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A STUDY ON THE USE OF UNFROZEN, DILUTED SEMEN  
FOR THE IN VITRO FERTILISATION OF BOVINE OOCYTES  
MATURED *IN VITRO*

ANGELA DAWN SEATON

1991

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A STUDY ON THE USE OF UNFROZEN, DILUTED SEMEN  
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A thesis presented in partial fulfilment  
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## ABSTRACT

The study investigated the use of unfrozen, diluted semen for in vitro fertilisation of bovine oocytes matured *in vitro*. In experiment 1, semen from each of two bulls was used on two consecutive days ("day-old" and "two-day-old" sperm) to explore the effect of sperm concentration on oocyte penetration rates. The sperm concentrations used were 0.125, 0.25, 0.5, 1.0, 2.0 and  $4.0 \times 10^6$ /ml. Penetration rates were uniformly high when day-old sperm was used, but low penetration rates were obtained below  $1 \times 10^6$ /ml with two-day-old sperm. Unfrozen sperm appeared to give better penetration rates than frozen-thawed sperm at concentrations of  $0.5$ - $2.0 \times 10^6$ /ml. There was no relationship between sperm concentration and incidence of polyspermy. In experiment 2, sperm from the same two bulls were used to investigate the presence of Caprogen extender in the fertilisation medium. Caprogen inhibited penetration when present in concentrations greater than 10ml/litre. Experiments 3 and 4 studied the effect of heparin on penetration rates. In experiment 3, sperm from one bull was used to inseminate oocytes in medium containing 0, 1, 5, 10, 20, 30 or 50 $\mu$ g/ml heparin. There was no relationship between penetration rates and heparin concentrations in the medium, and average penetration rates were high for all concentrations. In experiment 4, five bulls were used to investigate penetration rates at heparin levels of 0 $\mu$ g/ml (frozen-thawed and unfrozen sperm) and 10 $\mu$ g/ml (unfrozen sperm). The results obtained in experiment 3 with sperm from one bull were confirmed; penetration was obtained in the absence of heparin with all five bulls.

Good penetration can be obtained *in vitro* with unfrozen sperm, and its greater longevity and viability make it a useful alternative to frozen semen for both commercial and research in vitro fertilisation programmes.

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**LIST OF ABBREVIATIONS.**

AR	acrosome reaction
BO	Brackett's-Oliphant's medium
BSA	bovine serum albumin
cAMP	cyclic adenosine monophosphate
COC	cumulus-oocyte complex
DNA	deoxyribonucleic acid
FCS	foetal calf serum
FSH	follicle stimulating hormone
GAG	glycosaminoglycan
hCG	human chorionic gonadotrophin
HIS	high ionic strength (medium)
IVC	in vitro culture
IVF	in vitro fertilisation
IVM	in vitro maturation
LIC	Livestock Improvement Corporation
LH	luteinising hormone
m199	medium 199
OCS	oestrous cow serum
PBS	phosphate-buffered saline solution
PHE	penicillamine, hypotaurine, epinephrine
SAS	Statistical Analysis Systems
TALP	Tyrode's medium with acetate, lactate, pyruvate