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An *In-Vitro* Criterion for Comparative Assessment of Fertility Characteristics of Bull Semen in Kenya

Mutembei H.M.*, Origa R., Agumbah G.J.O.

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Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi, Kenya.

ABSTRACT

The goal of a contemporary dairy farmer is to maximise the efficiency of milk production. Reproductive efficiency of the cow and bull fertility is major component of efficient production. General macro- and micro semen characteristics such as volume, motility and concentration have been majorly used to assess bull fertility in Kenya. Confounding reports as to the correlation between these parameters and bull fertility in Kenya may be due to their subjectivity and variability. Therefore, the need to develop objective and consistent analysis methods was considered necessary for the present study. Room temperature and deep frozen semen samples from six bulls at the Central Artificial Insemination Station in Kenya were used. The volume, motility, concentration, morphology, cleavage, Blastocyst and non- return rates of all the bulls were assessed. Correlation analysis was done between the various parameters. Significant differences (P≤0.05) were observed between breeds as well as between individual bulls within the same breed. There was a positive correlation observed between cleavage and blastocyst formation rates with semen motility and a negative correlation with concentration. Correlation was also observed between percentage of morphological abnormalities and cleavage and blastocyst rate. The results suggest that no single parameter can be used on its own to objectively predict bull fertility. Therefore multiple characteristics should be assessed in order to try to predict fertility. In addition, motility, concentration, cleavage and blastocyst formation rates can be used to assess bull fertility according to this study.

Key words: Artificial Insemination, Bull, *In-vitro* fertilization, Cleavage and Blastocysts rate.
*Corresponding author.E-mail: hmutembei@uonbi.ac.ke

INTRODUCTION

Artificial insemination (AI) is a reproductive technology that allows for accelerated propagation of the genes of good quality bulls. It is the first generation reproductive biotechnology that has made profound contribution to the genetic improvement, particularly in dairy cattle (Faber et al., 2003). When AI is used in combination with sexed semen, this technology permits the production of offspring of the desired sex (that is, females for the dairy industry, or more males for the beef industry) (Faber et al., 2003; Bearden et al., 2004). AI is considered safer than natural mating particularly against the spread of infectious agents and it is also economical because a bull can be used to serve many cows (Bearden et al., 2004). The technique has been widely applied for genetic improvement of cattle for milk production and to some extent meat production (Sugulle et al., 2006). AI technique is also the basis on which embryo transfer is undertaken, thus enhancing the reproductive capacity of the female (Mutembei et al., 2015). Semen from bulls used in AI programs is evaluated for fertility using several methods. Mostly used is light microscopic evaluation of classical sperm parameters including sperm concentration, motility, morphology and viability (Zhang et al., 1999). At the central artificial insemination station (CAIS) in Kenya, semen collected from the AI bulls is evaluated both macroscopically and microscopically (Mwangi 2008). The former involves visual assessment of the volume, colour, viscosity, presence of any admixtures and pH. The concentration is assessed by the use of a spectrophotometer while motility is assessed under a microscope.

The motility is assessed in two ways, mass motility or wave motion and individual progressive motility. Mass motility is evaluated at a magnification of ×40 and scored

from an arbitrary scale of M (-) to M (+++), with M (-) denoting the absence of the mass waves and M (+++) denoting strong mass motion with dark cloud like waves. Individual progressive motility is evaluated at a magnification of ×400 and scored as a percentage. Semen samples with mass motility of at least M (++) and progressive motility of at least 70% is considered to be of good quality. Semen samples that qualifies for use in AI based on these criteria is processed and packaged into either 0.5 ml straws or 1.0 ml vials, which are then distributed for inseminations either as deep frozen semen (DFS), or room temperature semen (RTS), respectively. For DFS, further evaluations in terms of post-thaw motility a day after freezing are done. The acceptable post-thaw motility in Kenya is 50% and above (Mwangi, 1998; Mutembei et al., 2015). However, in other parts of the world post-thaw motility of up to 30% is acceptable (Whittier and Thomas, 2000). Fertility of AI bulls is often judged by the percentage of cows that do not return to heat 60 to 90 days post insemination (non-return rate, NRR).

This method is used worldwide in estimating fertility of AI bulls (Sarder, 2006). The danger in this is that a lot of economic losses are passed over to the farmers should a bull be passed and later detected to have low NRR. NRR of a bull can be attributed to several possible factors such as: (a) poor heat detection and timing of insemination, (b) the inseminators' skills and technique, (c) semen quality, (d) the body condition and (e) reproductive health of the cow/heifer. However, given the fact that semen from the bulls are handled randomly by several technicians and on cows of different body conditions, a given bull consistently showing high return rates whenever his semen is used would be indicative of such a bull having low fertility, despite having passed the motility and concentration tests. Thus, a criteria that could pre-test the fertility status of the bull semen before use in the field can help save on the farmers' burden, especially for a bull whose semen, though seemly fertile under current methods of semen evaluation, is indeed infertile with high return rates. Since such bulls have been noticed in Kenya (Mutembei et al., 2008), this study aimed at establishing the relationship between the evaluation methods currently used at our bull station in Kenya and an IVF evaluation of the semen as a fertility judgment of the bull. With this criteria for the bull semen on its ability IVF, cleavage and embryo development in the laboratory as the basis for pre-testing fertility, there will be value addition for the semen quality (Muasa et al., 2105; Muraya et al., 2015; Mutembei et al., 2015) for enhanced output of the Kenyan AI programs (Lawrence et al., 2015).

MATERIALS AND METHODS

Random sampling of experimental bulls was done at bull station in Kenya. Semen was collected from the sampled bulls, assessed for volume, colour, presence of admixtures, mass and individual progressive motility and concentration. Passed samples based on bull station criteria were processed, packaged and transported to the IVF laboratory for IVF, cleavage and embryo production. Two bulls of each of breeds (Friesian, Ayrshire and Jersey) were randomly chosen from a pool of forty nine Friesians, forty four Ayrshires and seven Jerseys. The bulls were prepared and semen collected from them using standard sized artificial vagina (IVM, France) as described by Mwangi (1989). Semen collection was done in the morning hours between 0700 and 0900. After collection, the semen was quickly transferred into racks immersed in water bath kept at 34ºC during which the macroscopic assessment of the volume, colour and presence of any admixtures was done and the results recorded for each bull. Samples with abnormalities of colour or with any admixtures were not used. Only bulls that produced an average volume of 3.5 to 7.2 ml were considered adequate for further processing. For microscopic evaluation, both mass and individual or progressive forward motility were determined. Mass motility was determined by placing a drop of fresh undiluted semen onto a warm glass slide and observing under a microscope at a low magnification of ×40 and graded on a scale of M (-), M (+), M (++) and M (+++) with M (-) denoting the absence of the mass waves and M (+++) denoting strong motion with dark cloud like waves. Individual progressive forward motility was determined by placing another drop of undiluted semen onto a warm glass slide and covering it with a warm cover slip.

This was observed under a microscope with a heated stage constantly kept 37^{o}C at ×400 magnification. Motility was scored subjectively by assessing the percentage of cells in a given field that are progressively moving to the nearest 5%. Semen with mass motility of M (++) or more and 70% and above individual progressive motility was further processed. The semen concentration was determined by mixing 0.02 ml of semen and 1.98 ml 10% sodium chloride in a cuvette. The mixture was then placed into a photoelectric calorimeter and the optical density was read through a light filter at 580 nanometres. The resultant figures were converted to the concentration using a standard pre-calculated chart as described by Mwangi (1989). To prepare room temperature semen (RTS) the collected sample was diluted to prepare samples with concentration of 3.0x10^{6} using an extender made from coconut water extract at a pH of 7.4. The diluent contained 28 ml egg yolk, 68 ml coconut water, 200 ml distilled water, 8.8 gr sodium citrate, 1.2 gr sulphanilamide, 0.24 gr penicillin, 0.54 gr streptomycin. These were then packaged into 1 ml clean glass ampoules covered with sterilized corks and transported in Styrofoam box to the IVF laboratory. Frozen semen was prepared by diluting the semen with bioxel (IVM France)

Table 1. Least square means for cleavage and blastocyst rate of all the bulls.

Bull	Breed	Semen type	Cleavage rate	Blastocyst rate
Impresario	Ayrshire	DF	26.61±5.52	7.03±2.38
Impresario	Ayrshire	RTS	11.14±6.73	7.26±2.69
Kios	Ayrshire	DF	36.18±4.91	11.71±2.38
Kios	Ayrshire	RTS	38.01±5.97	12.20±2.61
Gordon	Friesian	DF	10.39±6.20	3.43±2.81
Gordon	Friesian	RTS	31.53±7.07	14.63±2.81
Pegasus	Friesian	DF	32.94±6.01	4.30±2.72
Pegasus	Friesian	RTS	29.04±6.42	7.17±2.90
Darwin	Jersey	DF	40.53±5.64	6.13±3.07
Darwin	Jersey	RTS	15.69±7.79	6.94±3.44
Fame	Jersey	DF	23.84±6.09	0.85±2.69
Fame	Jersey	RTS	30.42±6.63	7.61±2.87

Table 2. Association between cleavage rate and other semen evaluation parameters.

Semen characteristics	Estimate± STD Error	Association range
Motility	1.72±0.10	1.82 - 1.62
Concentration	-2.36±2.27	-0.09 - -4.63
Percentage tail abnormalities	1.37±0.18	1.55 - 1.19
Percentage mid piece abnormalities	-1.81±0.08	-1.73 - -1.89
Percentage total abnormalities	2.07±0.05	2.12 - 2.02

Table 3. Association between blastocyst rate and other semen evaluation parameters.

Semen characteristics	Estimate± STD Error	Association range
Motility after swim up	2.41±0.03	2.44 - 2.38
Concentration	-1.04±0.31	-0.73 - -01.35
Percentage tail abnormalities	0.47±0.64	1.11 - -0.17
Percentage mid piece abnormalities	0.44±0.67	1.11 - -0.23
Percentage total abnormalities	-0.68±0.50	-0.18 - -1.18
Cleavage rate	0.06±0.02	0.08 - 0.04

extender containing glycerol, antibiotics and soya as a source of protein and packaged in 0.5 ml plastic straws. The straws were arranged in a rack placed 4 cm above liquid nitrogen and allowed to freeze at -112ºC in liquid nitrogen vapour for at least 7 min prior to plunging into liquid nitrogen tank at -196ºC. These were later transferred and transported to the laboratory in liquid nitrogen tank. Ova for IVF were collected from slaughter house in Nairobi and transported to the IVF laboratory, processed and *in-vitro* embryo production using both RTS and DFS was done as described in a previous paper (Muasa et al., 2015).

RESULTS AND DISCUSSION

The results of the cleavage rate (measure of fertilization rate) and blastocyst rate (measure of embryo development up to day 7) for all the bulls based on 50 (n= 50) oocytes are shown in Table 1. The sperm abnormalities detected during assessment are shown in Figures 1 and 2. Association between cleavage rate and semen evaluation parameters as assessed at the bull station are presented in Table 2. Association between blastocyst rate and semen evaluation parameters as assessed at the in-vitro laboratory are presented in Table 3. Significant difference (P≤0.05) in semen characteristics were noted between breeds and individuals as expected (Sarder, 2006). Also freezing affected sperm motility when compared to RTS (Watson, 1995). However, it is acceptable to compare RTS and DFS in an *in-vitro* set up because the semen undoes a swim up procedure which boosts the post swim up motility to similarly accepted levels (Parish et al., 1995). A relatively higher percentage

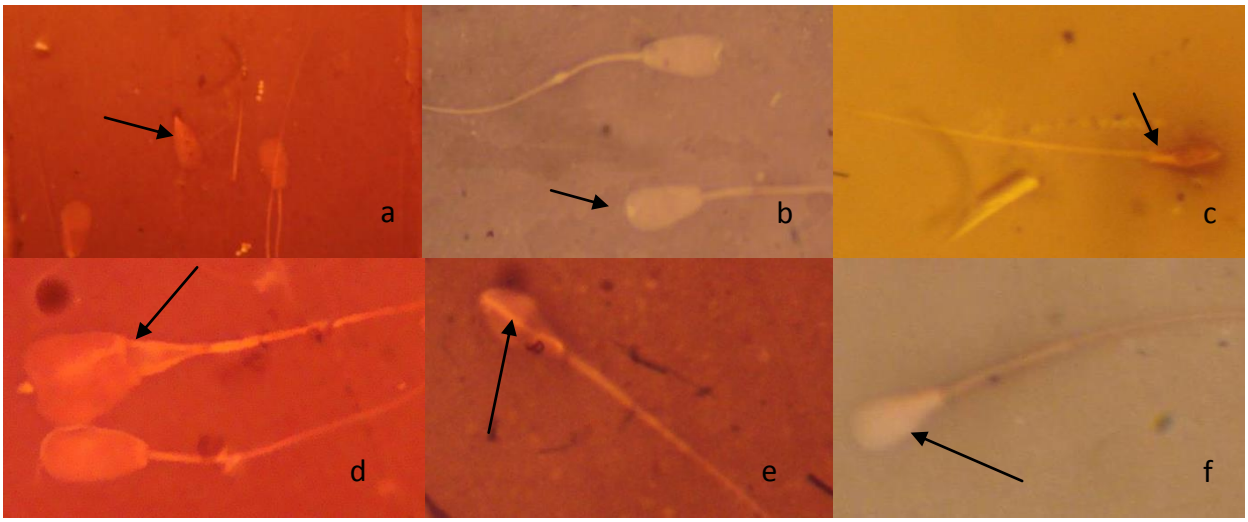

Figure 1. Spermatozoa head abnormalities at x400; (a) detached heads (b) knobbed acrosome defect (c) micro cephalic (d) Double head (e) Nuclear crest (f) Normal sperm.

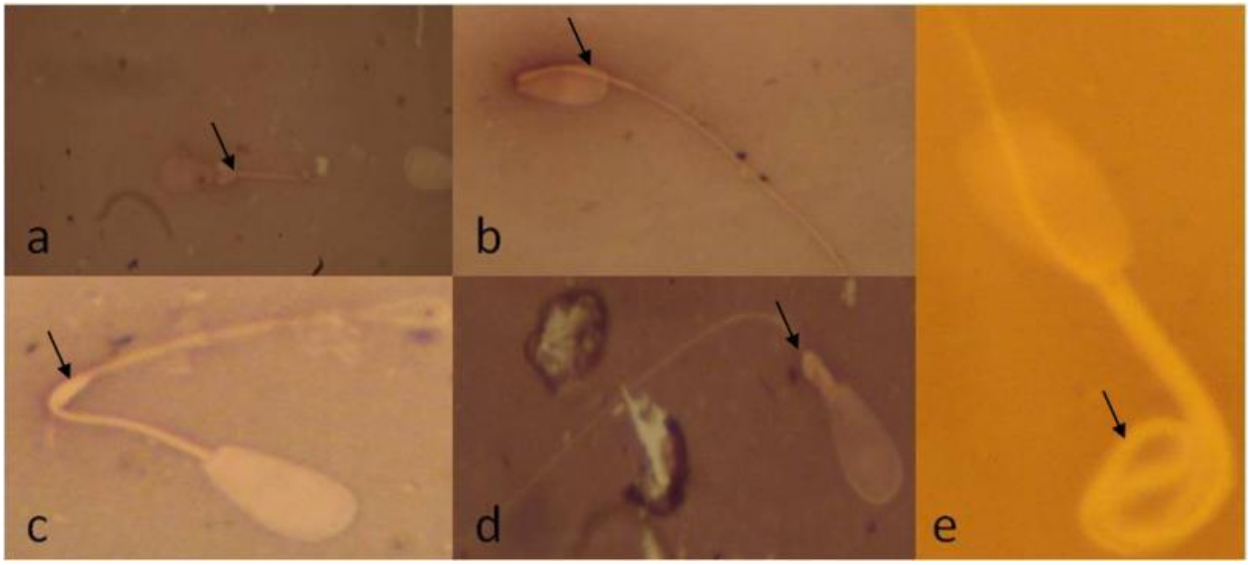

Figure 2. Spermatozoa mid-piece and tail abnormalities at x400; (a) proximal droplet, (b) malformed mid-piece; (c) Distal droplet, (d) open form distal mid-piece reflex (d) and (e) dag- like defect.

of abnormalities were observed in DF compared to the RTS semen due to the expected structural changes that also occur in the sperm cells during cryopreservation (Barth and Oko, 1989). However, this could not affect the *in-vitro* procedures because the defective sperms could not swim up take part in fertilization process (Zhang et al., 1999). It was noted that cleavage and blastocyst rate differed significantly (P≤0.05) between the breeds, individual batches of semen and between RTS and DFS. Blastocyst rate was higher in RTS compared to DFS. This may suggest that freezing causes changes in semen that may reduce fertility in the DF semen. This observation has been noted by previous authors (Larsson and Rodriguez-Martinez, 2000; Camargo et al., 2002; Mutembei et al., 2015).

The mortality rate of embryos is obviously reduced by the use of fresh rather than frozen semen (Larsson and Rodriguez-Martinez, 2000). Batches of semen are never prepared in identical conditions and one would expect differences in their cleavage and blastocysts rates as reported earlier (Knessl, 1993; Graham, 1997; Camago et al., 2005). The correlation between the assessment at the bull station and the output at the *in-vitro* laboratory based on cleavage and blastocyts rates suggest that no single parameter can be used on its own to objectively Predict bull fertility (Marko et al., 2006). Therefore

multiple characteristics should be assessed in order to try to predict fertility. From this study, motility, concentration, cleavage and blastocyst formation rates could be used to assess bull fertility for use in embryo production. The ability of the sperm to fertilize ova that then undergo cleavage and develop into an embryo can be dependent upon many factors (Mutembei et al., 2016). The expression of compromised genetic information from the spermatozoa can impair embryo quality (Lonergan et al., 1997; Mehmood and Saqlan, 2007) and interfere with an *in-vitro* embryo development (Saacke et al., 2000). As expected there was a positive correlation between the sperm motility and both cleavage and blastocyst rate (Larsson and Rodriguez-Martinez, 2000). However contrary to the other finding a negative correlation was noted between concentration and blastocyst rate. Danilda (2000) also noted that for optimum cleavage and blastocyst formation, the required sperm concentration to be used in IVF varied from bull to bull. There was a negative correlation between cleavage rate and the total percentage abnormalities and as expected there was a positive correlation between cleavage and blastocyst rate (Muasa et al., 2015; Mutembei et al., 2015).

CONCLUSION

The *in-vitro* evaluation criteria for bull semen based on cleavage and blastocyst rates are a possibility to pre-test the fertility of bulls in an AI station. However, no single semen parameter can be used on its own to objectively predict bull fertility. Therefore multiple characteristics should be assessed in order to try to predict fertility. The authors suggest that this kind of test could add value to the existing tests at bull AI stations.

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