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Low Temperature Sperm Selection Method to Support Bovine Breeding Industry

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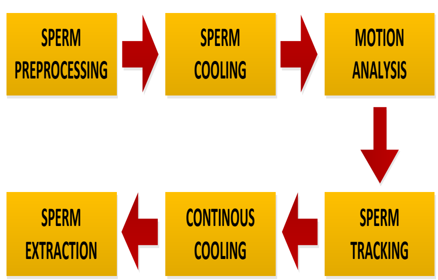
Graphical abstract



Abstract

This research proposed novel bovine sperm selection method based on sperm motility parameter to support bovine breeding industry. Sperm selection method consists of three main processes. First, decrease and holdbovine semen temperature at 4°C to reduce average sperm motility.Second, determine targeted sperm location which has highest motility within objective's field of view after general motility observed decreasedinto 5% from initial value.Third, track targeted sperm and maintain holding temperature continuously until targeted sperm immotile and ready to be aspirated.
Testing result showstemperature controller prototype can decrease bovine semen temperature safely without generate any intracellular ice. Micro actuator prototype can provide high motion performance exceed bovine sperm average velocity so it fully supporting motion detection software to perform real time bovine sperm tracking. Autofocus mechanism was succeedingincrease motion detection sensitivity using 4X, 10X and 40X objectives lens. All prototype devices developed in this research provide safely selection process to achieve high quality bovine sperm.

Keywords: Bovine sperm selection; sperm tracking; sperm cooling; sperm analysis

1.0 INTRODUCTION

This research proposeda novel sperm selection method to achieve high quality bovine sperm through semen temperature treatment.The basic principle of proposed method utilizes sperm motility response due to low temperature environment. High quality sperm has high motilityvalue and high endurance to ambient thermal influence[1-4]

Most of mammal sperm will decrease their motility into minimum metabolism state (self-immotile)whenplaced in low temperature environment between $0^o\ C$ to$10^o\ C$ [4]. In this temperature, any semen fluidcomponents still remain in liquid-phase providing safety in sperm cooling process.High quality sperm will have high metabolism endurance due to semen cooling process. This makes high quality sperm will have longer moving time than another average sperm.We study this phenomenon to build a novel sperm selectionmethod mainly focused on semen temperature manipulation and visual tracking algorithm.

Bovine sperm selection hardware consisted of microscope camera device, semen temperature controller, microactuator and motion detection software enhanced by autofocus system. Motion detection software will find, track and cue targeted sperm continuously until it self-immotile caused by low temperaturelong-term exposure. Sperm aspiration can be done after targeted sperm stop moving completely. Selected bovine sperm can be used for zone thinning (ZT), zone drilling (ZD), subzonal insemination (SUZI)orintra cytoplasmic sperm injection (ICSI) [5-6].

2.0 EXPERIMENTAL

2.1 General Design

Proposed bovine sperm selection procedure started by decreasing pre-processed bovine semen to certain holding temperature and certain cooling rate continuously. Temperature controller cooling down semen temperature into specific holding temperature close to water freezing point but still maintain all semen fluid components in liquid-phase state avoiding intracellular ice formation which harmful for sperm organelle. Minimum holding temperature proposed is 4^oC where water has highest density but still in liquid phase state [4] [7].

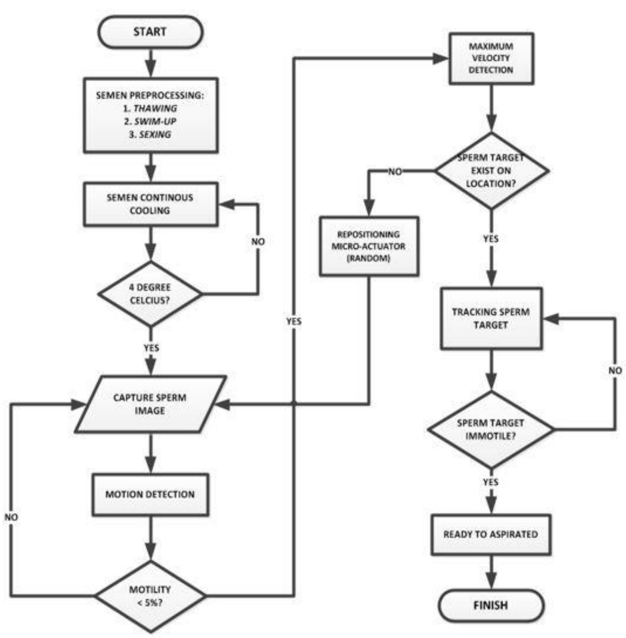


Figure 1 Detailed bovine sperm selection process

Software measuring all motion detected within objective viewpoint when semen temperature reach4^oC. If sperm average motility measuredbelow 5% from initial value then motion detection software will execute real time sperm tracking algorithm immediately to find highest motile sperm location, apply visual marking on object's centroid and calculate object boundary line. When targeted sperm crossing boundary line, motion detector software will command microactuator device to relocate last known crossing position to center of screen and motion detection software will recalculate to find highest motile sperm location again while semen temperature keep holding at 4^oC. This procedure looped infinitely until targeted sperm immotile and ready to be aspirated using micropipette.

2.2 Temperature Controller Design

Bovine semen temperature controller consists of hardware and software connected each other through serial communication.Proportional integralderivative (PID)control algorithm chosen to achieve best performance in this case [8]

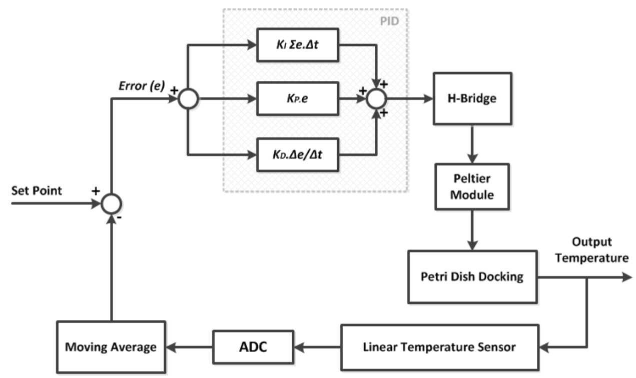


Figure 2 Semen temperature controller diagram

Semen temperature controller measuring initial semen temperature and calculating error value due to set point command using Equation 1.

$$u(t) = K_p e(t) + K_i \int_{t_0}^{t} e(t)\, dt + K_d \frac{d}{dt} e(t) \qquad (1)$$

u(t)is PID output control signal, $e(t)$ is error value due to set point, t is working time, K_p, K_i and $K_d$respectively are PID's proportional, integral and derivative constant. If Equation 1 will be used to control bovine semen temperature trough pulse-width modulation (PWM) thenEquation 1 must be transformed into Equation 2 as PID algorithm in digital control system [8]

$$u(t) = u(t-1) + K_p\big(e(t) - e(t-1)\big) + K_i T e(t) + \frac{K_d}{T}\big(e(t) - 2e(t-1) + e(t-2)\big) \qquad (2)$$

Here, $u(t)$is PWM control signal output, $e(t)$is error value between set point and real temperature measured,$t$is step time process,K_p is a PID proportional constant, K_i is a PID integrative constant and K_d is a PID differential constant. Temperature controller will translate PID result value $u(t)$ into certain PWM duty cycle (D) to generate equivalent direct current (DC) voltage as Equation 3.

$$\bar{V}(t) = DV_{cc} \qquad (3)$$

This is equivalent DC voltage injected into peltier module through H-bridge MOSFET. At the same time, temperature sensor measuring semen temperature value to get next error data.

2.3 Micro Actuator Design

Micro actuator device developed using a pair of precision hybrid linear actuator (HLA)module to convert rotation step into linear movement [9]. An anti-backlash system was applied to reduce error and enhance linear movement precision. In this research, a pair of micro stepper driver is used to control HLA movement by convertingactive electrical pulse into a high precision step movement. Figure 3 shows rotary-to-linear movement conversions.

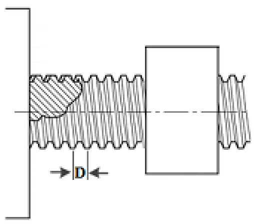


Figure 3 Rotary-to-linear movement conversions

Assumed if all rotary movement can be ideally converted into linear translation without any slip, we can calculate linear displacement using Equation 4

$$L(\theta) = D\left(\frac{\theta}{2\pi}\right) \quad (4)$$

$L(\theta)$is linear movement resultant by step angle changing on hybrid motor(θ) and D is gap between HLA's neighbor screw teeth. At another viewpoint, we can use also electrical pulse parameter (n) to generate certain linear step translation $L(n)$ using micro step motor driver de-numerator constant $K(m)$ as shown in Equation 5.

$$L(n) = \frac{Dn}{NK(m)} \quad (5)$$

$K(m)$ is an integer valuewhich directly affected to HLA's smoothness step.$K(m)$will divide HLA full step movement by $2^m \,|\, \{m = 1, 2, 4, 8 \,..\, 2^m\}$ to emulate smaller step and N represent full-step needed to make a complete rotation.

2.4 Motion Detector Design

Motion detection algorithm differentiates all gray scale pixels from sequenced microscope camera image (n) to image $(n-1)$ in real time process. This will generate new type image consists of absolute pixel differentiation between two sequenced images. Motion detection sensitivity can be calibrated by dividing differentiated image into kxl sub detection area. All pixels within sub detection area will be partially summarized to calculate local moving value.

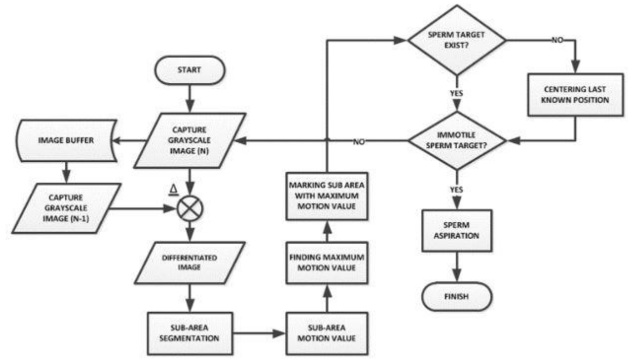


Figure 4 Sperm motion detection algorithm

Generally, motion detection software designed to recognize any moving particle within objective viewpoint. This software split streaming image into several sub detection area.If certain condition reached, motion detection software can determine area location which has highest motion activity and giving visual marking at targeted sub-area centroid and supervising centroid position due to image detection boundary at the same time

If targeted sub-area centroid moving within image detection boundary, software will doing nothing except giving a visual sign on targeted centroid.But if targeted sub-area centroid touching or moving across image detection boundary,system will relocate last known crossing position to center of screen and motion detection software will recalculate to find highest motile sperm location again. This procedure will be looped until targeted sperm immotile and ready to be aspirated.

2.5 Autofocus Design

Autofocus system is an optional design. It usedto provide high clarity images to enhance motion detection sensitivity. Focus defined asaverage of sum quadratic object edge achieved using first-order isotropic Gaussian detector [10-11]written as Equation 6

$$F(I,\sigma) = \frac{1}{wh}\left(\int_0^h \int_0^w [I(x,y) * G^1(x,y,\sigma)]\, dx\, dy\right) \quad (6)$$

where

$$G^1(x,y,\sigma) = -\left(\frac{y}{2\pi\sigma_x\sigma_y^3}\right) exp^{\left(-\frac{x^2}{2\sigma_x^2} - \frac{y^2}{2\sigma_y^2}\right)} \quad (7)$$

Here, $F(I,\sigma)$ is focal value of an image$I(x,y)$.w is image width, h is image height. σ is strength of Gaussian edge detector $G^1(x,y,\sigma)$

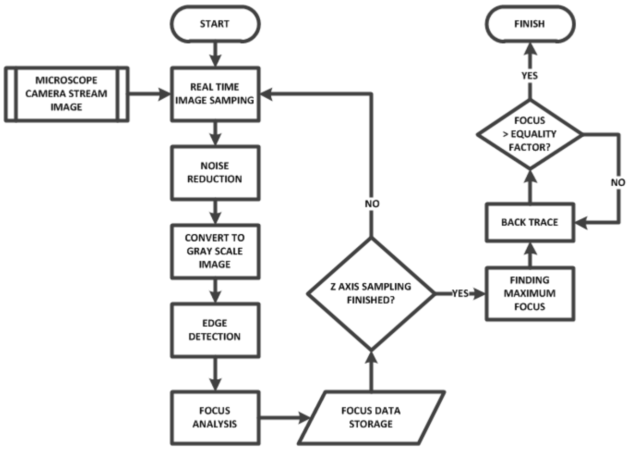


Figure 5 Autofocusalgorithm

Figure 5 shows autofocus algorithm [12] to find optimum positionat certain objective lens application(Z_{opt}). Autofocus system calculates all focal value for any elevation,generating focal function properties to find optimum objectives lens position providing maximum image clarity(I_{max}). After Z_{opt} founded, autofocus software will command focal actuator to place objective lens on targeted position and motion detector software can be started to find targeted bovine sperm.

3.0 RESULTS AND DISCUSSION

3.1 Temperature Controller Testing

Bovine's semen temperature controller tested using two methods. First, step function test to get temperature characteristicsresponse due to certain set point. This method appliespositive step function and negative step function to get transient and steady state temperature characteristic. Second, varying set point to certain temperature ranges to get steady-state response profile. This test aimed to measure hardware fidelity response due to any desired set point input.

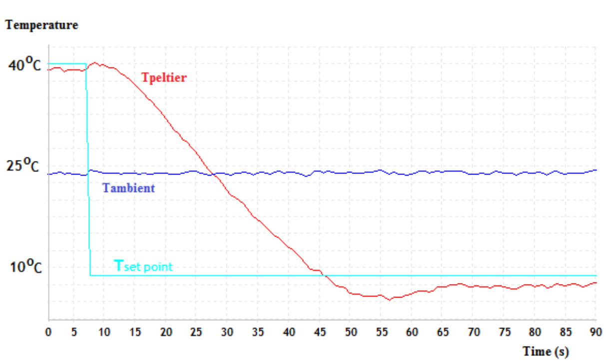


Figure 6 Cooling mode response ($40^oC$to 10^oC)

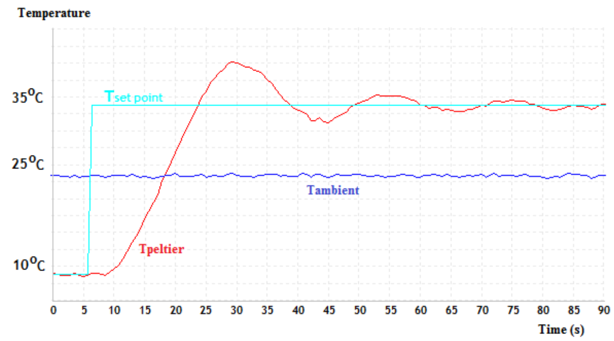


Figure 7 Heating mode response ($10^oC$to 35^oC)

Figure 6 and Figure 7 shows semen temperature controller can generate maximum cooling rate $-0.9^oC/s$ and maximum heating rate$+1.7^oC/s$. Maximum terminal temperature achieved in cooling mode is $-5,8^oC$. Overshoot highly visible when semen temperature controller operated on heating mode. PID control parameter set using optimum trial-error value $Kp = 25$, $Ki = 0.07$and $Kd = 10$.

Figure 8 shows semen temperature controller fidelity response due to certain set point command range. Semen temperature controller hardware has linear temperature response ranging from $-5^oC$to 50^oC in standard temperature and pressure($25^oC/1\,atm$) testing environment. Set point range above 50^oC was not performed because uncontrollable overshoot which potentially damagingpeltier modulein temperature controller.

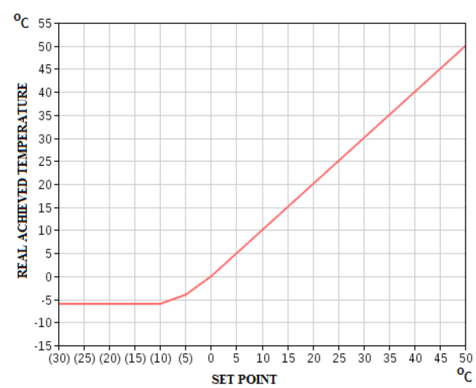


Figure 8 Set-point temperature fidelity response

3.2 Micro Actuator Testing

3.2.1 *Linearity Testing*

Linearity testing performed by actuating micro actuator independently in one axis direction then measuring final position using $10\,\mu m$ objective micrometers interpolated using image processing software. Figure 9 shows micro actuator linear testing result [12].

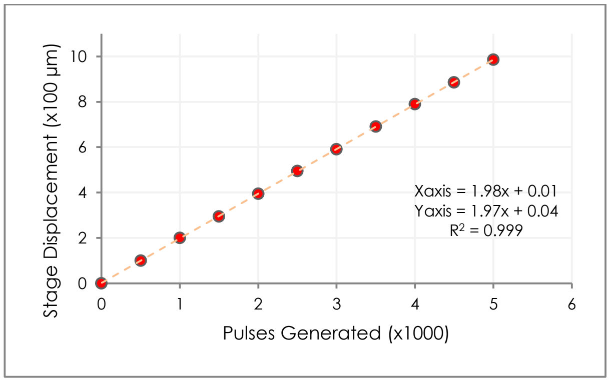


Figure 9 Micro actuator linear response

Figure 9 shows micro actuator has average horizontal micro step repeatability $0.198 \pm 0.001\ \mu m/step\ (y1)$ and vertical microstep repeatability $0.197 \pm 0.004\ \mu m/step\ (y2)$. Micro actuator has linear response with$R^2 = 0.999$. It can achieve maximum displacement speed$3,675\ \mu m/s$ at $18,519$ kHz signaling rate.

3.2.2 Hysteresis Testing

Hysteresis testing performed by moving micro actuator backward and forward $20x$repeatedly to obtain hysteresis response profile. Figure 10 shows hysteresis testing result measured by $10\ \mu m$objective micrometer resolutions interpolated using image processing software.

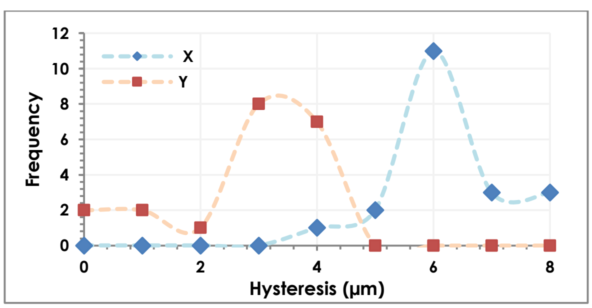


Figure 10 Micro actuator hysteresis response

Figure 10 showmicro actuator has average horizontal step hysteresis $5.99 \pm 1.09\ \mu m/step$ (diamond dots) and average vertical step hysteresis $2.36 \pm 1.28\ \mu m/step$ (square dots).

3.3 Motion Detector Testing

Motion detection software testing performed by an universal serial bus (USB) camera device and Brownian particle simulator. Motion detection software capturing random Brownian particle image were displayed onanother computer using USB camera device. At the same time, motion detection software performing real time image processing to find highest particle speed location. Table 1 shows motion detector testing result.

Table 1 Motion detection testing result

Particle	Software Tracking	Human Observation
1	Success, Stable	Clearly Visible
10	Success, Stable	Clearly Visible
50	Success, *Unstable*	*Adequate* Visible
100	*Failed*, *Unstable*	*Marginally* Visible

Table 1 show motion detection software success to identify fastest Brownian's particle up to50 random particles, above it motion detection software being unstable because uncertainty particle collision. Human eye can still identifymaximum Brownianparticle motion within 100 random particles. Although motion detection software just achieving 50% of human eye performance but motion detection software promising high reliability in continuous and heavy duty work.

3.4 Autofocus Testing

Autofocus testingis performed by capturing $10\ \mu m$ objective micrometers slide using various sampling displacement (Δz) at certain objective magnification. Image captured using OptiLab® Advanced microscope camera at $1024x768\ @24bit\ RGB$ resolution mode.

Table 2 Adaptive focus testing

Objective Lens	Adaptive Focus ($\varepsilon = 95\%$)
4x	Z_{opt} Locked, Stable
10x	Z_{opt} Locked, Stable
40x	Z_{opt} Locked, Stable
100x	Z_{opt} Locked, *Unstable*

Refer to Table 2, 100x objectives lens application cannot achieve stable Z_{opt} locking. Adaptive focus algorithm doesn't reliable and should be repeated several times to obtain Z_{opt} position because lack of intensity due to high power optic application. Autofocus performance can be enhanced by lowering back tracing similarity threshold (ε) below 90%

3.5 General Result

Bovine sperm selection procedure consisted of three main processes: (1) reduce and hold bovinesemen to certain temperature;(2) detect and recognize highest motile sperm within objective viewpoint and (3) track sperm at certain holding temperature until targeted sperm self-immotile. Figure 11 shows proposed hardware prototype and captured bovine sperm image within standard temperature and pressure($25^oC/1\ atm$) using 10X objective lens.

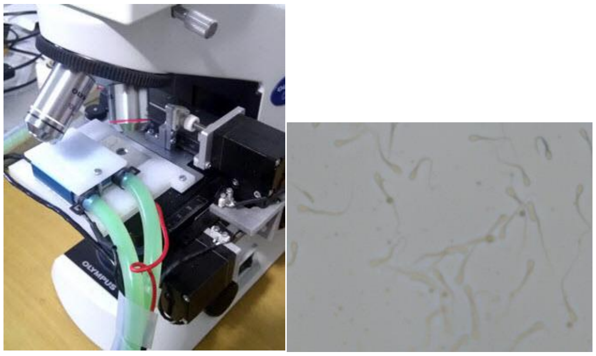


Figure 11 Sperm selection hardwareprototype (left) and captured bovine sperm (right)

Without presence of any cryopreservation solution, Mammal sperm still can be safely cooled using cooling rate between $-1^{\circ}\,\mathrm{C}/min$ to$-10^{\circ}\,\mathrm{C}/min$ and holding temperature between 0° C to 10° C[4][7][13]. Due to testing results, semen temperature controller performance exceeds all technical requirements to cooling down bovine semen safely. Maximum cooling rate can be provided by this temperature controller is$-54^{o}C/min$ with maximum negative temperature achieved is$-5.8^{o}C$.

Average velocity of fresh thawing bovine sperm is $23.33 \pm 1.42\ \mu m/s$ [13]. Due to motion testing result, micro actuator performance can fulfill minimum speed requirement providing real time sperm tracking therefore sperm tracking success factor entirely dependent on motion detector sensitivity.

Due to testing result, motion detection software performance is lower than professional observer when determining highest bovine sperm motility. Motion detection software performance also lower than standard computer-assisted sperm analysis (CASA)which can analyze 200 spermssimultaneously [2] but quite reliable to perform individual sperm selection aided using semen temperature controller.

Temperature controller will reduce bovine semen temperature and leave several active sperms which ready to analyzed using motion detection software. It helps motion detection software by lowering sperm candidate quantity through cooling process selection. In addition, autofocus algorithm also increasing motion detection sensitivity by enhancing sperm image contrast. Practically, application of 10X objective lens resulting optimum performance due to optical resolution, objective viewpoint area, dimension ratio and focus response profile.

4.0 CONCLUSION

This research developsnovel method achieving high quality bovine sperm to support bovine breeding industry. This method utilizes sperm motility decrementresponse when bovine semen applied into low temperature environment. Sperm selection procedure working autonomously makes active tracking until targeted sperm self-immotile due to long-term exposure of low temperature environment.

Temperature controller testing shows prototype device has linear set point response between $0^{o}C$ to $50^{o}C$ with maximum heating rate$+1.7^{o}C/s$ and maximum cooling rate $-0.9^{o}C/s$. It hasmaximum cooling terminal temperature $-5.8^{o}C$exceed mammal sperm safety cooling requirements ($-1^{o}C/min$ to $-10^{o}C/min$ at $0^{o}C$ to $10^{o}C$ holding temperature). Overshoot highly visible when semen temperature controller operated on heating mode. PID control parameter set using optimum trial-error value $Kp = 25$, $Ki = 0.07$and $Kd = 10$.

Microactuatorhas linear response ($R^2 = 0.999$) with average horizontal step $0.198 \pm 0.001\ \mu m/step$ and average vertical step $0.197 \pm 0.004\ \mu m/step$. It has average horizontal hysteresis $5.99 \pm 1.09\ \mu m$ and average vertical hysteresis $2.36 \pm 1.28\ \mu m$. Micro actuator prototype can achieve maximum displacement speed$3{,}675\ \mu m/s$ at $18{,}519$ kHz signaling rateexceed average velocity of fresh thawing bovine sperm($23.33 \pm 1.42\ \mu m/s$).

Motion detection software succeedsrecognizing fastest Brownian's particle up to 50 random particles, lower than average human eye which can recognizing up to 100 random particles. This detection performance is enough for proposed sperm selection method regarding sperm detection and tracking algorithm will be executed just when average sperm motility decreasedinto 5% from its initial value.

Autofocus algorithm was developed to increase motion detection software sensitivity. It work effectively using 4X, 10X and 40X objectives lens, but has low performance when applied to 100X objective lens because lack of intensity due to high power optic applicationPractically, application of 10X objective lens resulting optimum performance due to optical resolution, objective viewpoint area, dimension ratio and focus response profile.Finally, this proposed method still needs further development. Laser tweezers to capture selected sperm and ultra-sonication to immobilize targeted sperm permanently is promised technology supporting this novel bovine sperm selectionmethod.

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