

Computer-Assisted Analysis of Sperm Parameters after Selection of Motile Sperm by Either Percoll Gradient, Filtration, or Swim-up Procedures

C.N. Person, T.D. Lester, M.D. Person, and R.W. Rorie¹

Story in Brief

A computerized sperm analyzer was used to compare sperm parameters of frozen-thawed semen from 10 bulls, before and after selection of motile sperm, by either Percoll gradient, filtration, or swim-up procedures. Sperm parameters measured at 0, 4 and 8 h after selection included motility, progressive motility, velocity distribution, path velocity (VAP), progressive velocity (VSL), track speed (VCL), lateral amplitude (ALH), beat frequency (BCF), straightness (STR), linearity (LIN), head elongation and area. Percoll separation resulted in more total motile sperm recovered than either swim-up or filtration ($P = 0.001$). Percent motile, progressive, and rapid sperm did not differ ($P \geq 0.87$) across treatments. Across bulls, the percent motile sperm declined by approximately 50% from 0 to 4 h of culture, and by approximately another 33% from 4 to 8 h of culture. The VAP and VSL were similar ($P \geq 0.19$) across treatments, whereas VCL was greater ($P = 0.04$) for both filtration and swim-up than for Percoll. Sperm LIN and STR were similar across treatments ($P = 0.52$ and 0.93 , respectively), whereas ALH was greater for filtration and swim-up than for Percoll ($P < 0.01$). The BCF for filter-selected sperm was greater than that of either Percoll or swim-up selected sperm ($P < 0.01$). Sperm head elongation and area were also greater for filter-selected than either swim-up or Percoll ($P \leq 0.03$), whereas these parameters were similar for Percoll and swim-up ($P \geq 0.68$). Based on total motile sperm recovered, Percoll separation was superior to the other methods. Overall, results suggest that the method used for selection of motile sperm can influence some parameters related to motility and sperm head morphology.

Introduction

When performing *in vitro* fertilization on oocytes aspirated from ovarian follicles of superior donor cows, the producer may specify the use of semen that is of poor quality and thus, not well suited for *in vitro* fertilization. Semen is typically characterized based on sperm concentration, morphology, and motility. Computer-assisted sperm analysis provides objective information on a number of other sperm parameters that might be useful in characterizing semen and improving overall *in vitro* fertilization procedures.

Semen quality can be improved by utilizing procedures to select and concentrate the motile sperm. Three such techniques used for selection of motile sperm are Percoll gradient separation, filtration, and swim-up. Few studies have directly compared the efficiency of Percoll gradient, filtration, and swim-up procedures for recovery of motile sperm and what effects these selection procedures may have on various sperm function parameters. Because computer-assisted sperm analysis provides an objective repeatable measure of a number of sperm parameters, it might be able to detect differences in these parameters that are due to selection procedures but not obvious through routine analysis. Therefore, this study utilized a Hamilton-Thorne IVOS sperm analyzer to compare sperm parameters, related to motility and morphology before and after motile sperm selection procedures.

Experimental Procedures

Frozen-thawed semen from 10 bulls, varying from very good to poor quality, was washed by centrifugation in Sperm-TL medium to remove cryoprotectant and extender. Initial sperm parameters were then measured, using a Hamilton-Thorne IVOS sperm

analyzer. Within bulls, an equal number of sperm was assigned to the three motile sperm selection methods.

The Percoll gradient was prepared layering 2 ml of 45% Percoll - 55% Sperm-TL over 2 ml of 90% Percoll - 10% Sperm-TL, in a 15-ml conical centrifuge tube. (Parrish et al., 1995). Thawed semen was layered on top of the Percoll gradient and the tube centrifuged at 700x g for 30 min. As soon as the centrifuge stopped, the Percoll media and dead sperm were pipetted off. The sperm pellet containing motile sperm recovered from the bottom of the tube was re-suspended to approximately 80 million sperm/ml.

The sperm filter was prepared by placing 90 mg of Pyrex glass wool in the barrel of a 1 ml syringe and packing the glass wool to a depth of approximately 5 mm (Stubbings and Wosik, 1991). After rinsing the filter with Sperm-TL, semen previously washed in Sperm-TL was placed into the syringe and the motile sperm passing through the glass wool filter was collected in a 15 ml centrifuge tube. The collected sperm were then concentrated by centrifugation, the supernatant was removed and the sperm pellet re-suspended to approximately 80 million sperm/ml.

The swim-up procedure used for recovery of motile sperm was the same as reported by Parrish et al. (1995). For each bull, three culture tubes, each containing 1 ml of Sperm-TL, were equilibrated in a 5% CO₂ incubator at 39°C. Semen was carefully layered the medium in each tube and incubated in the incubator at 39°C for 1 h. After incubation, the upper 0.7 ml of medium in each tube containing the motile sperm was collected, pooled and centrifuged for 10 min at 200 x g. After removal of supernatant, the sperm pellet was re-suspended to approximately 80 million sperm/ml.

The motile sperm recovered by each selection method were maintained in a 39°C incubator with a humidified atmosphere of 5% CO₂ in air during the 8 h culture period. The IVOS system was used to measure sperm parameters immediately after selection and

¹ All authors are associated with the Department of Animal Science, Fayetteville.

again at 4 and 8 h post-selection. Within each sample, 12 different fields were scanned to determine averages for each sperm parameter. The sperm parameters measured after selection included motility, progressive motility, velocity distribution, path velocity (VAP), progressive velocity (VSL), track speed (VCL), lateral amplitude (ALH), beat frequency (BCF), straightness (STR), linearity (LIN), head elongation, and area (Table 1).

Data were analyzed with JMP software (SAS Institute, Inc., Cary, N.C.). Analysis of variance was used to compare the sperm parameters across treatments, within evaluation times and bulls. A multivariate analysis of variance was also used to compare total sperm across treatments by time in culture.

Results and Discussion

Mean values (across time) for each sperm parameter after selection of motile sperm by either Percoll gradient, swim-up, or filtration are presented in Table 2. Across bulls, Percoll separation resulted in more ($P = 0.001$) total motile sperm recovered than either swim-up or filtration, while swim-up did not differ from filtration for total motile sperm recovered. These results are in agreement with Sharma et al. (1997) who also reported Percoll gradient selection results in recovery of more motile sperm. Another study suggests that in addition to higher recovery rates, density gradient separation results in with better motion characteristics with increased hyperactivity and longevity (Tucker and Jansen, 2002). While Percoll gradient separation did result in more total motile sperm recovered, the percent of motile, progressive, and rapid sperm did not differ ($P = 0.87, 0.91, \text{ and } 0.94$, respectively) across treatments. Percoll gradient selection did result in more motile sperm categorized as slow than did swim-up ($P = 0.07$), while filtration was intermediate and similar to the other treatments. Across bulls, the percent motile sperm declined by $\sim 50\%$ from 0 to 4 hours of culture, and by approximately 33% from 4 to 8 h of culture.

Both sperm path and progressive velocity (VAP and VSL) were similar ($P \geq 0.19$) among treatments. Track speed (VCL) was greater ($P = 0.03$) for both filtration and swim-up than for Percoll, while linearity and straightness of sperm path were similar among treatments ($P = 0.52 \text{ and } 0.93$). Lateral amplitude (AHL) was greater for filtration and swim-up than for Percoll ($P < 0.01$). Beat frequency (BCF) was greater for filter selected sperm than either Percoll or swim-up ($P < 0.01$). Sperm head elongation and area were greater for filter-selected sperm ($P \leq 0.03$), but similar for Percoll and swim-up. These changes in sperm head length and area

may indicate that filtration helps to induce sperm capacitation.

Overall, these results suggest that the method used for selection of motile sperm can influence some of the parameters related to motility and sperm head morphology. Farrell et al. (1998) found that combinations of various sperm parameters, obtained from computerized analysis, such as ALH, BCF, LIN, VAP, VCL, VSL and STR, were highly correlated ($R^2 = 0.95 \text{ to } 0.98$) with bull fertility, as defined by 59-d non-return rates in dairy cows. Of these parameters, LIN, VAP, VSL and STR were not affected by sperm selection method, but ALH, VCL and BCF were increased by filtration. Therefore, further study is needed to determine if there is any advantage or one selection method over another, for in vitro fertilization rate.

The Hamilton-Thorne IVOS sperm analyzer is a reliable method for determining percent motility, percent progressive motility, and velocity parameters, due to its ability to detect even the slightest changes in sperm motion and to produce repeatable, reliable results without any technician bias. Additional studies will be conducted to determine if the sperm parameters measured by the IVOS system can be used to refine in vitro fertilization procedures and reduce bull to bull variability in fertilization rates.

Implications

Computer-Assisted Sperm Analysis is a useful tool for identifying differences in sperm parameters, related to motility and morphology. Characterization of semen, based on these parameters, could improve methods for assessing bull fertility and improve the efficiency of in vitro fertilization.

Literature Cited

- Farrell, P.B., et al. 1998. *Theriogenology* 49:871-879.
 Parrish, J.J., et al. 1995. *Theriogenology* 44:859-869.
 Sharma, R.K., et al. 1997. *Int. J. Fertil. Womens Med.* 42:412-417.
 Stubbings, R.B. and C.P. Wosik. 1991. *Theriogenology* 35:276.
 Tucker, K.E. and C.A.M. Jansen. 2002. In: *Proceedings 2nd International workshop for Embryologists.*

Table 1. Description of sperm parameters measured by the Hamilton-Thorne sperm analyzer.

Parameter	Description
Motile	Path velocity $\geq 30 \mu\text{m}/\text{sec}$ and progressive velocity $\geq 15 \mu\text{m}/\text{sec}$
Progressive	Path velocity $\geq 50 \mu\text{m}/\text{sec}$ and straightness $\geq 70\%$
Rapid	Progressive % with path velocity $> 50 \mu\text{m}/\text{sec}$
Medium	Progressive % with path velocity $< 50 \mu\text{m}/\text{sec}$ but $> 30 \mu\text{m}/\text{sec}$
Slow	Path velocity $< 30 \mu\text{m}/\text{sec}$ and progressive velocity $< 15 \mu\text{m}/\text{sec}$
Static	Sperm not moving at all.
Path velocity (VAP)	Average velocity of the smoothed (best fit) sperm path in $\mu\text{m}/\text{sec}$
Progressive velocity (VSL)	Average velocity ($\mu\text{m}/\text{sec}$) measured in a straight line from the beginning to the end of sperm track
Track speed (VCL)	Average velocity ($\mu\text{m}/\text{sec}$) measured over the actual point-to-point sperm track
Lateral amplitude (ALH)	Mean width (μm) of the head oscillation as the sperm swims
Beat frequency (BCF),	Frequency (MHz) of sperm head crossing the sperm average path in either direction
Straightness (STR)	Measures departure of average sperm path from straight line (ratio of VSL/VAP)
Linearity (LIN)	Measures departure of actual sperm track from straight line (ratio of VSL/VCL)
Elongation	Ratio (%) of head width to head length
Area	Average size (μm^2) of all sperm heads

Table 2. Mean values for sperm parameters after selection of motile sperm by either Percoll gradient, swim-up or filtration.

Item	Selection procedure			P value
	Percoll	Swim-up	Filtration	
Total motile sperm ($\times 10^6/\text{ml}$)	85.9 ^a	21.5 ^b	32.2 ^b	0.001
Total progressive ($\times 10^6/\text{ml}$)	72.2 ^a	18.5 ^b	24.3 ^b	0.001
% Motile	38.8	38.2	35.8	0.866
% Progressive	32.7	31.0	30.5	0.912
% Rapid	35.2	33.2	34.8	0.937
% Intermediate	3.7	2.6	3.6	0.268
% Slow	7.9 ^a	4.9 ^b	6.9 ^{ab}	0.075
Path velocity (VAP), $\mu\text{m}/\text{sec}$	100.0	117.2	107.0	0.210
Progressive velocity (VSL), $\mu\text{m}/\text{sec}$	91.5	105.5	88.6	0.191
Track speed (VCL), $\mu\text{m}/\text{sec}$	142.7 ^b	171.6 ^a	175.0 ^a	0.035
Linearity ratio	59.4	57.5	53.3	0.524
Straightness ratio	83.3	81.4	82.7	0.930
Lateral amplitude (AHL), μm	5.1 ^b	6.3 ^a	6.8 ^a	0.006
Beat frequency (BCF), MHz	21.6 ^b	22.6 ^b	28.7 ^a	0.007
Sperm head elongation	45.3 ^b	44.9 ^b	53.1 ^a	0.029
Sperm head area (μm^2)	4.9 ^b	4.8 ^b	7.6 ^a	0.001

a,b Means within rows with no superscript in common differ ($P < 0.05$).