

MODIFICATION OF FREEZING EXTENDER PH AND USE OF HEPES AS BUFFER ON POST-THAW BOAR SPERM QUALITY

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Introduction and Aims

During freezing certain substances may crystallize and precipitate, modifying the pH and affecting this to the membrane stability. The aim was to evaluate the pH pre-adjustment of freezing extender and use of HEPES as buffer to prevent pH changes during the process

Material and Methods

Cryopreservation

Pool sperm-rich fractions from 5 fertile boars (5 ejaculates/boar)

Treatments

Lactose-egg yolk-glycerol extender supplemented with HEPES and pH pre-adjusted

A: non-supplemented, pH 6.4

B: 10 mM, pH 7.2-7.4

C: 10 mM, pH 7.6-7.8

D: 20 mM, pH 7.2-7.4

E: 20 mM, pH 7.6-7.8

Freezing at 0.5 mL straws (1 x 10⁹ cells/mL) in a programmable freezer.

Thawing

Straws were thawed at 37°C for 20 sec

Samples were incubated during 30 min in a waterbath at 37°C

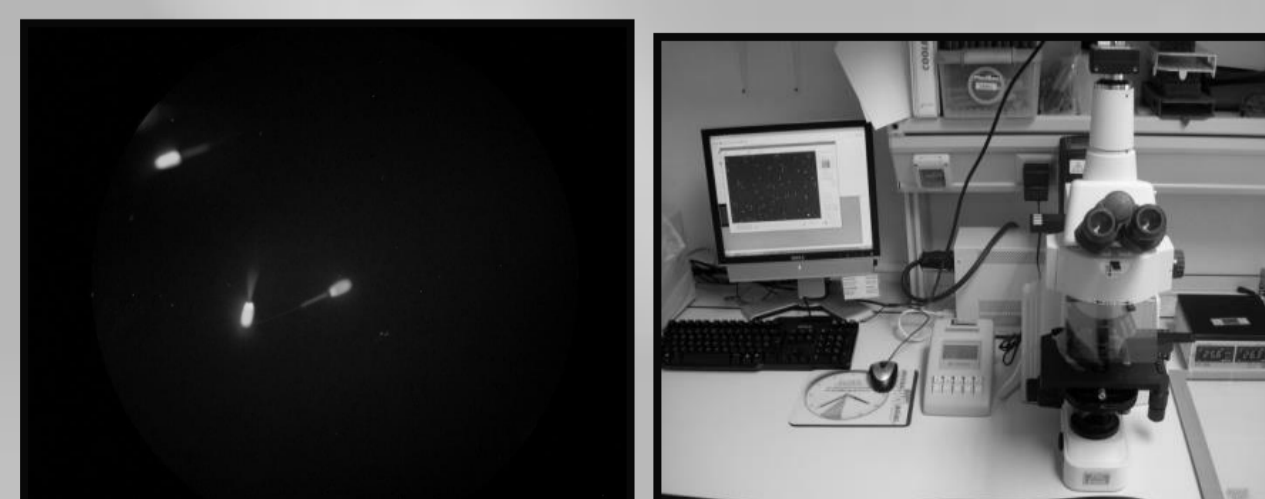
Sperm Assessment

Sperm motility



ISAS® (Proiser, Spain)

Live sperm (%LS)

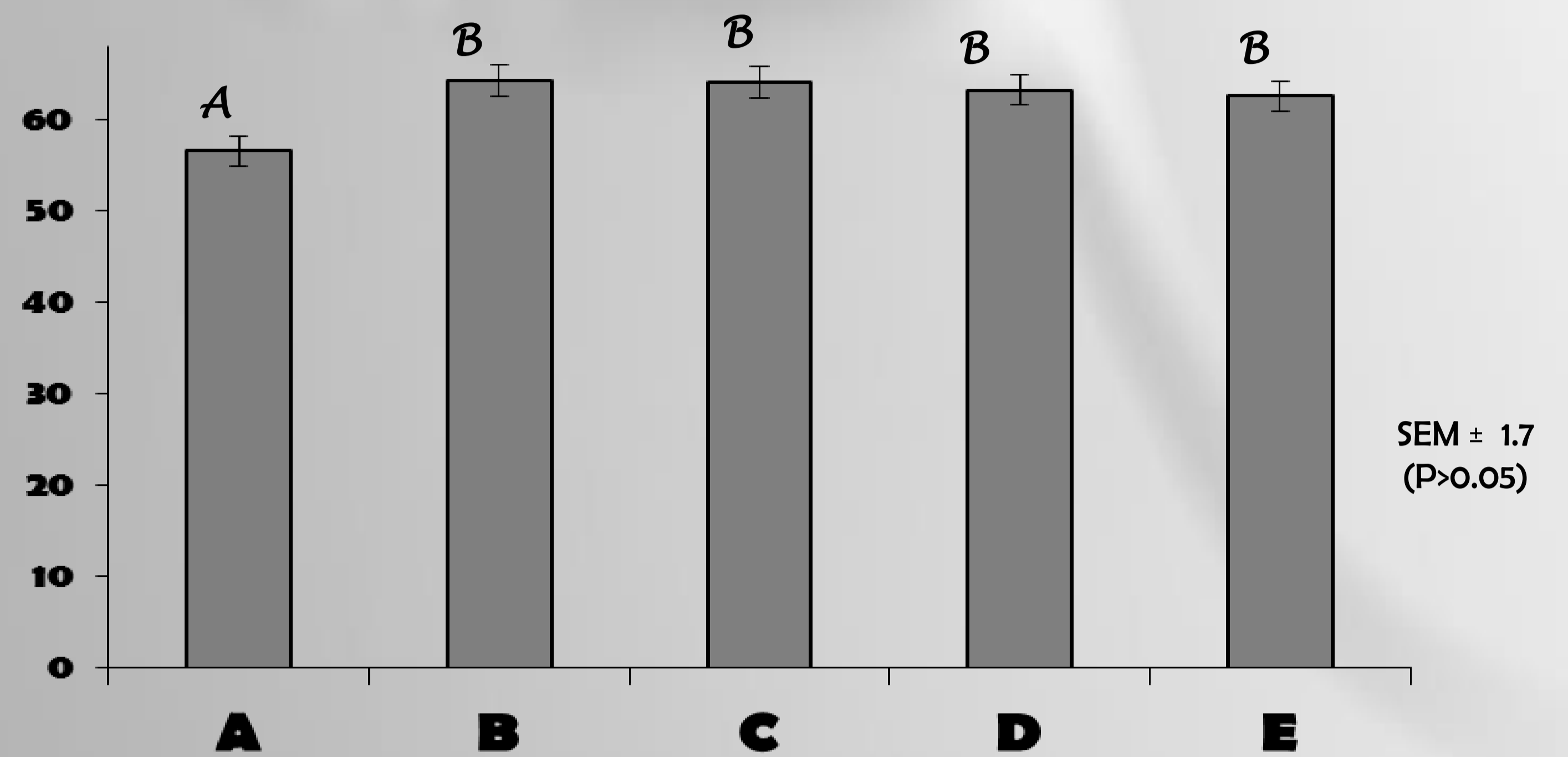


fluorescence microscopy (SYBR14/propidium iodide)

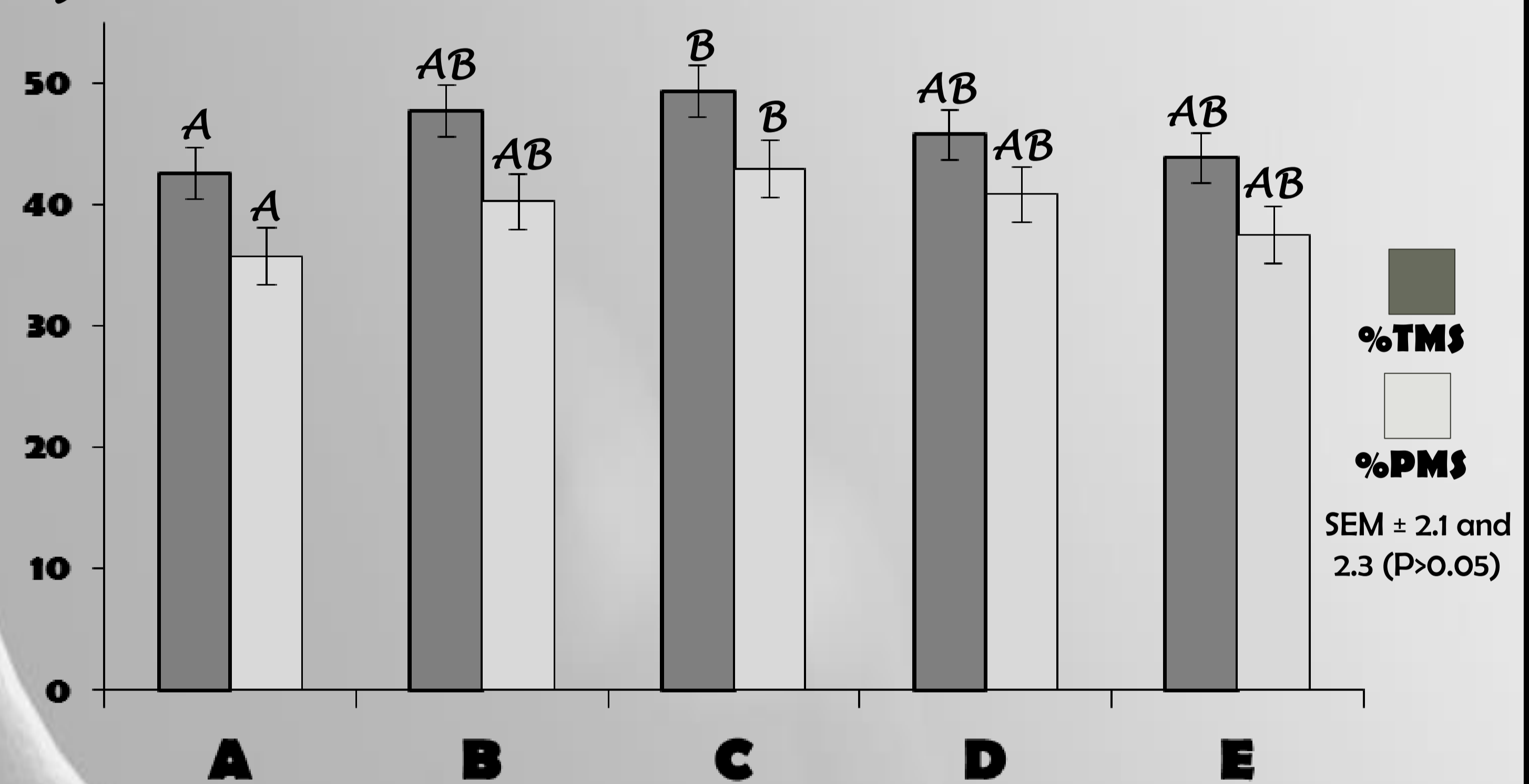
- Total motile sperm (%TMS)
- Progressively motile sperm (%PMS)
- Kinetic parameters (VCL and VSL)

Results

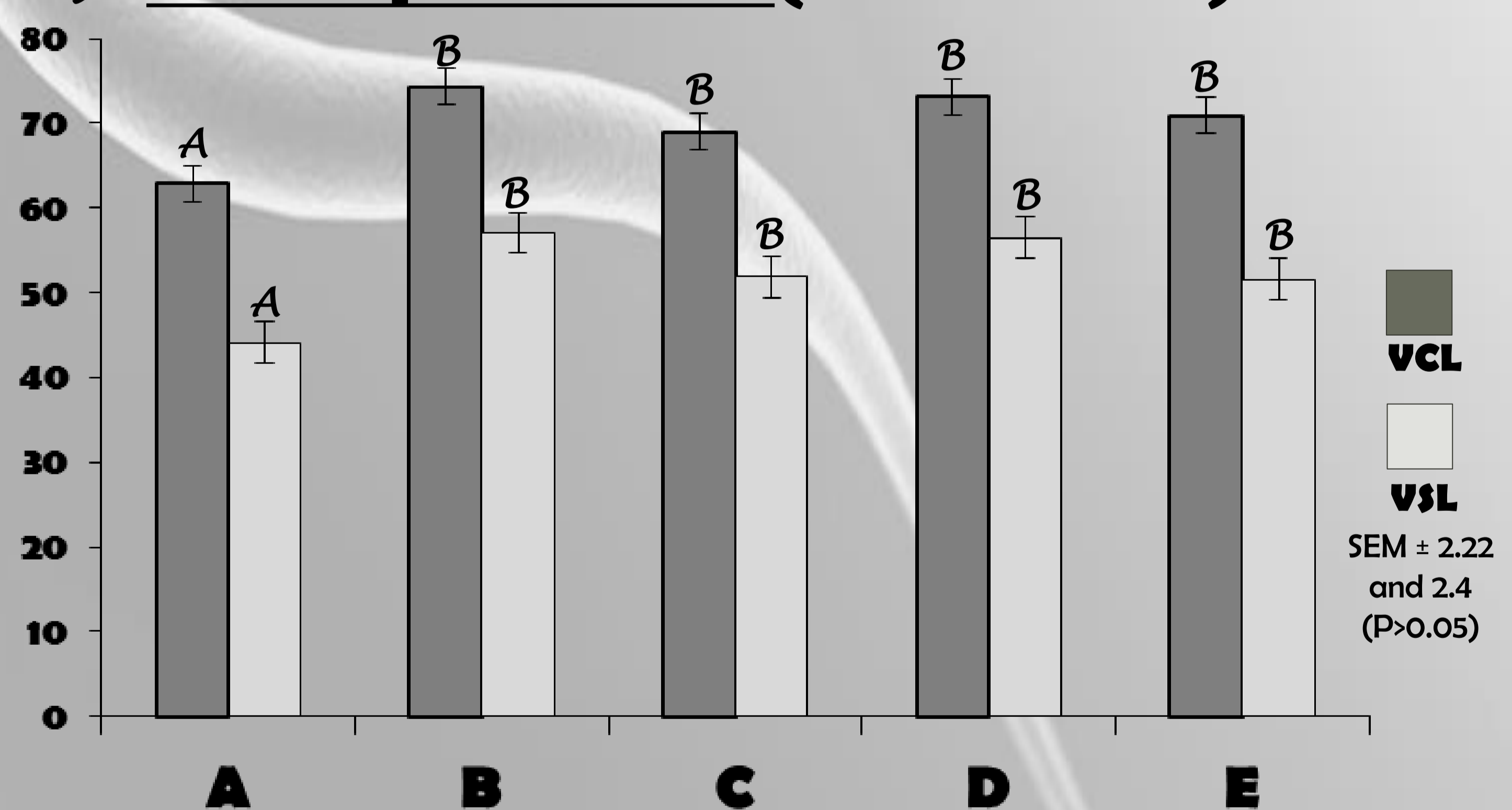
a) Live sperm (%)



b) Total and Progressively motile sperm (%)



c) Kinetic parameters (VCL and VSL)



All treatments were significantly different to the control (P<0.05) in %LS, VCL and VSL. But only treatment C was different to the control in %TMS and %PMS.

Conclusion: the pH pre-adjustment of freezing extender is able to improve the viability and motility, but high HEPES concentrations as buffer may decrease motility