Double freezing for future sperm banking

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Background

The costs involved in the collection and processing of semen from multiple bulls under evaluation in commercial insemination centers are enormous.

Currently in bovine insemination centers, for example, up to 40,000-50,000 straws are stored in liquid nitrogen from each bull under evaluation. It takes about 4 years and only 10% of the bulls are selected, while 90% of the doses are disposed.

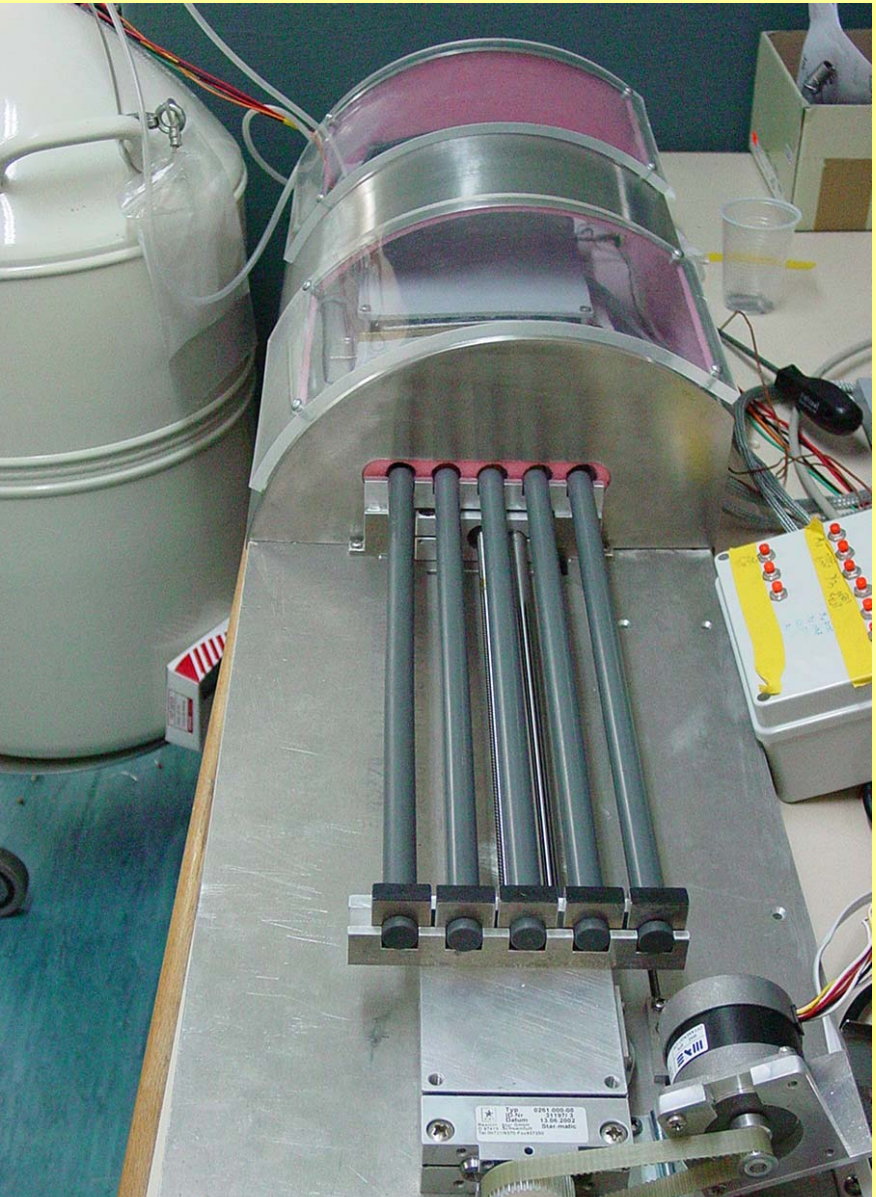
For this reason, it is necessary to reduce the costs without affecting the quality of the insemination dose or the conception rate.

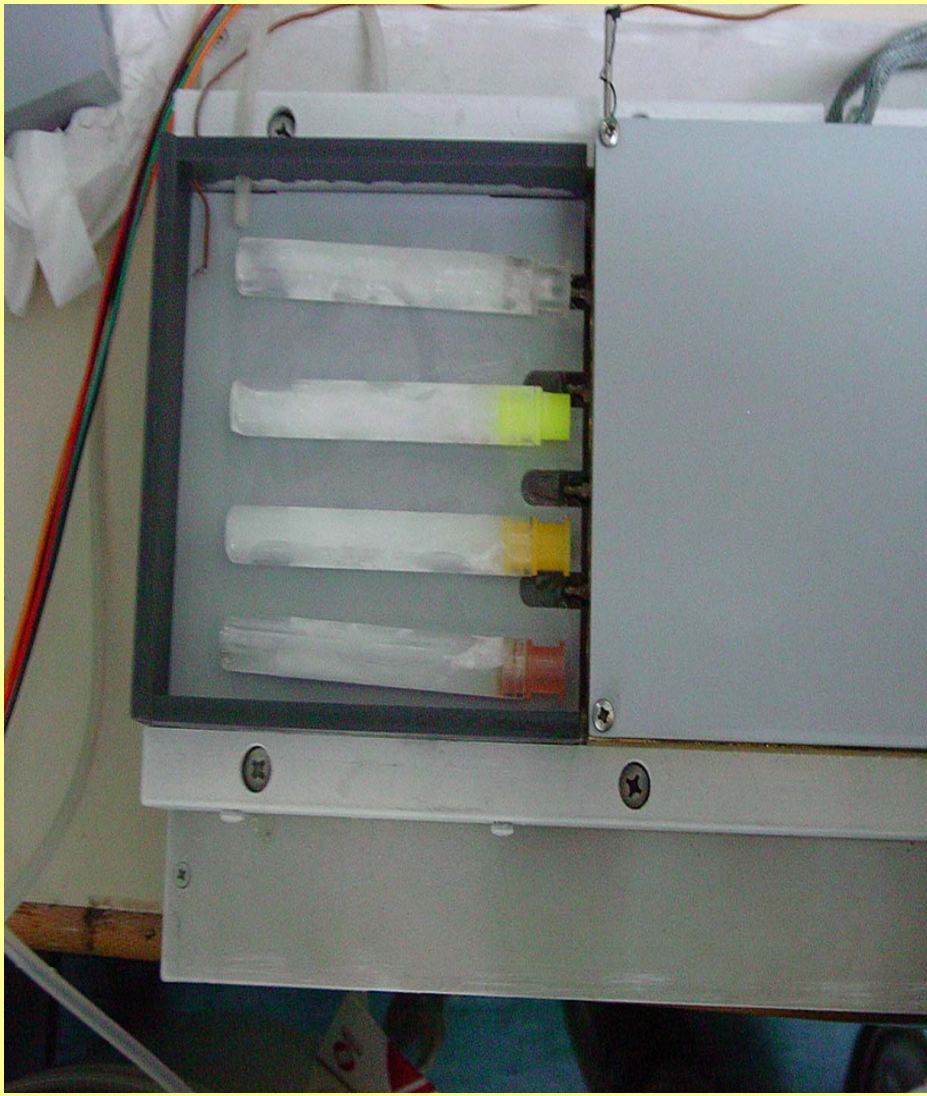
Double freezing

Double freezing can be a solution.

Semen is frozen in large volume and higher concentration in reusable 8 ml. tubes, to save costs, and then, for the selected bulls, it can be thawed, diluted, repackage in straw and frozen again.

Directional freezing





Objectives

1. To examine the changes in semen quality, frozen in large volume and high concentration, thawed, diluted, and refrozen again in straws (Double freezing).

2. To examine the influence of the double freezing technique on conception rate in dairy cows.

The experiment

Semen of four Israeli Holstein bulls was used for the experiment. Each ejaculate was split into two parts:

- One part was frozen in 0.25 ml. straws by the conventional freezing technique.
- The second part was frozen in 8 ml. cryogenic tubes by the directional freezing technique.

The experiment

The tubes were thawed, diluted and frozen again in 0.25 ml. straws (second freezing).

3610 cows and heifers were inseminate at random with semen frozen with one of the two techniques. 400 to 500 cows were assigned to each bull in each treatment.

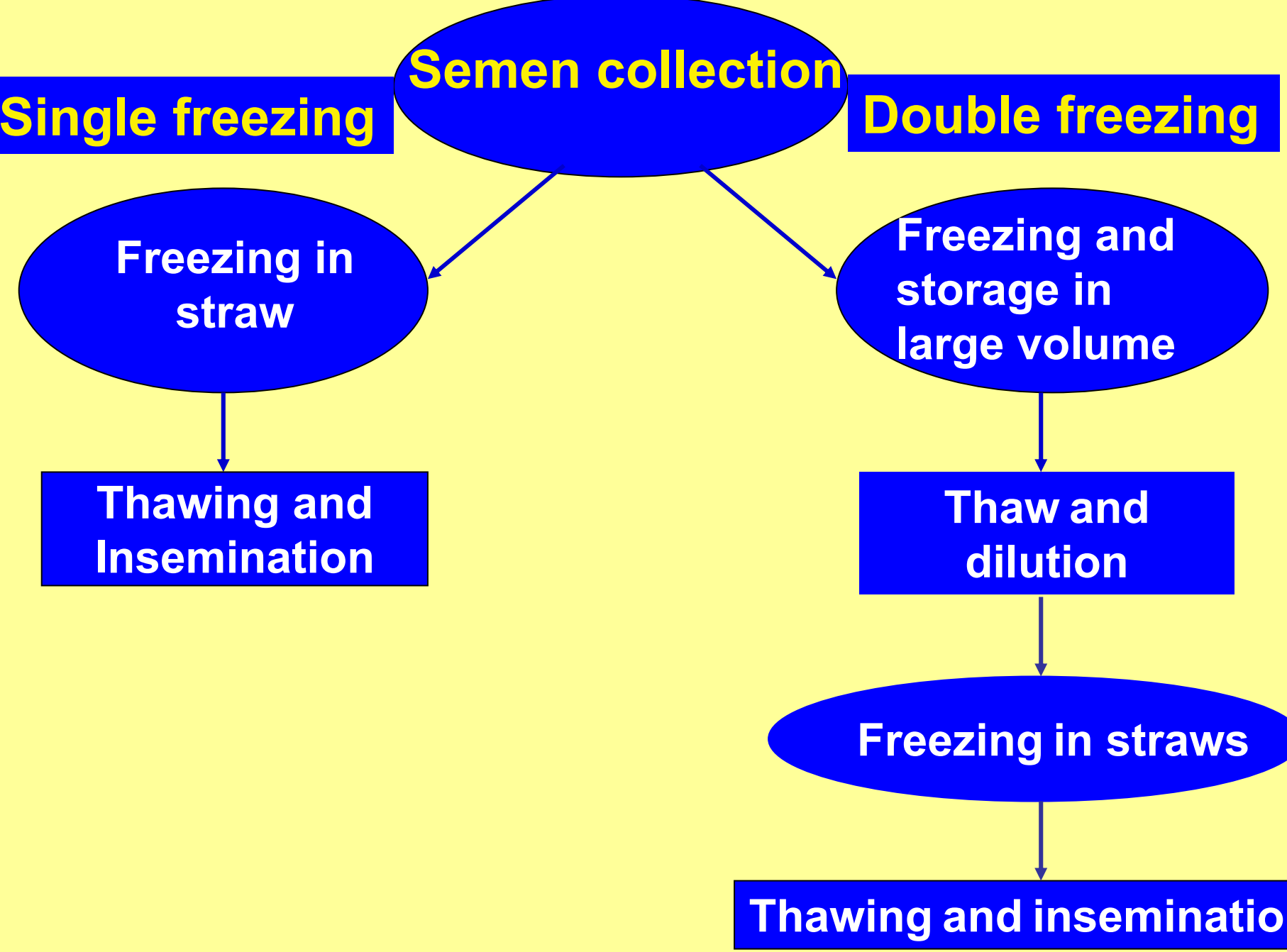
The tested parameters

In the laboratory:

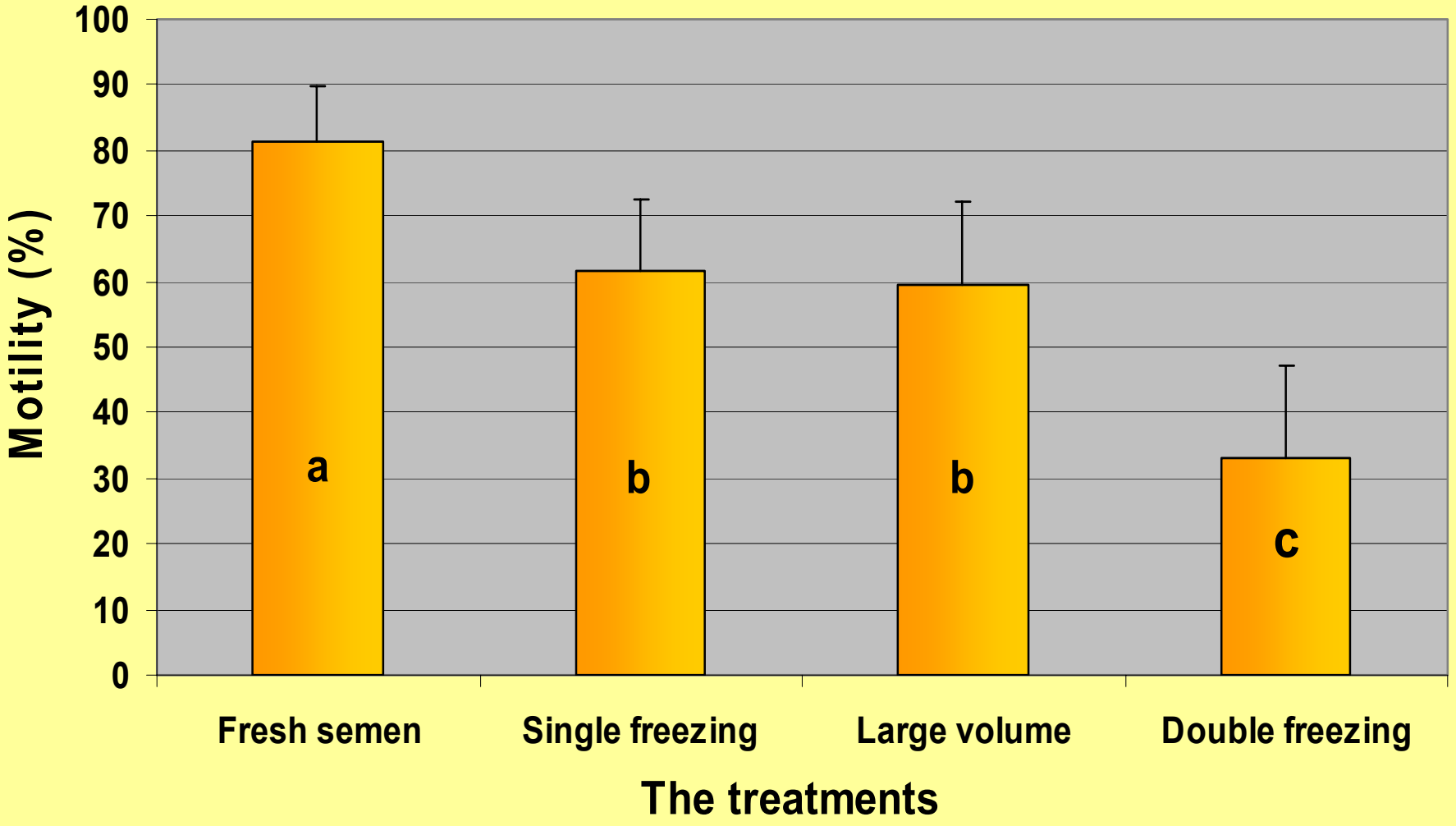
1. Motility of the sperm before and after freezing (single and double)
2. Membrane and acrosome integrity by fluorescent staining.

In the field:

Conception rate of cows and heifers, inseminated with semen frozen by one of the two techniques. Pregnancy diagnosis 42 days after insemination by rectal palpation.

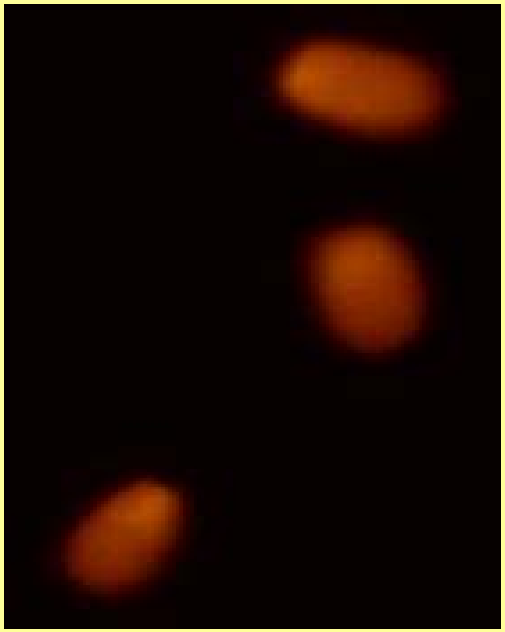

Semen collection
Single freezing
Double freezing
Freezing in straw
Freezing and storage in large volume
Thawing and Insemination
Thaw and dilution
Freezing in straws
Thawing and inseminatio

Comparative motility evaluation between treatments

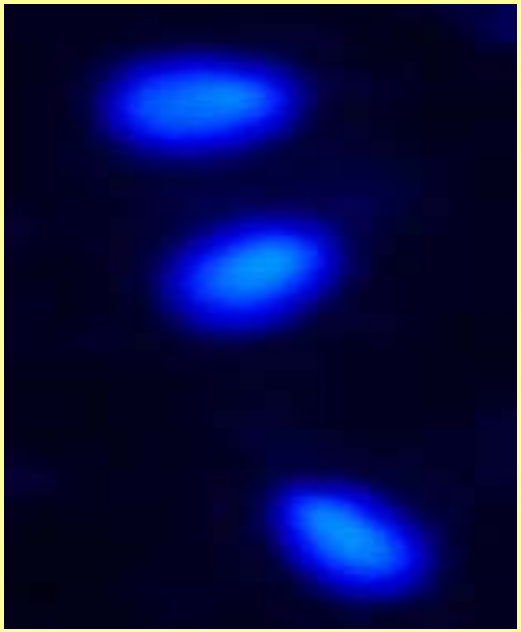


Columns with different letters are significantly different (P<0.001)

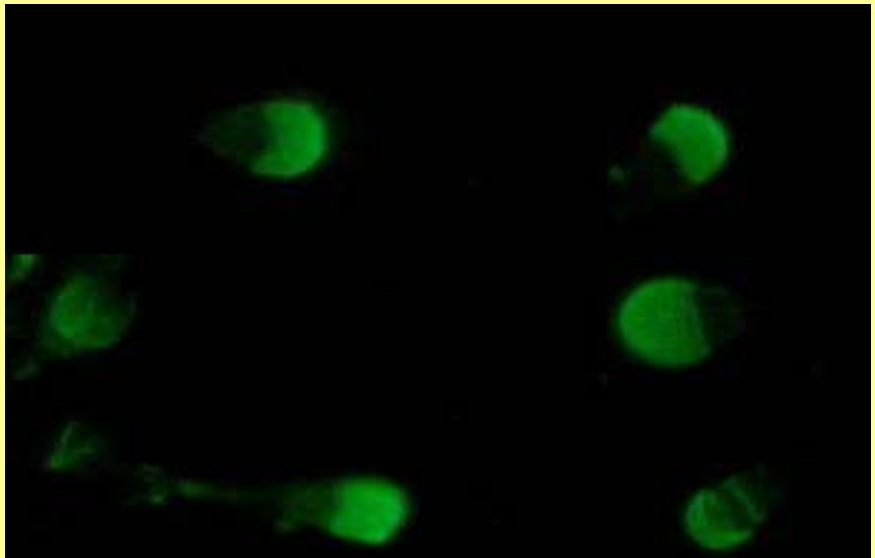
Propidium Iodide.

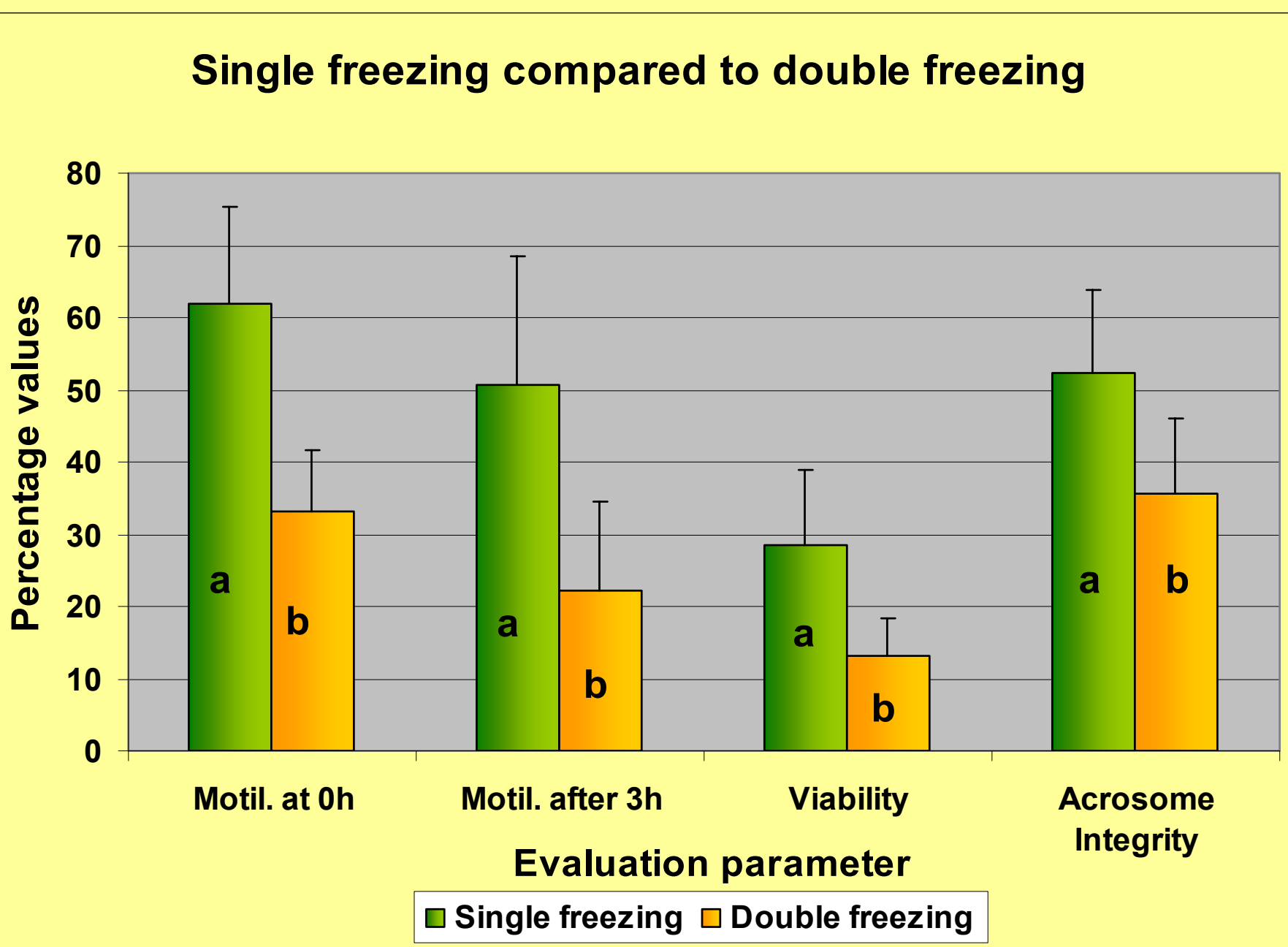


Hoechst 33342



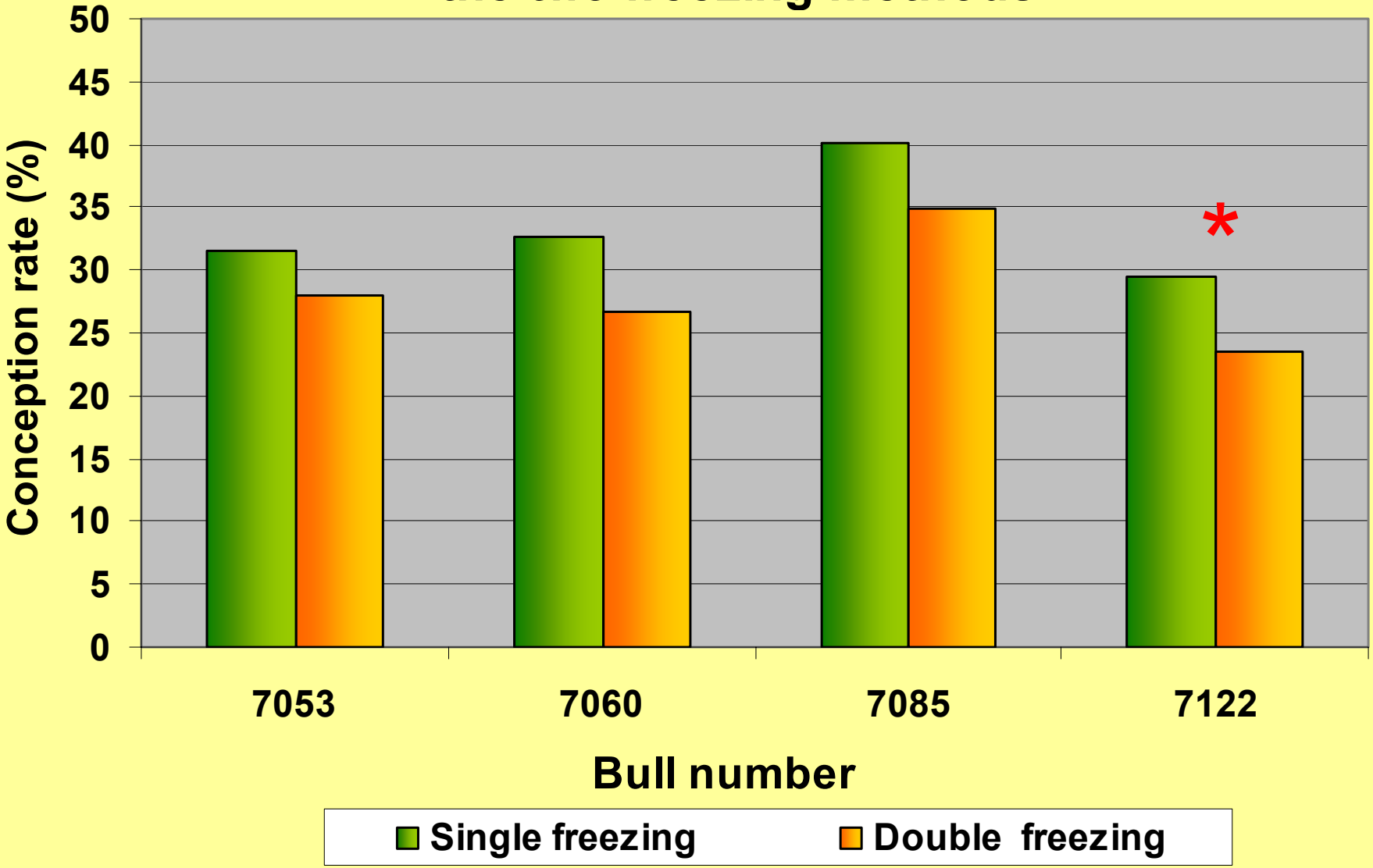
FITC-PSA





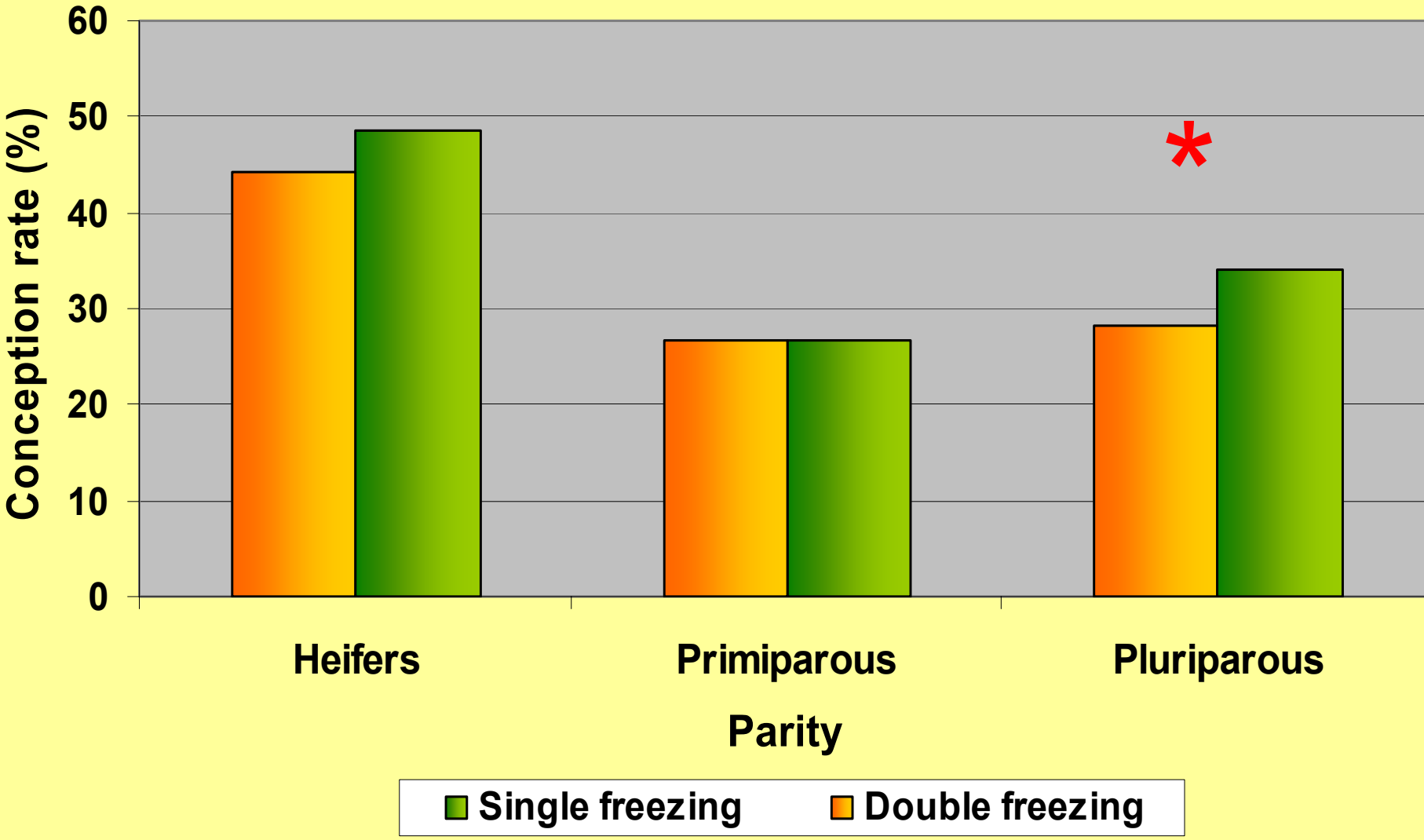
Columns with different letters are significantly different (P<0.001)

Conception rate by bull - comparison between the two freezing methods



* Indicate significant difference (P<0.05)

Conception rate by parity and semen freezing method



* Indicate significant difference (P<0.001)

Summary

1. In this study we have showed that acceptable conception rate can be achieved with the double freezing method.

2. The results confirm the working hypothesis that freezing in large volume and high concentration to reduce storage costs is possible.

3. General linear model evaluation showed that conception rate is influenced by freezing method, inseminating technician, bull and parity.

4. Parity influenced conception rate between freezing methods only in pluriparous cows

5. Future study will evaluate the double freezing technique with sex-sorted sperm.

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THANK YOU FOR YOUR ATTENTION