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 FOR THE IN VITRO FERTILISATION OF BOVINE OOCYTES MATURED *IN VITRO*

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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 $4.0x10^6$/ml. Penetration rates were uniformly high when day-old sperm was used, but low penetration rates were obtained below $1x10^6$/ml with two-day-old sperm. Unfrozen sperm appeared to give better penetration rates than frozen-thawed sperm at concentrations of $0.5\text{-}2.0x10^6$/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 50µg/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 0µg/ml (frozen-thawed and unfrozen sperm) and 10µg/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