



## Supplementary Material

# Short Communication: Optimization of Acridine Orange Staining for Buffalo Sperm Cryopreserved in Egg Yolk based Extender to Detect DNA Fragmentation

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**Supplementary Table S1.- Comparison of washing with phosphate buffered saline (PBS), normal saline (NS) and without washing of semen sample before smear preparation on presence of debris in slide and intensity of acridine orange staining.**

	Washing with PBS	Washing with Normal Saline	Without washing	P-value
Debris Presence	1.77±0.22 <sup>a</sup>	1.66±0.16 <sup>a</sup>	3.88±0.20 <sup>b</sup>	0.0002
Intensity of staining	3.37±0.18	3.77±0.22	3.55±0.17	0.369

Different superscripts (<sup>a-b</sup>) in a row show significant difference.

**Supplementary Table S2.- Effect of single and double washing of semen sample with normal saline before smear preparation on score of debris presence and intensity of staining (1-5).**

	Single Washing	Double Washing	P-value
Debris Presence	1.50±0.19	1.44±0.18	0.824
Intensity of staining	3.87±0.23	4.11±0.20	0.247

There is no difference between single and double washing.