

ESAR Conference 2023, Cryopig

Effect of maturation media on *in vitro* nuclear and cytoplasmic maturation of pig oocytes

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Introduction

Due to the controversial use of animal products, *in vitro* culture media are now tending to be synthetic. Porcine follicular fluid (pFF) is currently used in sow oocyte maturation media, but its composition is not well described and can vary among sample. Polyvinyl alcohol (PVA) seems to be a good substitute for pFF in media but its efficiency on nuclear and cytoplasmic maturation has not yet been proven.

Oocyte maturation includes both nuclear and cytoplasmic maturation, because the completion of nuclear maturation does not necessarily reflect normal cytoplasmic maturation. Oocyte nuclear maturation is characterised by the extrusion of the first polar body, whereas cytoplasmic maturation is reflected, among other things, by the maturation of cortical granules (CGs) distribution pattern in the oocyte cytoplasm.

Procedures

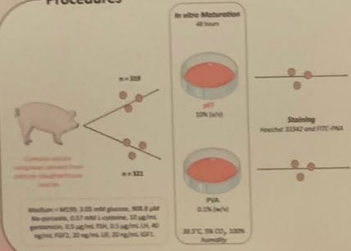


Figure 1. Porcine oocytes at different stages of maturation stained with Hoechst 33258 (blue) and FITC-PNA. (a) germinal vesicle, (b) metaphase I (metamere), (c) metaphase II (metamere). White arrows show the nucleus of oocyte. PB: the polar body x400.



Figure 2. The pattern of CGs distribution, by FITC-PNA staining. (a) Type I (completely matured), in which the CGs were distributed to the cytoplasmic cortex and formed a continuous single layer underneath the cell membrane. (b) Type II (incompletely matured) in which the CGs were localized in the cytoplasmic cortex and near the cell membrane. (c) Type III (metamere) in which the CGs were diffusely distributed among the cytoplasm. PB: the polar body x400.

Results

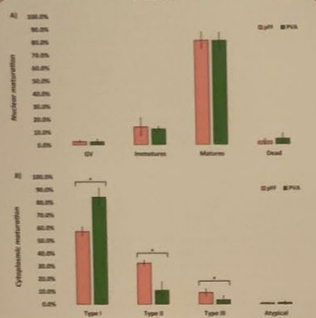


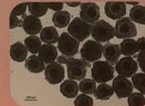
Figure 3. Effects of maturation media on *in vitro* nuclear (A) and cytoplasmic (B) maturation of porcine oocytes. GV: germinal vesicle; metamere: metaphase I and metaphase II; mature: from metaphase I to metaphase II; Type I: completely mature type; II: incompletely mature; Type III: metamere. * p < 0.05.

Interpretation

The distribution of CGs is not always representative of the nuclear status of the oocyte. This seems to indicate that cytoplasmic maturation occurs independently of nuclear maturation. Thus, the PVA group showed more nuclear and cytoplasmic synchronization than to the pFF group.



Oocytes from post-mortem ovaries with follicles 3-6 mm in diameter.



CGs before *in vitro* maturation.

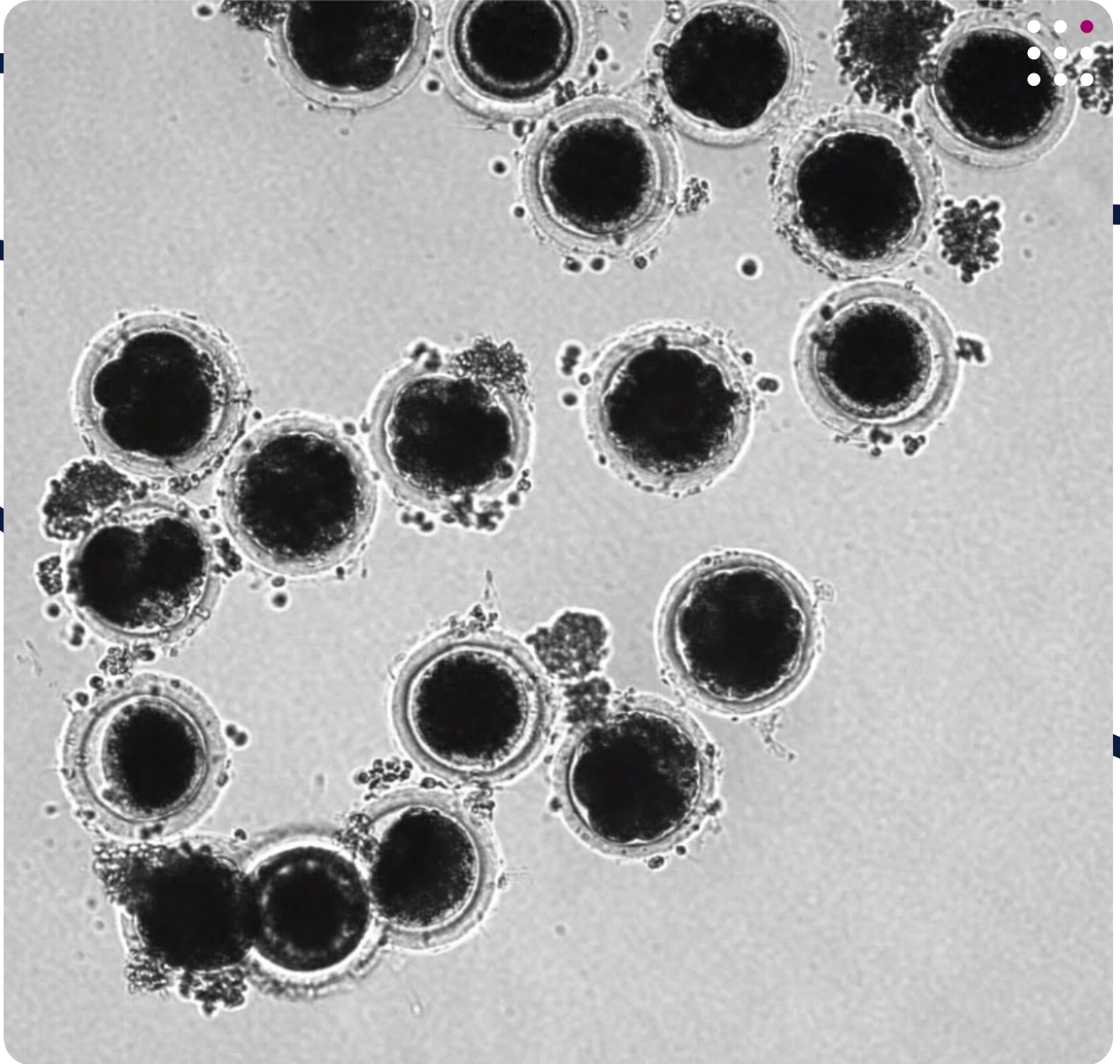
Conclusion

Since PVA leads to a similar nuclear maturation rate and a better cytoplasmic maturation rate, we can conclude that it seems to be a good substitute for pFF. Studies in non-human primates have shown that PVA can prevent thickening of the zona pellucida¹⁰. It is therefore possible that the same event may occur in porcine oocytes and become a future problem for *in vitro* fertilisation due to the high rate of polyspermy.

[1] Akbarova, Theriogenology 57 (2002) 257-273.
[2] Liu et al., Theriogenology 61 (2004) 986-993.
[3] Vandervoort et al., J Med Primatol 36 (2007) 55-56.



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Oocyte maturation includes both nuclear and cytoplasmic maturation, because the completion of nuclear maturation does not necessarily reflect normal cytoplasmic maturation⁽¹⁾. Oocyte nuclear maturation is characterised by the extrusion of the first polar body, whereas cytoplasmic maturation is reflected, among other things, by the cortical granules (CGs) distribution pattern in the oocyte cytoplasm⁽²⁾.

Procedures

In vitro Maturation
48 hours

Staining
Mowich 33342 and FITC-PNA

Cytoplasmic maturation

Figure 1: Porcine oocytes at different stages of maturation stained with Mowich 33342 (shades of grey): (a) germinal vesicle; (b) metaphase I (Immature); (c) metaphase II (mature). White arrows show the nucleus of oocyte. (b): First polar body. x20.

Figure 2: The pattern of CGs distribution, by FITC-PNA staining: (a) type I (completely mature) in which the CGs were distributed to the cytoplasmic cortex and formed a continuous single layer underneath the oolemma; (b) type II (incompletely mature) in which the CGs were localized in the cytoplasmic cortex and near the oolemma as small aggregates; and (c) type III (immature) in which the CGs were diffusely distributed among the cytoplasm. (b): First polar body. x20.

Results

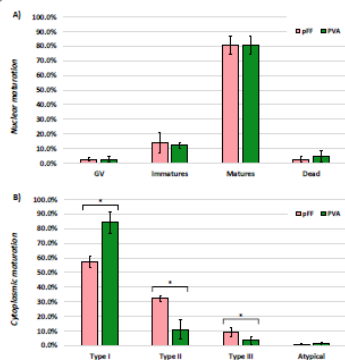
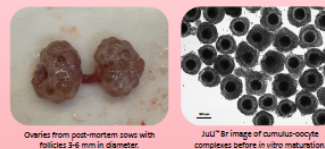


Figure 3. Effects of maturation media on *in vitro* nuclear (A) and cytoplasmic (B) maturation of porcine oocytes. GV: germinal vesicle; Immature: metaphase I and metaphase II; mature: from telophase I to metaphase II; type I: completely mature type; II: incompletely mature; type III: Immature. * p < 0.05.

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Studies in non-human primates have shown that PVA can prevent thickening of the zona pellucida⁽³⁾. It is therefore possible that the same event may occur in porcine oocytes and become a future problem for *in vitro* fertilization due to the high rate of polyspermy.

(1) Abayasinghe, Theriogenology 57 (2002) 293-279.
(2) Joly et al., Theriogenology 85 (2016) 396-407.
(3) Vandekerkhof et al., J Med Primatol 36 (2007) 10-16.