

# Viability of *Tritrichomonas foetus* Following the Freeze-Thaw Cycle Used for Freezing Bovine Semen

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### Summary and Implications

Although past research has proven the organism, *Tritrichomonas foetus*, can survive freezing technologies utilized in the 1950's, it is unknown if it can survive freezing in liquid nitrogen, the current freezing media for bovine semen. Live organisms were identified after thawing in five of the twelve straws evaluated. Between 0.5 and 6 live organisms were identified in the samples taken from these straws. The number of live organisms within each of these 0.5 mL straws was estimated to be between 25 and 300 live organisms. The ability of *T. foetus* organisms to survive current freeze-thaw techniques for bovine semen suggests that artificial insemination using semen from *T. foetus* positive bulls poses a significant risk for spreading this pathogen to other cattle in breeding herds.

### Introduction

*Tritrichomonas foetus*, the causative agent of bovine trichomoniasis, has been identified as a cause of bovine infertility for more than one hundred years. The widespread use of natural pasture mating in the beef cattle industry allows for transmission of the pathogen between breeding animals. Artificial insemination (AI) has been indicated as an alternative breeding method that decreases the spread of *T. foetus*. The objective of the study was to determine if *T. foetus* is capable of surviving the current freeze-thaw technology used to freeze commercial bovine semen. Current AI technology utilizes a variety of extenders for cryopreservation of bovine semen. An egg yolk citrate based extender is widely used throughout the industry and is considered an industry standard. Most of the published data regarding the ability of this organism to survive freezing was performed in the 1940's and 1950's. The freezing techniques, extenders, and cryoprotectant solutions used for those experiments are remarkably different from those used today.

**Table 1. Live *T. foetus* found post thaw.**

Day of Study	Straw number	Live Org. / 10µl	Live Org / straw	Total Cell Conc	% Live /straw
3	1	1	50	0.132 million/ml	0.076%
5	1	6	300	0.105 million/ml	0.571%
6	1	1	50	0.168 million/ml	0.060%
10	1	3	150	0.021/million/ml	1.429%

In the past, semen was frozen in glycerol, at a much different concentration compared to its current use, and stored at -95°C on dry ice, and *T. foetus* survived freezing and thawing at this temperature. Semen frozen commercially today is frozen in a lower concentration of glycerol and stored in liquid nitrogen at -195°C. It is unknown if *T. foetus* can survive at this temperature using commercially prepared extenders.

### Materials and Methods

A live culture of the *T. foetus* organism was used to inoculate egg yolk citrate extender (Trilady1@CSS, Minitube International; Tiefenbach, Germany) with glycerol as the cryoprotectant. The extender was added to the cell culture in a 2:1 ratio. The culture and extender mixture was then chilled at 4°C for 4 hours, loaded into thirty 0.5 mL Cassau straws used for artificial insemination, and then frozen and stored in liquid nitrogen (-195°C).

Two straws were thawed each day of the study in accordance with CSS (Certified Semen Services) in a 34°C to 36°C water bath for 1 minute. A 20 µL sample from each straw was visually evaluated for live motile *T. foetus* organisms using a phase-contrast microscope. A complete survey of the slide was accomplished using a measured grid method. Samples with positive *T. foetus* were calculated as to number of organisms per straw.

### Results and Discussion

On Days 3, 5, 6, and 10 of the study, live, motile *T. foetus* organisms were identified using the phase-contrast microscope at 200X and 400X objective. A total of five out of twelve straws had live *T. foetus* organisms identified. These results are significant as *T. foetus* organisms that survive freezing and thawing within bovine semen intended for use in artificial insemination pose a risk for transmission of this pathogen to breeding females. Cows have become infected with *T. foetus* with infectious doses as low as two hundred organism.

The next phase of research will be to artificially inseminate heifers with semen from *T. foetus* positive bulls that was frozen as described above. If infection can be produced from positive bulls, this will have a significant impact on the Custom Collection AI industry that does not currently test bulls for *T. foetus* prior to collection and use.