

## IVF FORM

Experiment	Date	Initial
Bull	Name:	Numbers:
Thawing (time)	Name:	Numbers:
Number of straws		
Water temperature		
Motility	After thawing:  After final dilution:	
Sperm wash	Wash twice by centrifugation at 328 x g for 5 minutes in 2-4ml BO-SemenPrep per 1-2 straws; Resuspend to the original volume with BO-SemenPrep (each straw has a volume of 0.25ml).	
Sperm count in Makler chamber	<p>Take a uniform (not vortexed) 25µl sample of the sperm suspension and mix with 25µm cold distilled water.            Mix the water/sperm well.            Take 10µl of the mixture and transfer to a Makler counting chamber.            The number of sperm in 10 squares corresponds to the number of sperm in millions per ml (x10<sup>6</sup>/ml) in the sperm suspension.            The dilution is 1:2 as 25µl of sperm suspension has been mixed with 25µl of cold water. Therefore, the number of sperm counted in 100 squares must be divided by 10, and multiplied by 2 to give the actual sperm concentration x10<sup>6</sup>/ml.</p> <p>Count in 100 squares:</p> <p>Count in 10 squares x dilution:</p> <p>Sperm x10<sup>6</sup>/ml:</p> <p>Finished (time):</p>	
IVF dishes	Make 4-wells with 400µl BO-IVF; add oocytes in ~60µl BO-IVF and ~40µl sperm dilution = 500µl	
End of IVM	Hours in IVM medium:  Cumulus expansion:  Viscoelasticity:	
Start of IVF	<p>Sperm volume: <math>\frac{2 \times 10^6 \text{ sperm/ml} \times 500\mu\text{l}}{\dots} =</math></p> <p>Final sperm concentration (in the well):</p> <p>Motility:</p> <p>Final sperm concentration usually <math>2.0 \times 10^6 / \text{ml} = 1 \times 10^6 / \text{well}</math></p>	