Short Communication

Culture of Ovine IVM/IVF Zygotes in Isolated Mouse Oviduct: Effect of Basal Medium

Abbas Farahavar 1, Abolfazl Shirazi 2,3, Hamid Kohram 1,4*, Ahmad Zareh Shahneh 1, Ali Sarvari 2, Mohammad Mehdi Naderi 2, Sara Borjijan Boroujeni 2, and Mahdi Zhandi 1

1. Department of Animal Science, Faculty of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
2. Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
3. Research Institute of Animal Embryo Technology, Shahrekord University, Shahrekord, Iran
4. Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Shahid Chamran, Ahvaz, Iran

Abstract

Background: The basal medium that supports Isolated Mouse Oviduct (IMO) is important for supporting embryo development and quality.

Methods: The culture of ovine IVM/IVF zygotes was done in IMO using SOFaaciBSA and SOFaBBSA as basal medium of IMO and in SOFaBBSA alone as control. For preparation of IMO mature inbred strain C57BL/6 female mice were synchronized and mated with vasectomized males. The females with vaginal plug were sacrificed and the zygotes were transferred to the isolated oviduct at 20 hpi. The oviducts were cultured with SOFaaciBSA and SOFaBBSA for 6 days. Another group of zygotes were cultured in SOFaBBSA alone as control.

Results: Culture of zygotes in the IMO with SOFaaciBSA and SOFaBBSA, did not significantly affect the development and quality of embryos (p>0.05). The hatching rate, total and trophectoderm cells number in IMO groups' blastocysts were significantly higher than SOFaBBSA alone. The morphological appearance of IMO blastocysts was superior to SOFaBBSA alone. When the quality of oocytes was poor, IMO could better support ovine embryo development either with SOFaaciBSA or SOFaaciBSA than SOFaBBSA alone and there was a significant difference in blastocyst formation at day 6 with SOFaBBSA alone.

Conclusion: The culture of ovine IVM/IVF zygotes in IMO using two highly efficient ruminant embryo culture media not only could support development of ovine embryos similar to the level in non IMO culture system (SOFaBBSA alone) but also could improve the quality of resulting embryos. Additionally, IMO could better support the development of ovine embryos derived from poor quality oocytes compared to the SOFaBBSA alone.

Keywords: Embryo, Mice, Organ culture technique, Ovine

Introduction

The main factor affecting the quality of the in vitro produced blastocyst is the post fertilization culture conditions 1. IMO culture system has been used successfully in the culture of mouse, rat 2, hamster 3 and pig 4 and bovine 5,6 embryos from the one-cell to the morula/blastocyst stage. Basal medium that support IMO and cultured zygotes, is important for embryo development 6,7 and quality 5.

In conventional production of ovine embryos in vitro, the original formulation of SOF from Tervit et al (1972) with some modification (SOFaBBSA) was used for high rate of blastocyst production. SOFaBBSA is a glucose containing medium but viability of isolated mouse oviduct and cultured embryos with SOFaBBSA is unclear.

The culture of ovine zygotes in IMO with two highly efficient ruminant embryo culture media (SOFaaciBSA and SOFaBBSA), viability of explanted oviduct, cultured zygotes development and quality of resulted embryos have not been studied to date.

Material and Methods

Except where otherwise indicated, all chemicals were obtained from the Sigma (St. Louis, MO, USA).

In vitro maturation and fertilization

Sheep ovaries were collected from slaughterhouse and the follicular fluid was collected in HEPES-buffered TC199. The selected Cumulus Oocyte Complex (COCs) were matured for 24 hr in bicarbonate buffered TC199 supplemented FBS, 0.1 IU/ml FSH and 0.4 mM sodium pyruvate. For IVF, matured oocytes were fertilized with Shall breed ram spermatoza at concentration of 2×10⁶ spermatoza per ml. Semen from the same ram was used for all experiments.

Culture of zygotes in vitro

In vitro culture of zygotes was carried out in SOFaBBSA and used as control. The culture was done in a humidified atmosphere of 7% O₂, 7.5% CO₂ and 85.5% N₂ at 39°C. Charcoal stripped fetal bovine serum (10% v/v) was added to each droplet 48 hr after start of culture (72 hpi).
Culture of zygotes in the isolated mouse oviduct

Zygotes were transferred to the mouse oviduct (approximately 5-7 per oviduct) at 20 hpi. The oviducts were incubated on to a Costar Transwell membrane (non-tissue culture treated, Nucleapore polycarbonate membrane, 3.0 µm pore size, 24 mm diameter, Costar 3414, Corning, NY). Approximately 1.5 ml of equilibrated medium had been placed in the compartment below the membrane insert. Plates were incubated at 39°C under an atmosphere of 8% CO2 in air with maximum humidity. Embryos were recovered 6 days later (i.e., day 7 post insemination) by flushing with handling medium.

Mouse oviduct isolation

All animal experiments were performed in accordance with Avicenna Research Institute Ethical Committee Guidelines. For preparation of IMO mature inbred strain C57BL/6 female mice were synchronized and mated with vasectomized males. The females with vaginal plug were sacrificed and the zygotes were transferred in to the isolated oviduct at 20 hpi. The oviducts were cultured with SOFaaBSA and SOFaBSA for 6 days.

Embryo quality evaluation

**Morphological evaluation:** Embryo diameter at hatching was assessed by a microscopic grade. International Embryo Transfer Standards (IETS) were used for embryo evaluation by morphological methods. There are three grades of quality namely: excellent, good, fair and poor.

**Embryonic cells:** For differential staining of Inner Cell Mass (ICM) and Trophoderm cells (TE) blastocysts were incubated in 0.2% Triton X-100 for 20 s and for staining of TE, immediately were transferred into 30 µg/ml Propidium Iodine (PI) solution for 1 min. For staining ICM were incubated in ethanol containing 10 µg/ml bisbenzimide (Hoechst) for 15 min on ice. Embryos were immediately mounted on glass slides and examined under an epifluorescence microscope.

**Statistical analysis**

Data analyses were carried out by one-way ANOVA using the SAS (Statistical Analysis System, 9.1 versions) software and GLM procedure. Shapiro-Wilk test was used for determining normality of data. A p-value of 0.05 by Tukey test and was considered significant. To present a true mean value of data the transformed means were recomputed. Moreover for representing the standard deviation, confidence limits of the transformed data were computed and then converted to linear scale.

**Results**

**Embryo development**

This is the first study which showed that IMO could support ovine embryo development when cultured with two highly efficient ruminant embryo culture media (SOFaaBSA and SOFaBSA) as basal medium (Figure 1). There were no significant differences (p>0.05) in cleavage rate between experimental groups. Culture of zygotes in the IMO with SOFaaBSA and SOFaBSA, did not affect the proportion of zygotes development to the blastocyst stage at days 6, 7 and 8 compared to the respective control (p>0.05). There was a significant difference (p<0.05) between hatching rate of IMO group’s blastocysts compared with SOFaBSA (Table 1). Results of this study showed that when the quality of oocytes are poor, IMO could support better ovine embryo development either with SOFaBSA or SOFaaBSA than SOFaBSA alone (Table 2).

**Embryo quality**

**Morphological evaluation:** We generally had recovered more excellent embryos (51-57%) from the IMO (either with SOFaaBSA or SOFaBSA) and showing significant difference with SOFaBSA alone (10.34%) (Figure 2, Table 3). The hatching of IMO embryos occurs at a smaller diameter (225±.42 and 240±8.1 vs. 257±6.42 µm) than SOFaBSA alone (Table 3). The IMO groups’ blastocysts had

![Figure 1. Ampulary segment of mouse oviduct containing ovine embryos 6 days after culture with, A) SOFaBSA; B) SOFaBSA](http://www.ajmb.org)

| Culture medium | No. of zygotes | Cleaved no. (%) | D6 no. (%) | D7 no. (%) | D8 no. (%) | Hatched no. (%) |
|----------------|----------------|----------------|------------|------------|------------|----------------|
| SOFaBSA alone | 150            | 133 (89.88±3.45) | 29 (21.89±1.07) | 48 (24.84±1.24) | 56 (38.5±1.82) | 26 (46.42%) |
| IMO with SOFaaBSA | 78           | 64 (82.73±2.75) | 21 (29.83±1.43) | 28 (32.46±1.62) | 29 (40.63±1.91) | 21 (72.41%) |
| IMO with SOFaBSA | 94           | 75 (80.83±2.76) | 20 (22.4±1.1) | 26 (26.±1.3) | 29 (30.8±1.49) | 22 (75.86%) |

*A IMO: Isolated mouse oviduct. Values in the same columns with different superscripts differ significantly (p<0.05). Results were reported as Lsmeans±SD
** Hatching rate analyzed by chi-square test

Avicenna Journal of Medical Biotechnology, Vol. 5, No. 2, April-June 2013
brighter appearance, with more prominent inner cell mass compared with blastocysts produced in SOFaaBSA alone (Figure 2).

**Embryonic cells**

There was no significant difference in the numbers of total embryonic cells and TE and ICM cells number of embryos cultured in IMO with SOFaaBSA. The numbers of total embryonic cells of embryos cultured in IMO with SOFaaBSA and SOFaaBSA were significantly higher (p<0.05) than SOFaaBSA alone. The numbers of TE cells in IMO groups were significantly higher (p<0.05) than control. There was a tendency (p<0.05) to increase the number of ICM cells of IMO groups in comparison to control (Table 4). The ratio of ICM:TE did not show significant difference between groups. The normal allocation and integrity of inner cell mass between IMO produced blastocyst and SOFaaBSA alone after differential staining was significantly different (Figure 3).

**Discussion**

Culture of ovine IVM/IVF zygotes was done in IMO using two highly efficient ruminant embryo culture media as basal media for support viability of the explanted mouse oviduct and cultured ovine embryos development and quality. SOFaaBSA that was used as first basal medium of IMO was a glucose free medium which had been used by Holm et al (1999) for high bovine blastocyst development in vitro and supplemented with sodium citrate and myo-inositol. Citrate stimulates fatty acid synthesis and is a chelator of metal ions (e.g., Ca2+), a feature that may be of importance for maintaining junctional integrity and thus of importance for compaction and blastocoel formation. However, it is possible that addition of citrate to SOFaaBSA further enhanced blastocyst development.

Myoinositol or its metabolites are essential components in cellular signalling and some metabolites may have a direct mitogenic effect. The embryotrophic properties of some batches of human serum have been correlated with high concentration of myoinositol. In the present study, there was no significant difference in blastocyst yield at days 6, 7 and 8 between zygotes cultured in IMO either with SOFaaBSA or SOFaaBSA and SOFaaBSA alone (Table 1). It has been reported that the post fertilization culture environment, within certain limits, does not affect blastocyst development. The development of immature bovine and ovine oocytes to the blastocyst stage following maturation, fertilization and culture in vitro is generally limited to a frequency of about 40%. There is now a growing amount of evidence to suggest that while culture conditions during in vitro embryo production can impact the developmental potential of the early em-
hatching rate 15. As a result, the higher hatching rate oxygen tension during IVC could negatively affect is postulated that culture of ovine zygotes in IMO

differences between IMO produced embryos (Table 3). It has been shown that pres-
vitro

An aberrant ICM:TE ratio has been suggested to be related to the large offspring syndrome of in vitro-produced embryos 22. Therefore we did not observe significant differences in ICM:TE ratio between groups, but embryos cultured in IMO (either with SOFaaBSA or SOFaaciBSA) had approximately 2 fold higher total embryonic cells than SO-

bryo, the intrinsic quality of the oocyte is the key factor determining the proportion of oocytes develop-
op to the blastocyst stage 1,13. Results of our study were in agreement with published documents.

The hatching rate in our study was significantly different in IMO groups compared to the SOFaaBSA alone. In fact, 72-76% of the embryos cultured in IMO had been hatched from the zona pellucida by day 8, which was similar to the rate found in vivo 14 . In our study culture of zygotes in IMO was done in 20% O2 atmosphere, while O2 percentage in conventional IVC (control) was 7% O2. It was reported that oxygen tension during IVC could negatively affect hatching rate 15. As a result, the higher hatching rate in IMO groups may be related to the other unknown beneficial effects of mouse oviduct on ovine embryo development in IMO.

Post fertilization embryo culture condition affects embryo morphology. It has been shown that presence of serum in embryo culture medium has provided materials linked to abnormal embryo mor-

Table 4. Ovine blastocyst cell numbers derived from IVM/IVF zygotes cultured in different conditions

| No. blastocysts | SOFaaBSA alone | IMO with SOFaaciBSA | IMO with SOFaaBSA |
|-----------------|----------------|---------------------|------------------|
| Total cell number | 74.26±16.12 b | 136.48±33.88 a | 146.67±36.96 a |
| No. ICM cells | 14.07±1.87 | 28.56±4.81 | 29.76±5.07 |
| No. TE cells | 59.25±24.36 b | 106.33±25.03 a | 114.36±27.35 a |
| ICM:TE ratio | 0.23±0.017 | 0.27±0.017 | 0.26±0.017 |

Values in the same row with different superscripts differ significantly (p<0.05). Results were reported as Lsmeans±SD.

The embryonic cell numbers, especially the ratio of ICM:TE cells, is one of the criteria for assessment of blastocyst quality 18. Aberrant allocation of ICM and TE cells in embryos at preimplantation stages may cause placental abnormalities and early fetal loss 19. The environment during early embryonic development clearly plays a crucial role in determining the cell numbers of the embryo. Small differences between in vitro and in vivo derived embryos already occur during development up to the 8-cell stage, but development in vivo during the first 100 hr post ovulation up to the fifth cell cycle is decisive for the total cell number of the embryo and the ICM in the expanded blastocyst at 7 days post ovulation 11,20. It was suggested that contact with the maternal tract might be of importance to switch on certain genes that encode for developmentally important processes, such as tight junction formation in the case of inner cell mass allocation 21. Therefore, it is speculated that in IMO groups, the embryonic cells are more stimulated to cleave because of their interaction with the oviductal cells. The embryonic cells number was not different between SOFaaciBSA and SOFaaBSA with IMO (Table 4). Therefore it can be concluded that SOFaaciBSA and SOFaaBSA can support ovine embryo cells division when cultured in IMO.

An aberrant ICM:TE ratio has been suggested to be related to the large offspring syndrome of in vitro-produced embryos 22. Therefore we did not observe significant differences in ICM:TE ratio between groups, but embryos cultured in IMO (either with SOFaaBSA or SOFaaciBSA) had approximately 2 fold higher total embryonic cells than SOFaaBSA alone.

**Conclusion**

The culture of ovine IVM/IVF zygotes in IMO using two highly efficient ruminant embryo culture media not only supported development of ovine embryos as well as embryo culture in vitro alone but also the quality of resulted embryos improved. Ani-
normal preparation and synchronization cost and difficulty of embryo transfer may be some of the limitations of this work.

**Acknowledgement**

We would like to thank Presidential Office Deputy of Science and Technology, Iran National Science Foundation (INSF) for financial support (Proposal No: 89003548).

**Reference**

1. Rizos D, Ward F, Duffy P, Boland MP, Lonergan P. Consequences of bovine oocyte maturation, fertilization or early embryo development in vitro versus in vivo: implications for blastocyst yield and blastocyst quality. Mol Reprod Dev 2002;61(2):234-248.
2. Whittingham D. Development of zygotes in cultured mouse oviducts. I. The effect of varying oviductal conditions. J Exp Zool 1968;169(4):391-397.
3. Minami N, Bavister BD, Iritani A. Development of hamster two-cell embryos in the isolated mouse oviduct in organ culture system. Gamete Res 1988;19(3):235-240.
4. Krisher RL, Petters RM, Johnson BH, Bavister BD, Archibong AE. Development of porcine embryos from the one-cell stage to blastocyst in mouse oviducts maintained in organ culture. J Exp Zool 1989;249(2):235-239.
5. Rizos D, Pintado B, de la Fuente J, Lonergan P, Gutiérrez-Adán A. Development and pattern of mRNA relative abundance of bovine embryos cultured in the isolated mouse oviduct in organ culture. Mol Reprod Dev 2007;74(6):716-723.
6. Sharif H, Vergos E, Lonergan P, Gallagher M, Kinis A, Gordon I. Development of early bovine embryos in the isolated mouse oviduct maintained in organ culture. Theriogenology 1991;35:270.
7. Sparks AE, Gwazauskas FC, McGilliard ML. Culture of one-cell bovine embryos in explanted mouse oviduct and bovine oviductal epithelial cells. Theriogenology 1992;37(3):587-594.
8. Goodridge A. Regulation of fatty acid synthesis in isolated hepatocytes. Evidence for a physiological role for long chain fatty acyl coenzyme A and citrate. J Biol Chem 1973;248(12):4318-4326.
9. Gray CW, Morgan PM, Kane MT. Purification of embryotrophic factor from commercial bovine serum albumin and its identification as citrate. J Reprod Fertil 1992;94(1):471-480.
10. Holm P, Booth PJ, Schmidt MH, Greve T, Callesen H. High bovine blastocyst development in a static in vitro production system using SOFaa medium supplemented with sodium citrate and myoinositol with or without serum proteins. Theriogenology 1999;52(4):683-700.
11. Downes C, MacPhee CH. Myo-inositol metabolites as cellular signals. Eur J Biochem 1990;193(1):1-18.
12. Chiu T, Tam PP. A correlation of the outcome of clinical in vitro fertilization with the inositol content and embryotrophic properties of human serum. J Assist Reprod Genet 1992;9(6):524-530.
13. Lonergan P, Rizos D, Gutiérrez-Adan A, Fair T, Boland MP. Oocyte and embryo quality: effect of origin, culture conditions and gene expression patterns. Reprod Domest Anim 2003;38(4):259-267.
14. Bindon B. Systematic studies’ of preimplantation stages of pregnancy in the sheep. Aust J Biol Sci 1971;24(1):131-147.
15. Yuan YQ, Van Soom A, Coopman FO, Mintiens K, Boerjan ML, Van Zeveren A, et al. Influence of oxygen tension on apoptosis and hatching on bovine embryos cultured in vitro. Theriogenology 2003;59(7):1585-1596.
16. Holm P, Booth PJ, Callesen H. Kinetics of early in vitro development of bovine in vivo- and in vitro-derived zygotes produced and/or cultured in chemically defined or serum-containing media. Reproduction 2002;123(4):553-565.
17. Van Soom A, Bols PEJ, Vanroose G, de Kruijf A. A comparison between in vivo and in vitro produced bovine embryos: Scientific and practical implications. Proceeding of the 12th International Congress on Animal Reproduction; 1992 Aug 23-27; The Hague, The Netherlands. 1531-1533 p.
18. Van Soom A, Boerjan M, Ysebaert MT, De Kruijf A. Cell allocation to the inner cell mass and the trophectoderm in bovine embryos cultured in different media. Mol Reprod Dev 1996;45(2):171-182.
19. Im GS, Seo JS, Hwang IS, Kim DH, Kim SW, Yang BC, et al. Development and apoptosis of pre-implantation porcine nuclear transfer embryos activated with different combination of chemicals. Mol Reprod Dev 2006;73(9):1094-1101.
20. Viuff D, Hendriksen PJ, Vos PL, Dieleman SJ, Bibby BM, Greve T, et al. Chromosomal abnormalities and developmental kinetics in in vivo-developed cattle embryos at days 2 to 5 after ovulation. Biol Reprod 2001;65(1):204-208.
21. Knijn HM, Gjøret JO, Vos PL, Hendriksen PJ, van der Weijden BC, Maddox-Hyttel P, et al. Consequences of In Vivo Development and Subsequent Culture on Apoptosis, Cell Number, and Blastocyst Formation in Bovine Embryos. Biol Reprod 2003;69(4):1371-1378.
22. Thompson JG, Gardner DK, Pugh PA, McMillan WH, Tervit HR. Lamb birth weight is affected by culture system utilized during in vitro pre-elongation development of ovine embryos. Biol Reprod 1995;53(6):1385-1391.

*Avicenna Journal of Medical Biotechnology, Vol. 5, No. 2, April-June 2013*