COMPARABLE DEVELOPMENTAL COMPETENCE OF *IN VITRO* FERTILIZED AND PARTHENOGENETICALLY ACTIVATED OVINE EMBRYOS

S. RAJA[1*], S. BALASUBRAMANIAN[2], G. DHINAKAR RAJ[3] AND C. VEERAPANDIAN[4]

Department of Veterinary Gynaecology and Obstetrics
Veterinary College and Research Institute, Thanjavur - 614 625

Received: 23.12.2015 Accepted: 26.04.2016

ABSTRACT

The present research throws light on utilizing parthenogenetically activated (PA) embryos instead of *in vitro* fertilized (IVF) embryos because the developmental rates of 2-, 4-, 8-16 cell and morula stages were similar (p>0.05) between IVF and PA embryos of sheep.

Keywords*:* Embryo, *In vitro* fertilization (IVF), Oocyte, Ovine, Parthenogenetic activation (PA)

In vitro fertilized (IVF) and *in vivo* derived embryos usage for research purpose and embryonic stem cell isolation has caused ethical concern in human beings. To replace this issue, production of parthenogenetic activation (PA) of oocyte has become a potential alternative tool. The interest in parthenogenetic ESCs (PESCs) is focused on cell replacement therapies because of their advantages that include efficiency of production of PA embryos similar to IVF embryos, histocompatibility and preclusion of ethical issues (Sritanaudomchai *et al.,* 2010). Hence, the present study was designed to assess the *in vitro* developmental competence of preimplantation ovine embryos produced by *in vitro* fertilization (IVF) and parthenogenetic activation (PA).

For the retrieval of cumulus oocyte complexes (COCs**),** sheep ovaries were obtained from local abattoir in medium containing 0.9% normal saline with penicillin (100 IU/ml) and streptomycin (50 mg/ml) at 30-35°C within 2h of slaughter. Ovaries were washed 2-3 times under running tap water and rinsed 5 times in 0.9% normal saline at 37-38°C. Using slicing technique, COCs were retrieved (Wani *et al*., 2000). Immature oocytes were isolated and graded as Grade A, B and C based on their cumulus cells investment and cytoplasm homogeneity (Wani *et al*., 2000).

The developmentally competent COCs were selected based on brilliant cresyl blue (BCB) test (Rodriguez-Gonzalez *et al.,* 2002). Briefly, the COCs were rinsed 3 times in modified Dulbecco's phosphate buffered saline (mDPBS) followed by incubation in 26µM BCB diluted in mDPBS for 90 min. Subsequently, COCs were rinsed 3 times in mDPBS and based on their cytoplasm coloration, they were classified as oocytes with a blue cytoplasm or grown oocytes (BCB +ve) and oocytes without a blue cytoplasm (colourless) or growing oocytes (BCB -ve).

Only BCB +ve COCs were subjected to *in vitro* maturation medium supplemented with hormones and growth factor and matured for 24h at 38.5ÚC in a humidified atmosphere of 5% CO_2 in air (Wani *et al.,* 2000). The maturation rate of oocytes was morphologically evaluated as degree (2, 1 and 0) based on the degree of cumulus expansion and extrusion of the first polar body (Kobayashi *et al.,* 1994). *In vitro* matured oocytes were randomly and equally assigned for IVF and PA groups.

For IVF group, 2-5µl of motile sperm suspension obtained by swim up technique (Parrish *et al.,* 1988) was used to inseminate the oocytes in IVF droplets (10-15 oocytes/75 µl droplet) to achieve the final concentration

[1]Assistant Professor; [2]Controller of Examinations; [3]Director, Translational Research Platform for Veterinary Biologicals, TamilNadu Veterinary and Animal Sciences University, Chennai - 600 051; [4]Professor and Head, Department of Veterinary Gynaecology and Obstetrics, Madras Veterinary College, Chennai - 600 007;
*argoraja@gmail.com

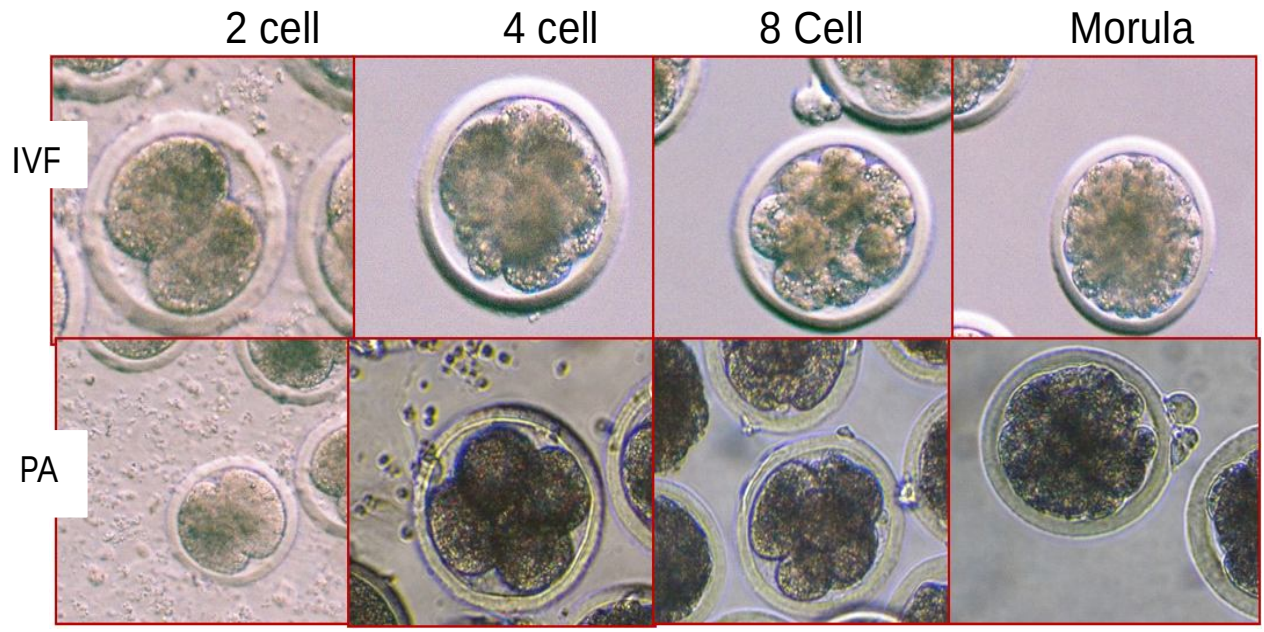

Figure: The developmental competence of *in vitro* Fertilized and parthenogenetically activated ovine embryos (200x)

of 2 million sperm/ml and co-incubated for 24h. For PA group, matured oocytes were exposed for 5min in 5µM ionomycin in TCM 199 supplemented with 10% FBS followed by 3h in 2mM 6-dimethylaminopurine (6-DMAP) in TCM 199 supplemented with 10% FBS (Bebbere *et al.,* 2010). In both the IVF and PA groups, the presumptive zygotes were transferred separately into pre-equilibrated 50µl IVC droplets (10-15 zygotes/droplet) containing two-step synthetic oviduct fluid medium and developmental competence of embryos at various stages viz. 2-, 4-, 8-16 cell and morula was recorded (Figure). The statistical analysis was carried out using chi-square test.

Six replicates were carried separately for IVF and PA groups (327 oocytes each). The developmental rates of 2-, 4, 8-16 and morula stages in IVF groups were 82.48±0.33, 66.46±0.30, 56.32±0.24 and 42.33±0.33%, respectively. Similarly, the developmental rates in PA group were 83.12±0.62, 67.50±0.95, 55.07±0.47 and 43.63±0.49%, respectively. The developmental rates of embryos at all these stages were similar (p>0.05) in IVF and PA groups. In a previous study, the cleavage rate and 8-cell stage percentage was 81.4±1.6 and 38.1±2.3 in PA embryos and 85.1±1.5 and 68.2±2.3 in IVF bovine embryos, respectively (Gomez *et al.*, 2009). However, in the present study, IVF and PA groups had almost similar cleavage and further developmental rates. Hence, in terms of developmental competence, it could be inferred that PA embryos can be used as an alternative source to IVF embryos for the production of embryonic stem cells, although further investigation on molecular study related to gene expression is warranted.

REFERENCES

Bebbere, D.L., Bogliolo, F., Ariu, S., Fois, G.G., Leoni, S., Succu, F., Berlinguer, N. and Ledda, S. (2010). Different temporal gene expression patterns for ovine pre-implantation embryos produced by parthenogenesis or *in vitro* fertilization. *Theriogenology,* **74**(5): 712-723.

Gomez, E.J.N., Caamano, P., Bermejo-Alvarez, C., Diez, M., Munoz, D., Martin, S., Carrocera. S. and Gutierrez-Adan, A. (2009). Gene expression in early expanded parthenogenetic and *in vitro* fertilized bovine blastocysts. *J. Reprod. Dev.,* **55**: 607-614.

Kobayashi, K.S., Yamashita. C. and Hoshi, H. (1994). Influence of epidermal growth factor and transforming growth factor-on *in vitro* maturation of cumulus cell enclosed bovine oocytes in a defined medium. *J. Reprod. Fertil.,* **100**: 439-446.

Parrish, J.J., Susko-Parrish, J., Winer, M.A. and First. N.L. (1988). Capacitation of bovine sperm by heparin. *Biol. Reprod.,* **38**: 1171-1180.

Rodriguez-Gonzalez, E.M., Lopez-Bejar, E., Vellila, B. and Paramio, M.T. (2002). Selection of prepubertal goat oocytes using the brilliant cresyl blue test. *Theriogenology,* **57**: 1397-1409.

Sritanaudomchai, H., Ma, H., Clepper, L., Gokhale, S., Bogan, R., Hennebold, R., Wolf, D. and Mitalipov. S. (2010). Discovery of a novel transcriptional analysis of parthenogenetic embryonic stem cells. *Hum. Reprod.,* **25**: 1927-1947.

Wani, N.A., Wani, G.M., Khan, M.Z and Salahudin.S. (2000). Effect of oocyte harvesting technique on *in vitro* maturation and *in vitro* fertilization in sheep. *Small. Rum. Res.,* **36:** 63-67.