

Comparison of commercial flushing and holding media on embryo production in equine ICSI cycles: a pilot study of 137 OPU procedures in 76 mares

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INTRODUCTION

Oocyte holding begins shortly after ovum pick-up (OPU) and precedes in vitro maturation (IVM), helping to preserve oocyte viability and prevent premature meiotic resumption. The composition of holding media influences meiotic progression and developmental competence of oocytes. Heparin has also been incorporated into OPU flushing solutions to reduce clotting of follicular fluid, although its effects on embryo development remain uncertain.

OBJECTIVE

To compare two commercial flushing and holding strategies in clinical equine ICSI cycles, including their effects on oocyte recovery, maturation and blastocyst production.

MATERIALS & METHODS

LABORATORY WORKFLOW

1 Ovum pick-



2 Oocyte search



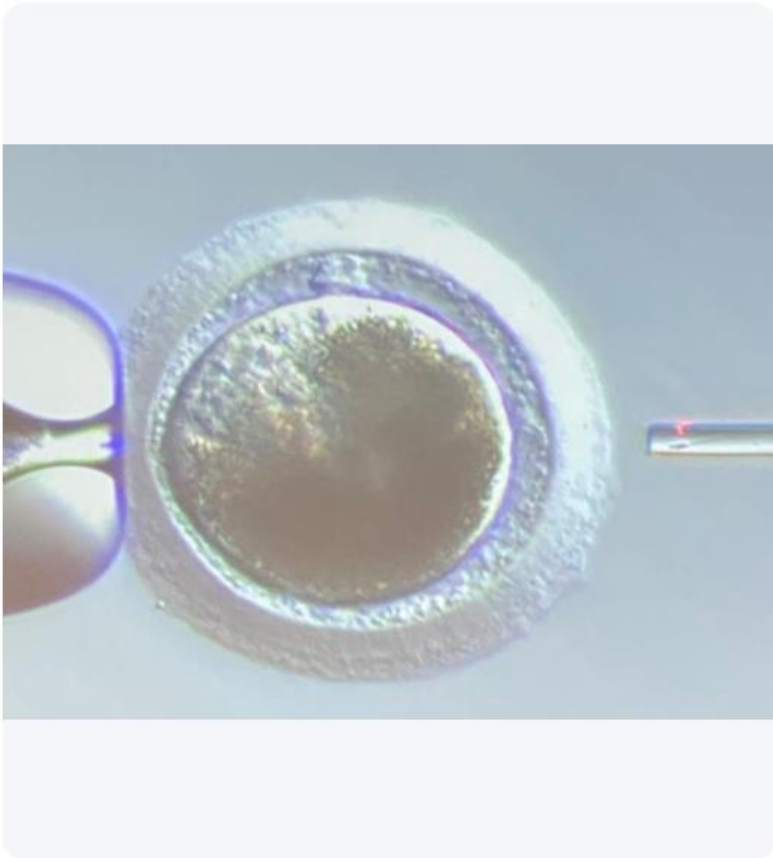
3 Holding



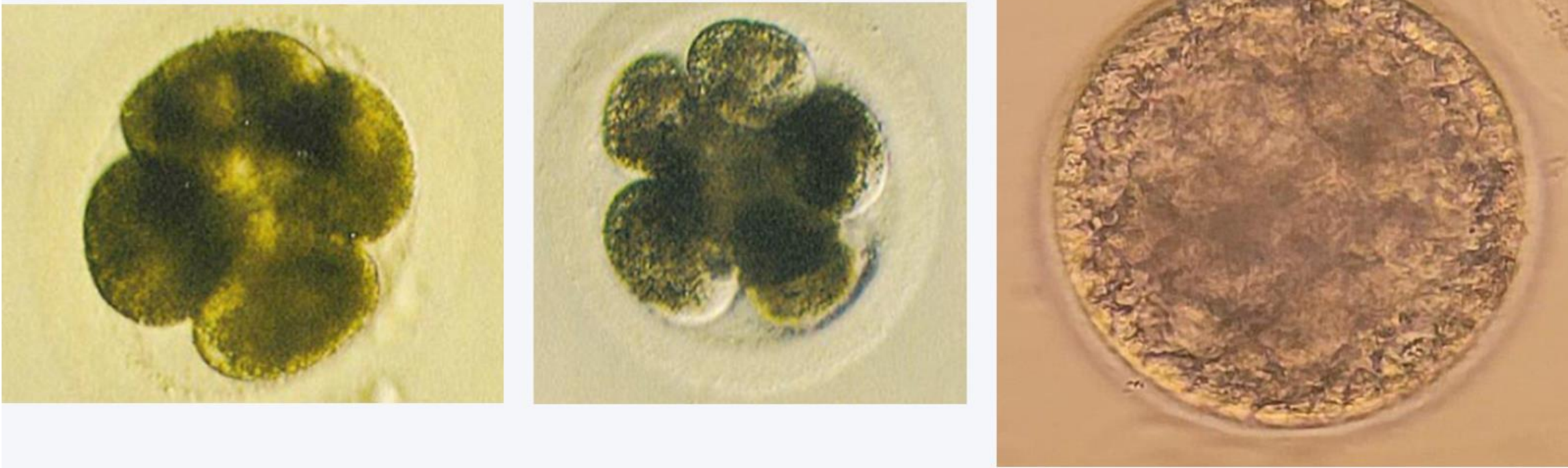
4 IVM



5 ICSI



6 Embryo development



137 OPU procedures
across 76 mares

Group A · n = 73

Group B · n = 64

BOTUPHARMA
PROTOCOL

Flushing solution
Botuflush OPU[®]
(4mL added in 1L
Ringer Solution)

Additives
Surfactant +
anticoagulant
(heparin)

Holding medium
BotuOocyte[®]

ABT PROTOCOL

Flushing solution
ABT Complete Flush[®]

Additives
None (heparin must be
added)

Holding medium
**ABT360[®] Holding
Medium**



GROUP A · FLUSHING
SOLUTION
Botuflush OPU[®] ·
(4mL added in 1L Ringer
solution)



GROUP A · HOLDING
MEDIUM
BotuOocyte[®]

RESULTS

OOCYTE RECOVERY



No adverse effect on oocyte recovery

MATURATION RATE



MII OOCYTES · ABSOLUTE COUNT



CLEAVAGE OUTCOMES



Numerically higher MII oocyte count and cleavage rate, without statistical difference

BLASTOCYST FORMATION · ABSOLUTE COUNT



BLASTULATION RATE · PER MII OOCYTE



Both outcomes showed numerical trends toward Group A without reaching statistical significance

137

OPU PROCEDURES
IN 76 MARES

+7.0 pp

MATURATION RATE
GROUP A VS B (P = 0.048)

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ENDPOINTS WITHOUT
STATISTICAL DIFFERENCE

CONCLUSION

The combined use of BotuOocyte[®] holding medium with a surfactant- and anticoagulant-supplemented flushing solution during OPU improves oocyte maturation and results in blastocyst production comparable to conventional protocols, without adversely affecting oocyte recovery. Importantly, the Botuflush OPU[®]-based protocol represents a practical and efficient alternative, supporting its use as a reliable option for routine equine assisted reproduction programmes.



Outcomes analyzed using **negative binomial generalized linear models** with cluster-robust standard errors at mare level