

# Effect of canine sperm concentration using INRA96 extender on sperm quality after cooling for 24 hours

## Background

- Shipping cooled canine semen for artificial insemination by veterinarians helps eliminate both time and geographical constraints often experienced by dog breeders.
- The INRA96 extender is a popular equine milk-based extender that works well in canines at a more cost-efficient price than current canine-specific extenders for mixed private practices.
- Currently, there are no standard protocols describing the ideal concentration to extend canine semen in preparation for cooled shipment.

## Hypothesis

- Lower sperm concentrations ( $50 \times 10^6$  sperm/mL) will result in better total motility and progressive motility after being chilled for 24 hours.

## Methodology

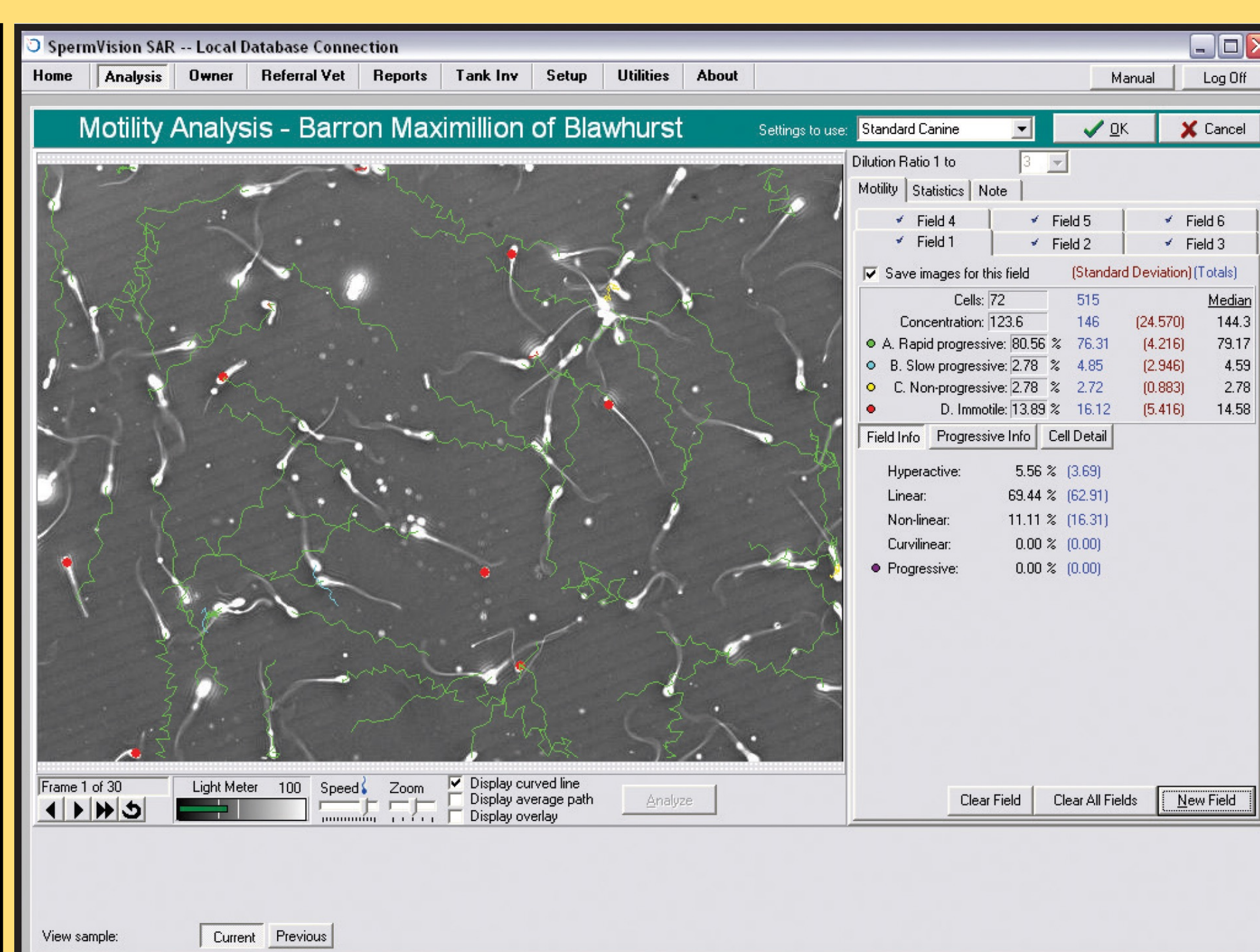
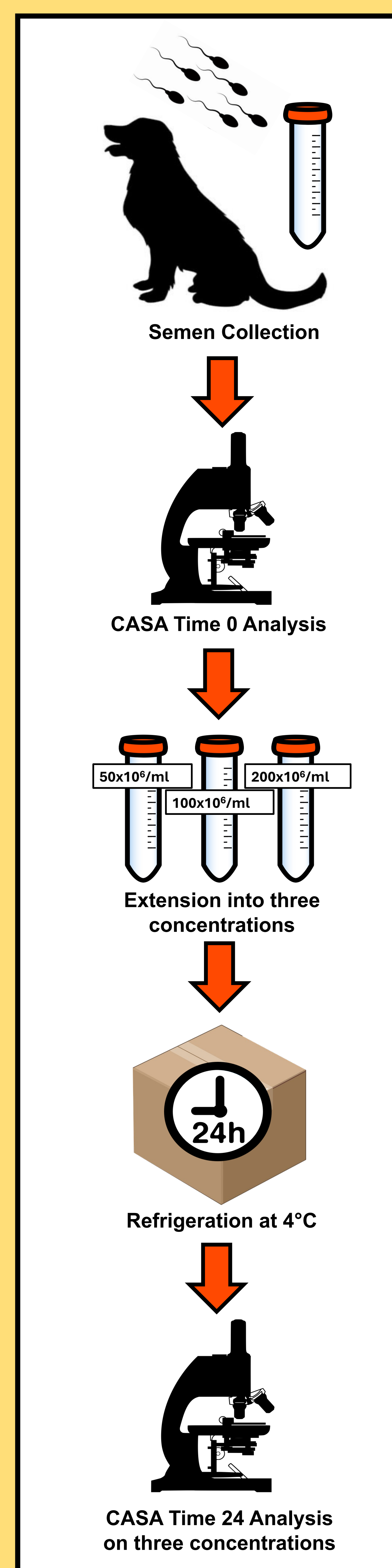


Figure 2: Example of Sperm Vision® a CASA software<sup>1</sup>

- The sperm-rich fraction was collected from the ejaculates of 11 male dogs by manual stimulation with a final total sperm number of at least  $352 \times 10^6$  per collection.
- Total motility and progressive motility was measured using a computer-assisted sperm analysis (CASA) at time of semen collection (time 0 = T0).
- If needed, semen was centrifuged and the pellet re-extended into three concentrations: 50, 100, and  $200 \times 10^6$ /mL to a final volume of 1 mL placed into an aliquot respecting the recommended semen:extender ratio of 1:3.
- The aliquots were placed into a standard canine semen transport box with a freezer pack to simulate shipping conditions for 24 hours at approximately 4°C.
- Total motility and progressive motility of all 3 aliquots were measured using the CASA after 24 hours (time 24) in the shipping box.

Figure 1: Schematic of methodology

## Results

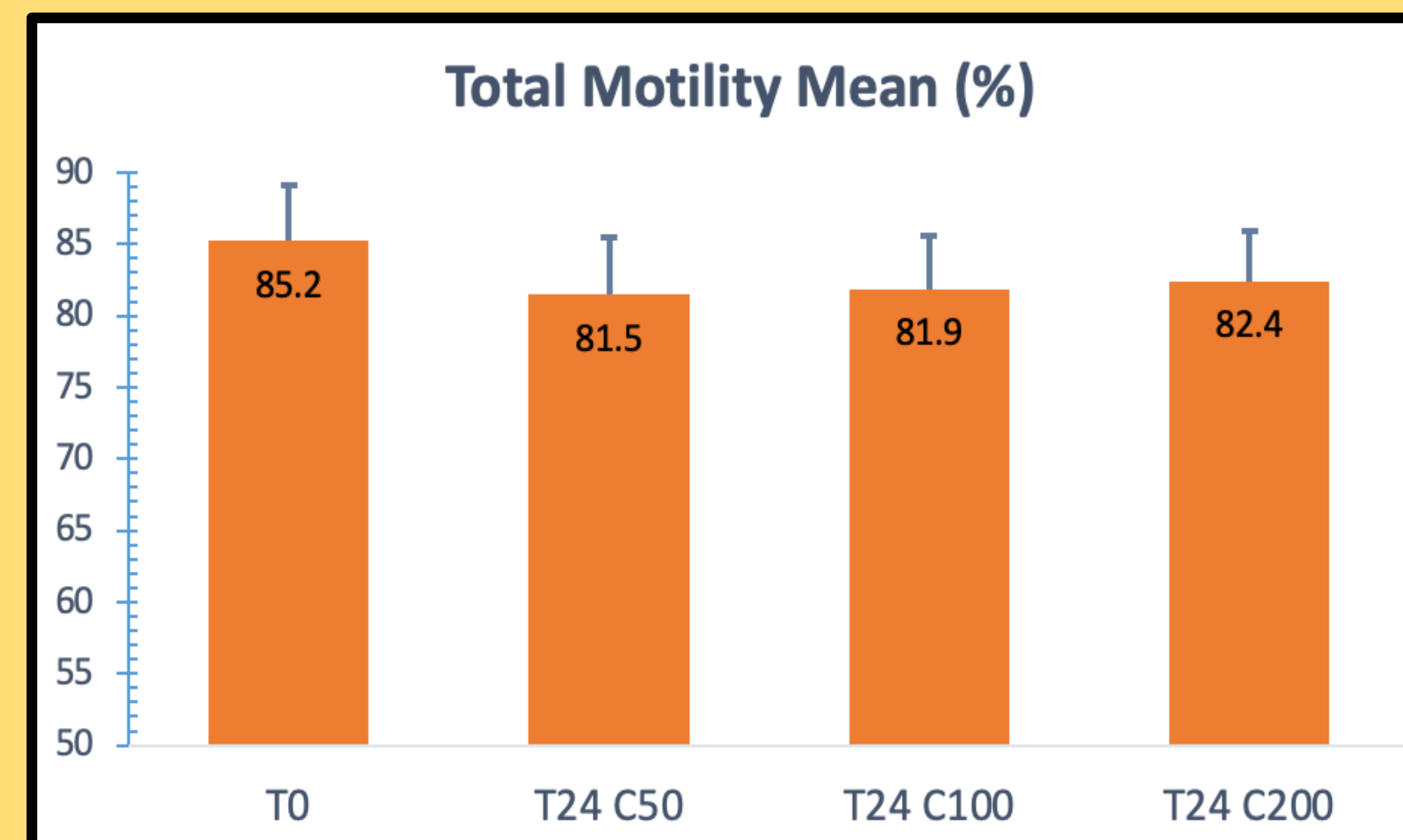


Figure 3: Mean of total motility at T0 and at 50, 100, and  $200 \times 10^6$ /mL at T24; SEM

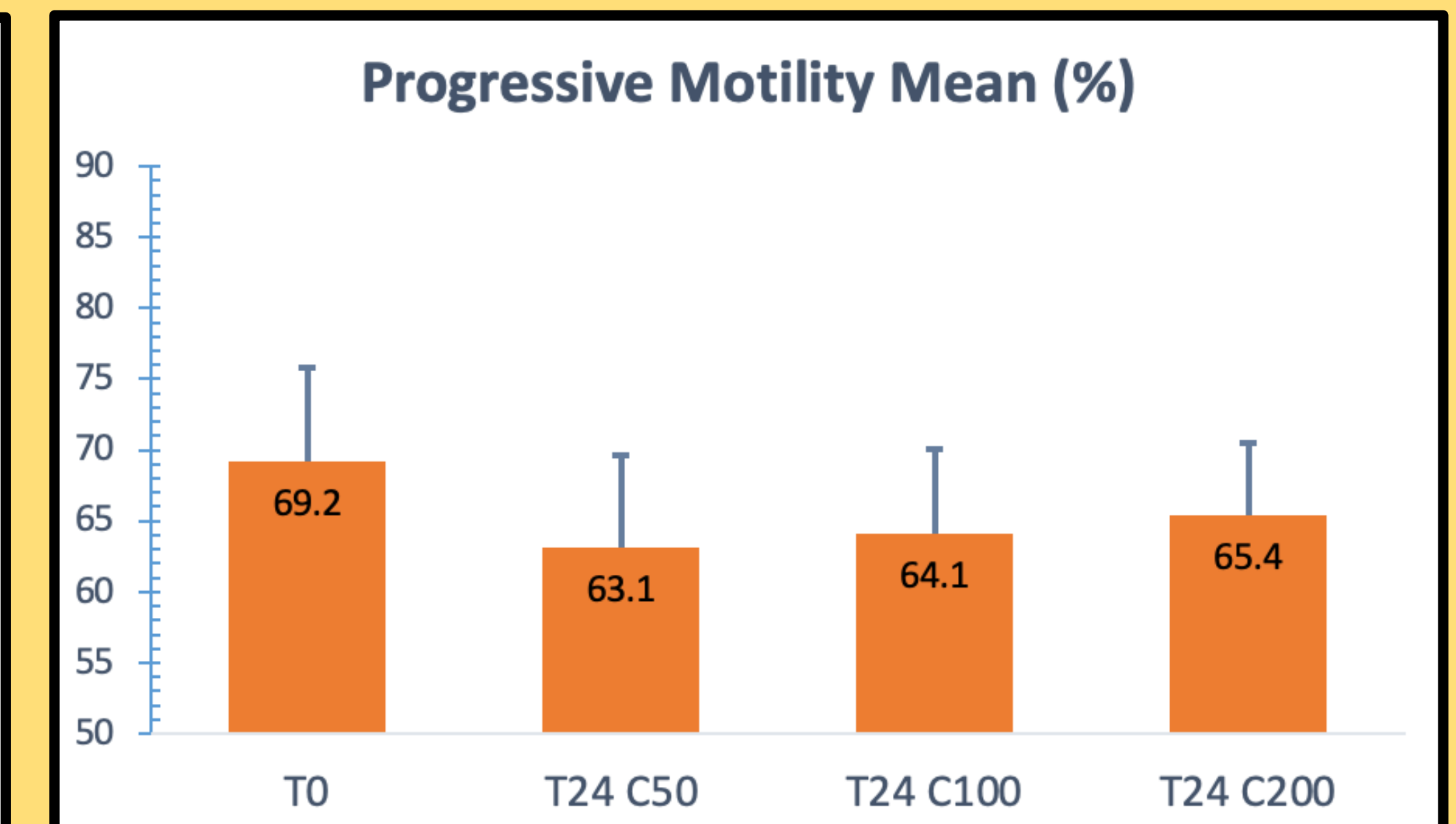


Figure 4: Mean of total progressive motility at T0 and at 50, 100, and  $200 \times 10^6$ /mL at T24; SEM

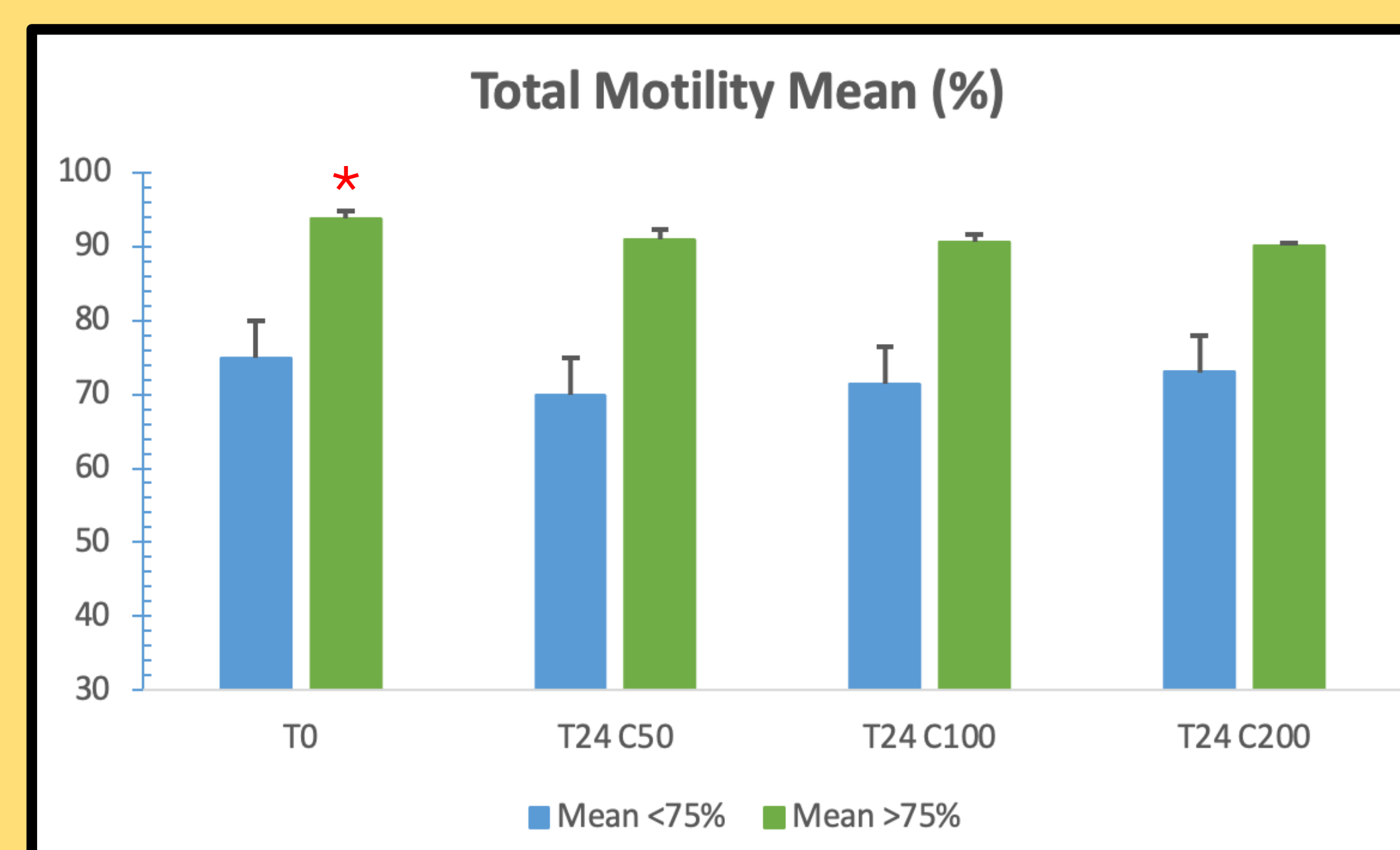


Figure 5: Mean of total motility of dogs with >75% (n=6) and <75% (n=5) progressive motility at T0 among the different concentrations at T24; SEM. There is a significant difference in motility between the higher and lower motility groups, but not between concentrations or time points within either group.

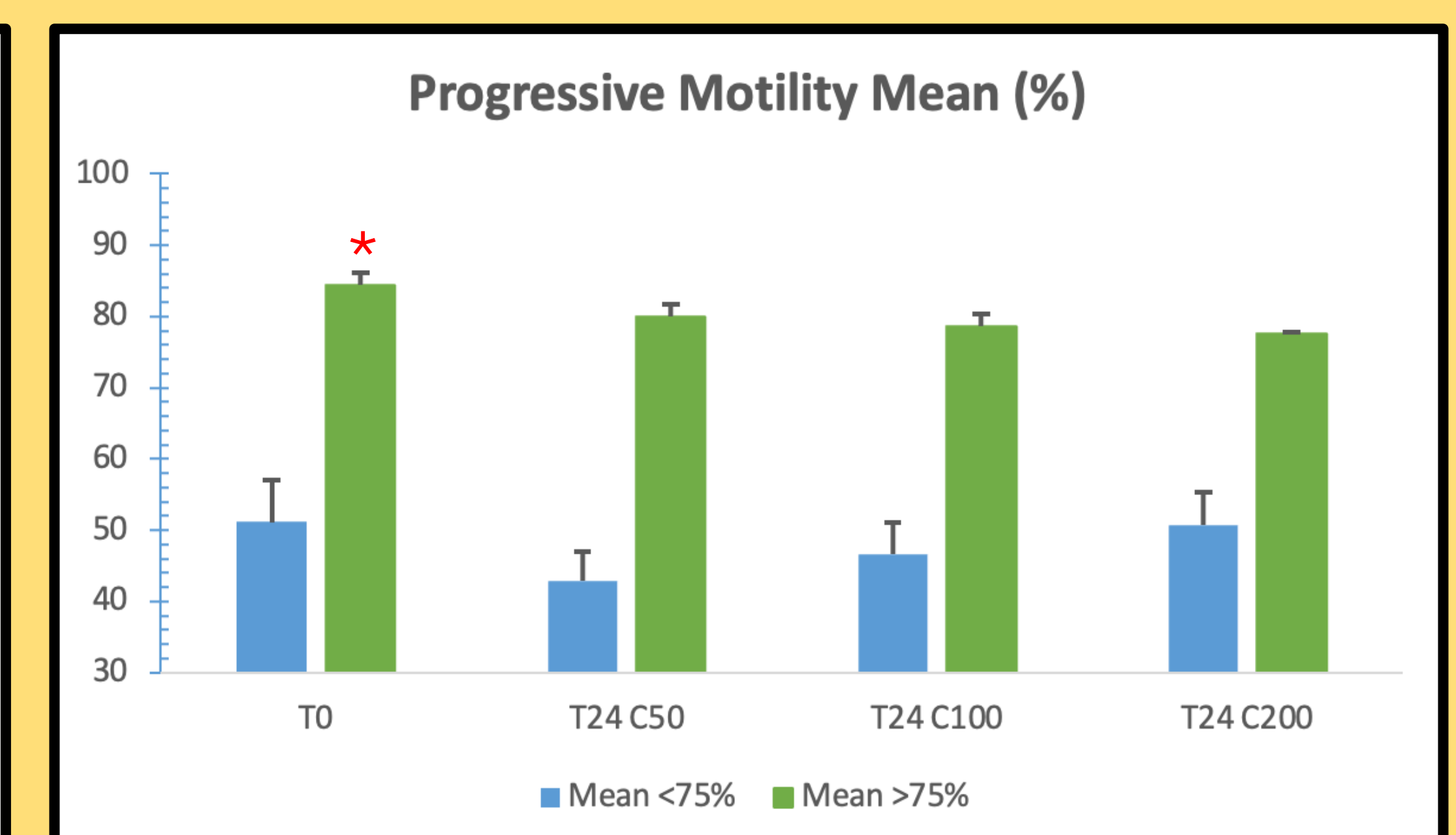


Figure 6: Mean of total progressive motility of dogs with >75% (n=6) and <75% (n=5) progressive motility at T0 among the different concentrations at T24; SEM. There is a significant difference in motility between the higher and lower motility groups, but not between concentrations or time points within either group.

## Conclusion

- Contrary to our initial hypothesis, different sperm concentrations did not result in different total motility and progressive motility after being chilled for 24 hours.
- There was no difference in total or progressive motility between concentrations or time points, and these results indicate that INRA96 is a safe extender for shipping cooled canine semen over 24 h.
- There is a high variability between animals and further studies with more animals are needed to see if differences between times or concentrations would appear.

## Limitations

- INRA96 is a milk-based extender so it is unknown if similar results would be found if other extenders are used, especially ones that are not milk-based.
- High variation among individuals account for the possible lack of differences among concentrations. A higher sample size could potentially surpass this issue.
- A fertility test would provide better clarification on the effect of different concentrations on pregnancy rates and/or litter sizes.

## Acknowledgements

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- References:**
  - 1. Minitube. Sperm Vision® SAR, CASA software with PC and monitor. Minitube.com. Accessed July 29, 2024. [minitube.com/catalog/en/spermvision-sar-p1171/](https://minitube.com/catalog/en/spermvision-sar-p1171/).