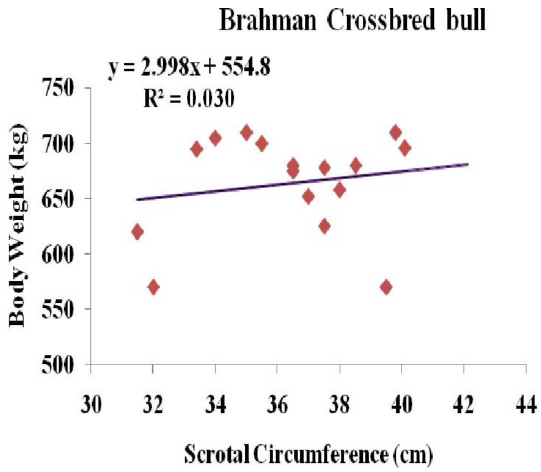

Figure 3. The relationship between body weight and scrotal circumference of Brahman crossbred breeding bulls.

Conclusion

It may be concluded from the present study that the measurements of scrotal circumference, body weight and their relationships were favorable for breeding purposes. Otherwise, most of the value of sperm motility and velocity parameters assessed by CASA was desirable and positively correlated with related parameters. From the results of the evaluation of Brahman crossbred bulls semen, it seems to be very potential in respect of fertility criteria compared to different published literatures. Moreover CASA system proved its usefulness and authenticity in routine evaluation of Brahman crossbred bull semen which might have positive significant effect on non-return rate of cows.

Summary

Findings of this research suggested that assessment of motile spermatozoa in a semen sample as well as other sperm motion characteristics derived from observations of individual cells assessment by CASA have been found to be more efficient in predicting potential fertility of semen. These parameters are probably important for the progression of spermatozoa into cervical mucus and the penetration of zona pellucida of oocytes. In addition to the use of computerized technique to predict semen fertility, CASA can be a useful tool to study the effects of various *in vitro* procedures on sperm motility as well as phenomenon of sperm hyperactivation.

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