

## Abstract

- The aim of the study was to design a protocol for the thawing of semen that would allow cooled transported frozen-thawed semen to be used effectively.
- The study was expected to provide information regarding the effectiveness of cooling semen after it has been thawed and diluted for shipment.
- A single ejaculate from six stallions was frozen in two commercially available extenders, FR5 and lactose-EDTA (L-EDTA). Each of the samples was subject to various thawing treatments and temperatures. The straws were cooled and held at 5°C for 24 hours until analysis.
- Analysis of post-thaw sperm motility parameters was carried out using computer-assisted semen analysis.
- When thawing sperm for cooled shipment, it is recommended that straws be thawed at 72 degrees for 7 seconds, followed by 37 degrees for 30 seconds. Diluting the sperm exhibited no benefit to sperm thawed at this temperature.

## Introduction

When using cooled semen there are times when a mare is ready to ovulate but the stallion is overbooked, or unavailable for collection. In addition, many breeding farms lack the facilities to receive frozen semen. With the acceptance of frozen-thawed semen use, a stallion's semen may be banked for use in these circumstances and shipped as cooled semen..

Thawing and handling procedures of frozen stallion semen are unfamiliar to some owners and practitioners. The aim of this study is to design a protocol that will allow sperm to be thawed effectively for breeding use, following cooled shipment.



## Materials and Methods

Six light horse stallions aged 9-22 with varying semen quality and semen freezing capacity were used in this study. A single ejaculate was collected from each stallion using a CSU model artificial vagina. The ejaculate was diluted at a ratio of 1:1 (vol:vol) with an antibiotic-free skim-milk based extender and centrifuged at 1000x gravity. The sample was divided and the appropriate quantity of FR5 or L-EDTA freezing diluents was added to each to give a final sperm concentration of 200 x 10<sup>6</sup> cells/ml. The sperm were packaged into 0.5ml straws for freezing and maintained at -196°C until thawing. The sperm were thawed as outlined in table 1.

Table 1. Thawing treatments

Treatment number	Thawing temperature	Thawing time	Further treatment
1	37°C	30 seconds	Analysed at time 0 **
2	37°C	30 seconds	Left undiluted in straws, cooled to 5°C for 24 hours
3	37°C	30 seconds	Diluted to 50x10 <sup>6</sup> sperm/ml, cooled to 5°C for 24 hours
4	72°C	7 seconds	37°C for 30 seconds, Analysed at time 0 **
5	72°C	7 seconds	37°C for 30 seconds, left undiluted in straws, cooled to 5°C for 24 hours
6	72°C	7 seconds	37°C for 30 seconds, diluted to 50x10 <sup>6</sup> sperm/ml, cooled to 5°C for 24 hours
7	5°C	5 minutes	Analysed at time 0 **
8	5°C	5 minutes	Left undiluted in straws, Cooled to 5°C for 24 hours
9	5°C	5 minutes	Diluted to 50x10 <sup>6</sup> sperm/ml, cooled to 5°C for 24 hours

Thawing procedures were carried out by placing straws in a water bath, set to the required temperature, for the stipulated time. Samples to be diluted were diluted in INRA96 and then re-packaged into straws. The samples were then cooled by placing the straws into an Equine Semen Transporter where they were cooled to 5°C and maintained for 24 hours until analysis. Samples were placed in 20 °C air for 15 minutes before analysis. Samples were analysed using a computer-assisted semen analysis system. The percent total motility (TOT), percent progressive motility (PROG) and average path velocity (VAP) were recorded.

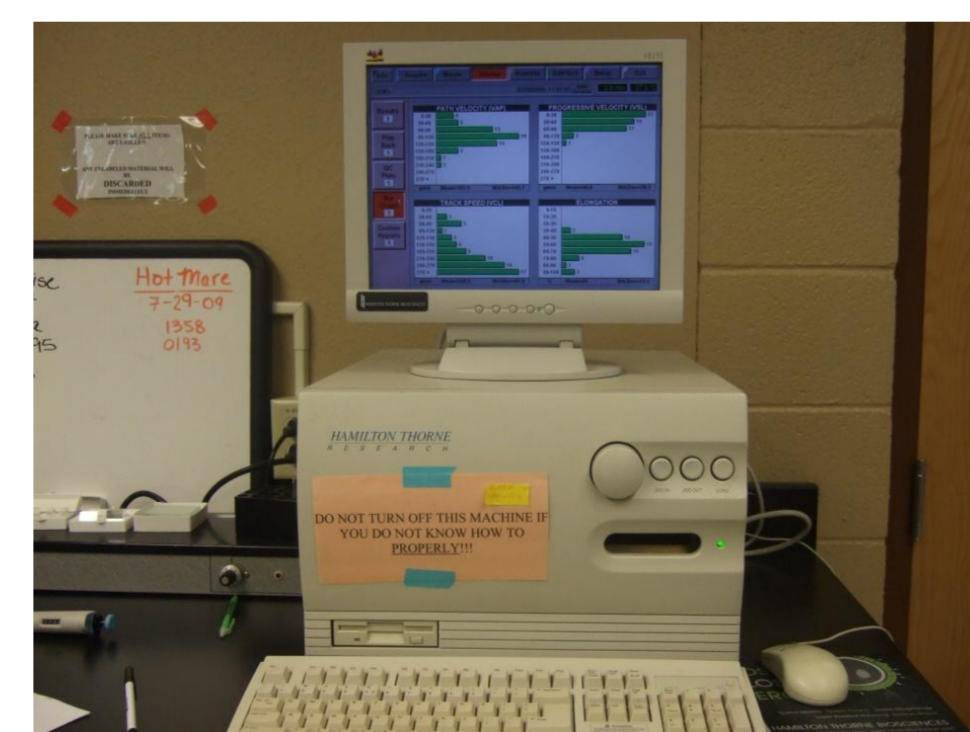


Figure 1. CASA (Hamilton-Thorn HT-IVOS Motility analyser, Hamilton-Thorne Research, Danvers, MA)

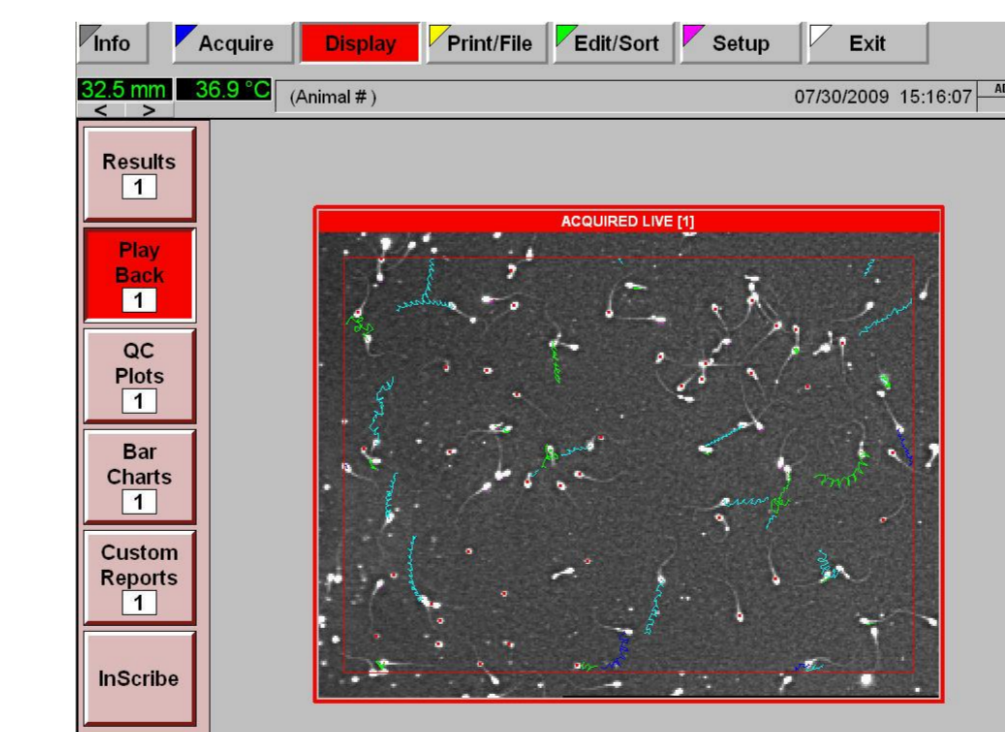


Figure 2. CASA screen capture measuring motility parameters of the spermatozoa

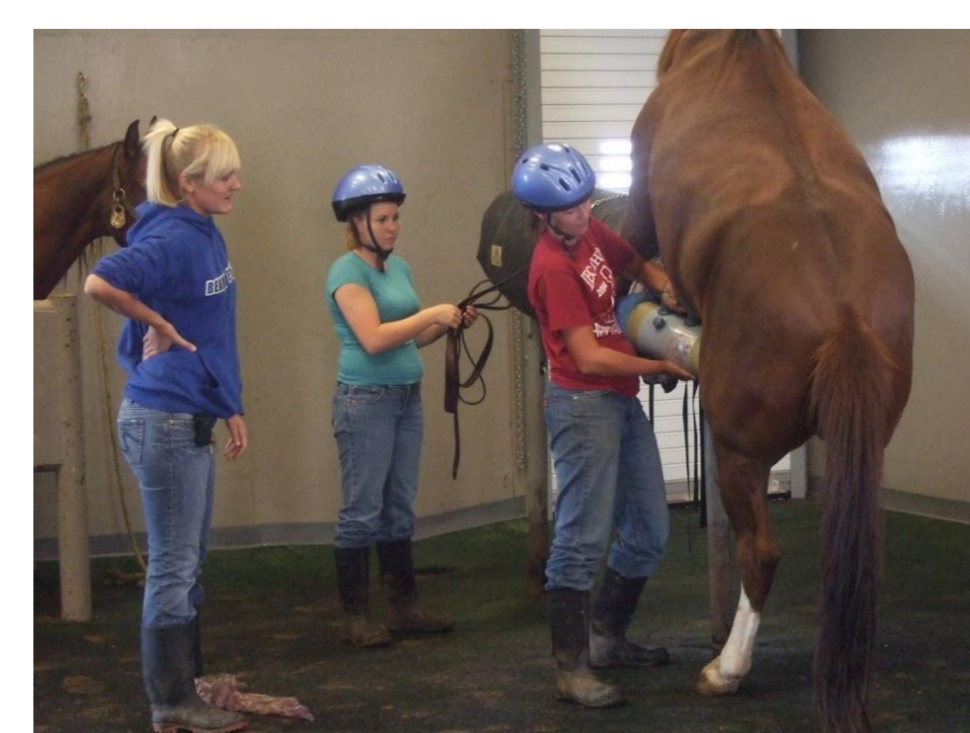


Figure 3. Stallion collection

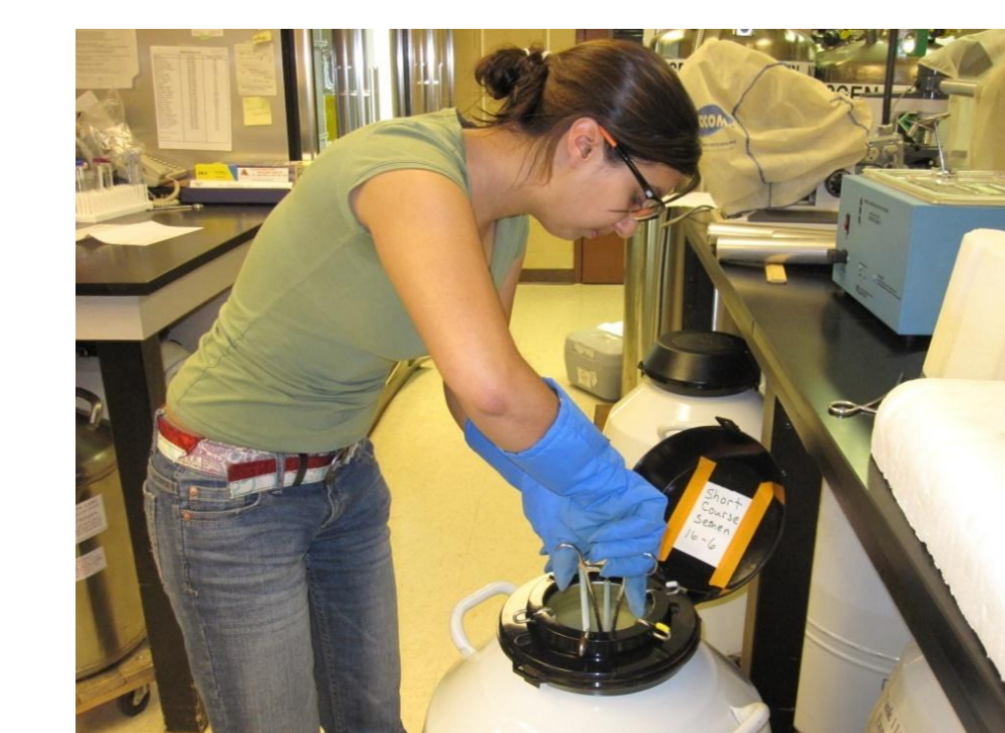


Figure 4. Semen handling

## Results

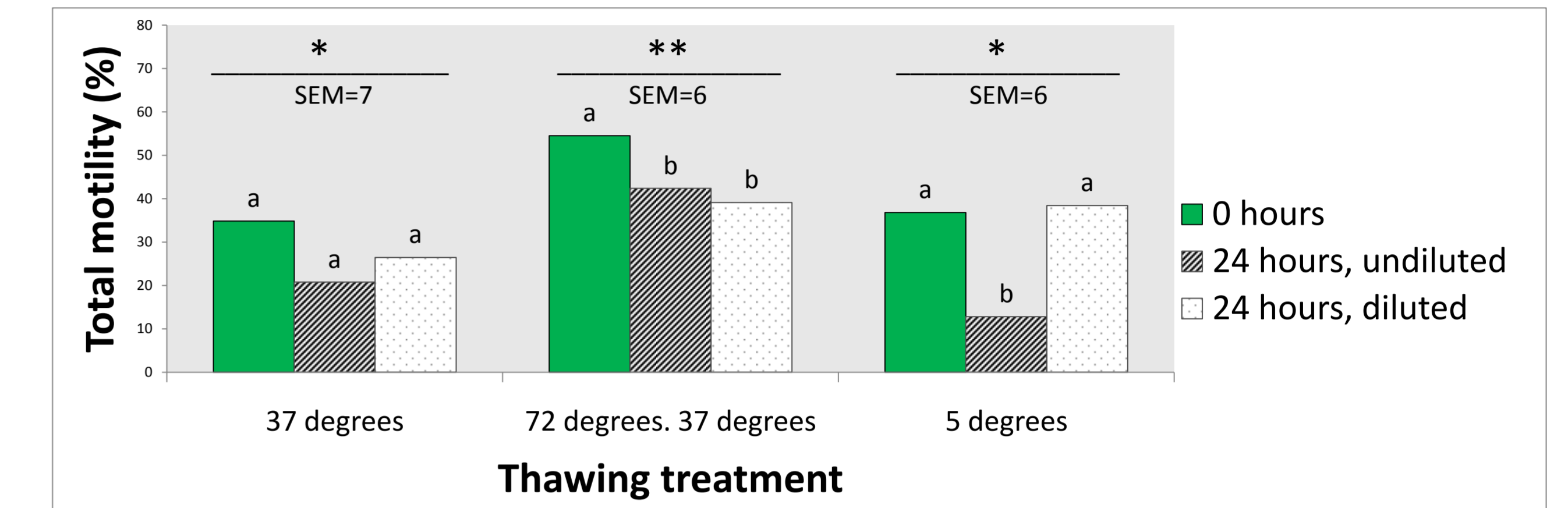


Figure 5. Post-thaw total motility of stallion spermatozoa thawed at 37, 72 and 5 degrees at 0 hours, 24 hours undiluted or 24 hours diluted. Results with different alphabetical superscripts denote a significant difference between treatments (P<0.05). Double asterisk treatments were found to be significantly different to other treatments.

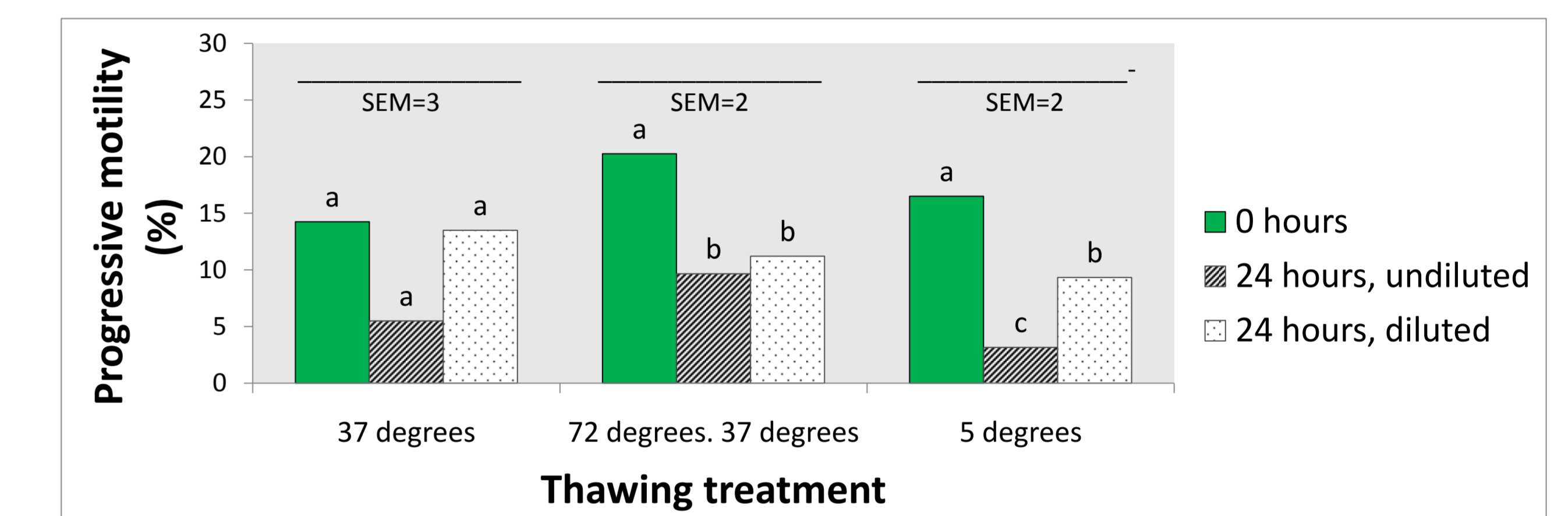


Figure 6. Post-thaw progressive motility of stallion spermatozoa thawed at 37, 72 and 5 degrees at 0 hours, 24 hours undiluted or 24 hours diluted. Results with different alphabetical superscripts denote a significant difference between treatments (P<0.05).

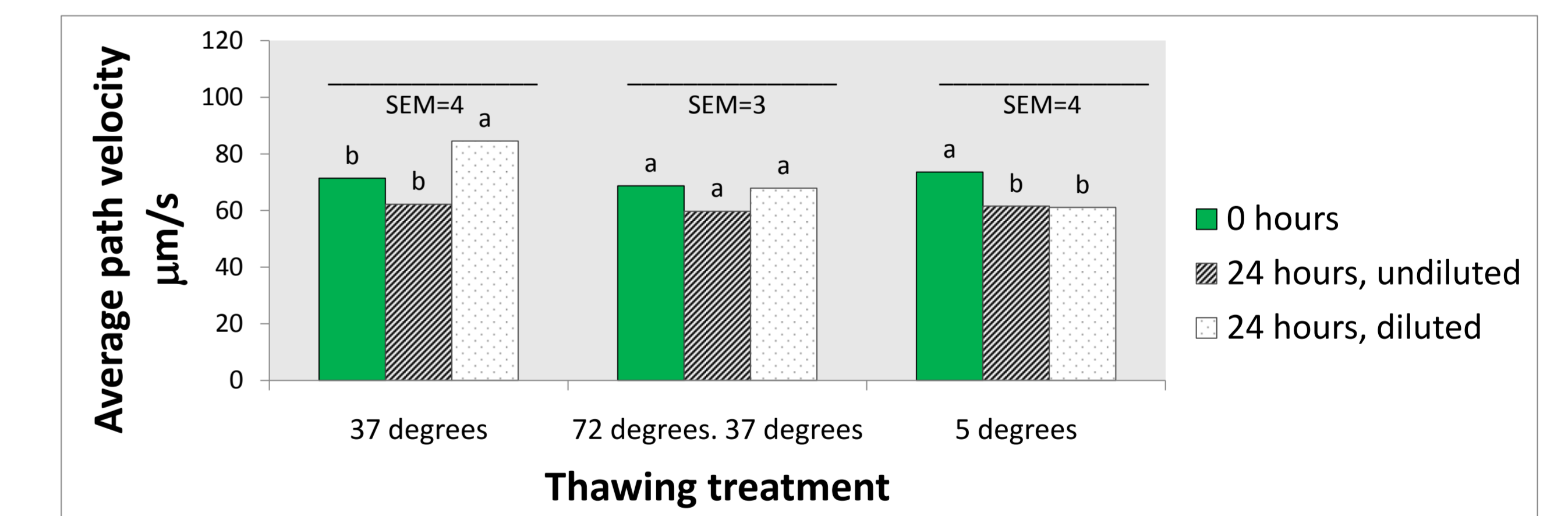


Figure 7. Post-thaw average path velocity of stallion spermatozoa thawed at 37, 72 and 5 degrees at 0 hours, 24 hours undiluted or 24 hours diluted. Results with different alphabetical superscripts denote a significant difference between treatments (P<0.05).

## Discussion and Conclusion

- Cryopreserving stallion spermatozoa in FR5 or L-EDTA diluent resulted in similar total and progressive motility.
- Thawing at 72 degrees resulted in significantly higher total motility of spermatozoa than other treatments.
- Within thawing treatments, diluting samples had little effect on the motility of spermatozoa thawed at 72 or 37 degrees, however diluting sperm thawed at 5 degrees significantly improved both total and progressive sperm motility.
- When thawing sperm for cooled shipment, it is recommended that straws be thawed at 72 degrees for 7 seconds, followed by 37 degrees for 30 seconds and shipping the sperm. Diluting the sperm exhibited no benefit to sperm thawed at this temperature.

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