ULTRASTRUCTURAL CHANGES INDUCED DURING *IN VITRO* CULTURE OF BOVINE EMBRYOS

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Introduction

In vitro fertilised and cleaved bovine oocytes are generally cultured up to morulae / blastocysts stages in a defined medium for up to eight days and it is possible that ultrastructural changes are induced in them by the artefactual environment. These changes may contribute towards low viability of *in vitro* produced embryos as several reports indicate a low production rate (20-30%) of viable embryos (Lonergan et al. 1994). The objective of this study was to examine ultrastructural changes *in vitro* produced morulae in relation to the ultrastructure of *in vivo* produced morulae obtained from Kedah Kelantan (KK) cows following superovulation.

Materials and Methods

Two KK cows aged 3-4 years were estrus synchronised and superovulated. Luteolysis was induced on Day 11 by administering $PGF_{2\alpha}$ and cows were observed for estrus at 48 hours later. Cows were artificially inseminated 48 h, 60 h and 72 hours after $PGF_{2\alpha}$ injection. Ova ($n \approx 7$, 5 morulae and 2 blastocysts) were collected from two cows by flushing the reproductive tract 6-8 days after estrus. *In vitro* embryos (morulae) were produced using method of Yoshioka et al. (1997) with some modifications in the preparation of sperm mixture. Highly enriched population of motile spermatozoa were separated using PerCeption kit (Pacific andrology Inc.). Good quality morulae obtained from *in vivo* and *in vitro* were fixed for 1-2 h in 2.5% glutaraldehyde in 0.2 M sodium cacodylate (pH 7.2 at 4^0C) followed by post-fixation in cacodylate-buffered 1% osmium tetroxide, dehydrated in ascending concen-trations of acetone and embedded in resin mixture. Thin sections were stained with saturated solution of uranyl acetate and lead citrate and were examined in a Phillips CM 12 transmission electron microscope.

Results and Discussion

Zona pellucida is a protective covering around the embryo and helps in maintaining the internal environment of the embryo. It also helps during the compaction of blastomeres. Reduction in its thickness and the presence of numerous spaces *in vitro* produced morulae indicated that it was undergoing the process of degeneration which might in turn affect the growth and compaction of blastomeres. TEM observations on *in vitro* produced morulae exhibited the presence of asymmetrical blastomeres with most of the nucleoli having a reticular morphology. While in case of *in vivo* produced morulae some of the blastomeres were flattened on one side which was due to the compaction of blastomeres. Adoption of reticular morphology by the nucleoli both *in vitro* and vivo produced morulae was due to their involvement in the synthesis of embryonic RNA. The cytoplasm of blastomeres *in vitro* produced morulae appeared electron dense due to the presence of granules. Similar observations *in vitro* derived ruminant embryos have been reported (Gardner et al. 1994). There was a reduced incidence of mitochondria and presence of condensed, vacuolated and electron dense mitochondria with numerous without the bounding membrane *in vitro* produced morulae. This might suggest that the organelle was undergoing the process of degeneration as a result of artefactual environment. These changes in mitochondria may alter the metabolic capability and consequently the viability of *in vitro* produced embryos (Dorland et al. 1994). Reduced mitochondrial development in the trophectoderm and ICM cells of *in vitro* derived ovine blastocysts and significant increase in the incidence of mitochondrial degeneration in embryos cultured in the presence of serum have been reported earlier (Dorland et al. 1994). In addition, large number of lipid droplets and vacuoles were observed in the cytoplasm of blastomeres of *in vitro* produced embryos. Similar changes were also observed by Thompson et al. (1995) who attributed these changes to the effect of serum present in the culture medium. The degenerative features *in vitro* produced morulae indicate that these changes were induced by the *in vitro* culture medium or the artefactual environment as these were not present *in vivo* produced morulae. The other marked difference between *in vitro* and *in vivo* produced embryos was the absence of multivesicular bodies (MVB) in the former. The MVB are probably involved in the transportation of enzymes and materials to different parts of the cell. their absence *in vitro* produced embryos may affect the transportation of materials.

Conclusions

Degenerative changes induced in various organelles of the *in vitro* produced morulae a medium or artefactual environment might have contributed towards lowering the viability of embryos.

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