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## Reproduction, Fertility and Development

## Supplementary Material

## Developing flow cytometry for precise evaluation of amphibian sperm viability: technical report

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Table S1. Generalized linear mixed model results testing for a correlation between microscopy and flow cytometry (bead) based methods of calculating sperm concentration. Significant terms are shown in bold.

Fixed Effect	Estim ate	S.E.	df	t	p value
(Intercept)	0.30	0.81	21	0.38	0.71
Flow- bead concentration	0.92	0.11	21	8.02	<0.001
Filtration (unfiltered vs. Filtered)	0.36	1.1	21	0.33	0.75
Concentration x Filtration (unfiltered)	-0.04	0.16	21	- 0.26	0.80
Random Effect	Varia nce	S.D.			
Individual identity	0.00	0.00			

Table S2. Generalized linear mixed model results testing for a correlation between microscopy and flow cytometry (volume) based methods of calculating sperm concentration. Significant terms are shown in bold.

Fixed Effect	Estimate	S.E.	df	t	p value
 (Intercept)	-0.73	0.83	21	- 0.88	0.39
Flow- volume concentration	1.10	0.12	21	9.09	<0.001
Filtration (unfiltered vs. Filtered)	0.35	1.16	21	0.30	0.77
Concentration x Filtration (unfiltered)	-0.04	0.17	21	- 0.23	0.82
Random Effect	Variance	S.D.			
 Individual identity	0.00	0.00			

Fixed Effect	Estimate	S.E.	df	t	p value
(Intercept)	6.81	0.07	21.0	103.7	<0.001
Filtration (Unfiltered vs. Filtered)	0.11	0.03	73.0	3.67	<0.001
Method (Flow- bead vs. Scope)	0.28	0.03	73.1	9.45	<0.001
Method (Flow – volume vs. Scope)	0.06	0.03	73.1	1.90	0.06
Filtration (unfiltered) x Method (flow – bead)	-0.07	0.04	73.0	-1.74	0.09
Filtration (unfiltered) x method (flow – volume)	-0.09	0.04	73.0	-2.10	0.04
Random Effect	Variance	S.D.			
Individual identity	0.07	0.26			

Table S3. Generalized linear mixed model results testing for an effect of microscopy vs. flow cytometry methods on measures of sperm concentration. Significant terms are shown in bold.

Fixed Effect	Estimate	S.E.	df	t	p value
(Intercept)	43.0	24.6	28.3	1.75	0.09
Flow viability	0.35	0.32	28.3	1.11	0.28
Filtration (unfiltered vs. Filtered)	-16.1	24.0	14.8	- 0.67	0.51
Viability x Filtration (unfiltered)	0.23	0.31	14.8	0.75	0.47
Random Effect	Variance	S.D.			
Individual identity	31.2	5.6			

Table S4. Generalized linear mixed model results testing for a correlation between microscopy and flow cytometry based methods of calculating sperm viability. Significant terms are shown in bold.

Fixed Effect	Estimate	S.E.	df	t	p value
(Intercept)	70.5	1.62	38.8	43.6	<0.001
Filtration (unfiltered vs. Filtered)	1.35	1.60	49.1	0.85	0.40
Method (flow vs. Scope)	6.46	1.59	49.5	4.07	<0.001
Filtration (unfiltered) x method (flow)	-1.92	2.23	49.1	- 0.86	0.39
Random Effect	Variance	S.D.			
Individual identity	23.44	4.84			

Table S5. Generalized linear mixed model results testing for an effect of microscopy vs. flow cytometry methods on measures of sperm concentration. Significant terms are shown in bold.