Research article

Effect of Culture Media on Fertilization Rate in Bovine and Caprine Embryos Production

SARUTTIWONG BOONKONG
Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand

CHAINARONG NAVANUKRAW*
Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand
Agricultural Biotechnology Research Center for Sustainable Economy (ABRCSE), Khon Kaen, University, Khon Kaen, Thailand
Email: chanav@kku.ac.th

JIRATTI THAMMASIRI
Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand

WINAI JAIKAN
Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand

SUTHIPONG URIYAPONGSON
Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand

Received 19 December 2011 Accepted 7 February 2012 (*: Corresponding Author)

Abstract The objective of this experiment was to determine the effect of culture media (Tyrode Albumin Lactate Pyruvate-In Vitro Fertilization-Synthetic Oviduct Fluid; TALP-IVF-SOF and SAGE media^{TM}) on fertilization rate in bovine and caprine embryos production. Thai crossbred bovine ovaries (n=40) were collected at a local abattoir located in Khon Kaen municipality. Caprine ovaries (n=22) were collected by the surgery method at the Department of Animal Science, Faculty of Agriculture, Khon Kaen University. In order to compare the culture media, oocytes were recovered and randomly cultured in TALP-IVF-SOF and SAGE media^{TM} during in vitro oocytes maturation and fertilization as described by the standard protocols for in vitro maturation, in vitro fertilization and in vitro culture, respectively. Oocytes, obtained from ovaries with corpus luteum (CL) and without CL, were recovered and determined as recovery rate prior in vitro culture. The results revealed that the recovery rates of bovine and caprine oocytes were not significantly different between ovaries with CL (58.5 and 27.6%) without CL (43.5 and 29.4%) respectively; bovine oocytes had significantly more layers of cumulus than caprine oocytes. IVF and IVC, developed from two cells to blastocyst stage, derived from SAGE media^{TM} were better than that of TALP-IVF-SOF.

Keywords culture media, in vitro fertilization, embryo, beef, goat

INTRODUCTION

In vitro production techniques of bovine and caprine embryos become widely used to genetic improvement. The improvement of bovine and caprine embryo culture systems is highly desirable in term of the production of preimplantation stage embryos for biotechnological studies and for the embryo transfer. A standard medium for in vitro maturation is tissue culture (Lonergan et al., 1994). TALP-IVF-SOF is the medium commonly use for embryo fertilization and embryo culture in vitro. SAGE Media^{TM} is also available as a commercial product. This culture media has been modified by addition of amino acids. The effect that the culture media may have on the developing embryos between different media is unknown.

Thus, the objective of this study was to determine the effect of culture media on the fertilization rate in bovine and caprine embryos production.

MATERIAL AND METHODS

The experimental procedures were approved by the Animal Ethic committee of Khon Kaen University (Reference No. 0514.1.12.2/88). Bovine ovaries were collected at a local abattoir located in Khon Kaen municipality and caprine ovaries were collected by surgery method at the Department of Animal Science, Faculty of Agriculture, Khon Kaen University. In order to compare the culture media, oocytes were recovered and randomly cultured in two media: TALP-IVF-SOF (Table 1) and SAGE media™ (CooperSurgical Inc., Trumbull, CT) as shown in Table 2.

Oocyte collection in bovine and caprine: Bovine oocyte collections were recovered from slaughtered bovines at Khon Kaen municipality and transfered in 0.9% saline to the laboratory within 1h (Yang et al., 1990). Follicles were aspirated with an 18 gauge needle using vacuum suction.

Caprine oocyte collections were recovered from live goats by surgery method at the Department of Animal Science, Faculty of Agriculture, Khon Kaen University and transferred in 0.9% saline to the laboratory within 1 h (Yang et al., 1990). Follicles were aspirated with a 21 gauge needle using vacuum suction.

Table 1 Chemical composition of culture media (TALP-IVF-SOF)

Composition	TALP-IVF	SOF-IVC
NaCl (mM)	114.00	-
KCl (mM)	3.20	-
NaH_2PO_4 (mM)	0.40	-
$CaCl_26H_2O$ (mM)	2.00	-
HEPES (mM)	0.50	-
Glucose (mM)	5.00	-
PVA (mg/mL)	1.00	-
Lactate (sodium salt) (mM)	13.00	-
Pyruvate (mM)	10.40	-
Penicillamine (mM)	3.35	-
Gentamicin (µg/mL)	50.00	-
Sodium pyruvate (g)	-	0.33
$NaHCO_3$ (g)	-	1.00
Glutamine (g)	-	1.50
Glucose (g)	-	10.00
Serum (mg/ml)	-	0.10
EDTA (g)	-	0.10
Streptomycin (g)	-	0.10

Table 2 Chemical composition of culture media SAGE Media™ Product

Catalog Number SAGE Media™Product	ART-1020/1021 Fertilization (HTF)	ART-1026/1027 Cleavage	ART-1029 Blastocyst
Basic Media Components	X	X	X
Non-Essential Amino Acids	X	X	X
Essential Amino Acids			X
Vitamins			X
Magnesium Sulfate Heptahydrate Magnesium Chloride	X	X	X
Potassium Phosphate, Monobasic Anhydrous	X		X
Calcium Lactate (L+)	X	X	X
Taurine	X	X	X
Citric Acid	X	X	
EDTA	X	X	

In vitro maturation (IVM): Oocytes were placed in maturation medium (TCM-199) under mineral oil (Rose et al., 1992). Maturation proceeded for 22-24 h at 38.5 ºC in an environment of 5% CO_2 in air.
In vitro fertilization (IVF): Frozen semen was thawed at 37 ºC for 30 second and washed by centrifuging at 700 g for 5 minutes to remove any remaining semen extender (Gandhi et al. 2000). Before transfer to fertilization drops, oocytes were transferred into drop of fertilization medium under mineral oil. Oocytes were coincubated with spermatozoa for 22-24 h at 38.5 ºC in an environment of 5% CO_2 in air.
In vitro culture (IVC): At 24 h post-insemination, the presumptive embryos were washed in culture medium and embryos were placed into culture medium under mineral oil at 38.5 ºC in an environment of 5% CO_2 in air. Cleavage rate was recorded at 24, 48, 72, 96 and 120 h post-insemination.
Statistical analysis: Data were analyzed using Chi-square test (Steel et al., 1997). Statistical analysis was performed in all tests at a 95% confidence interval.

RESULTS

Studies on the influence of ovaries with CL (ovaries within the development of CL) and without CL showed that ovaries with CL and without CL were not significantly different (P>0.05) (Table 3).

Table 3 Recovery rate of oocyte obtained from bovine ovaries

Ovary	Number		% Recovery of oocyte
	Follicle	Oocyte	
with CL	123	72	58.54 (72/123)
without CL	147	64	43.54 (64/147)

Table 4 The recovery rate of oocyte obtained from caprine ovaries

Ovary	Number		% Recovery of oocyte
	Follicle	Oocyte	
with CL	94	26	27.66 (26/94)
without CL	17	5	29.41 (5/17)

Studies on the influence of ovaries with CL (ovaries within the development of CL) and without CL showed that ovaries with CL and without CL were not significantly different (P>0.05) (Table 4).

Morphology of bovine oocytes and caprine oocytes

Study of the morphology of bovine and caprine cumulus cells have shown that bovine oocytes had significantly more layers of cumulus than caprine oocytes and can effect fertilization of oocytes.

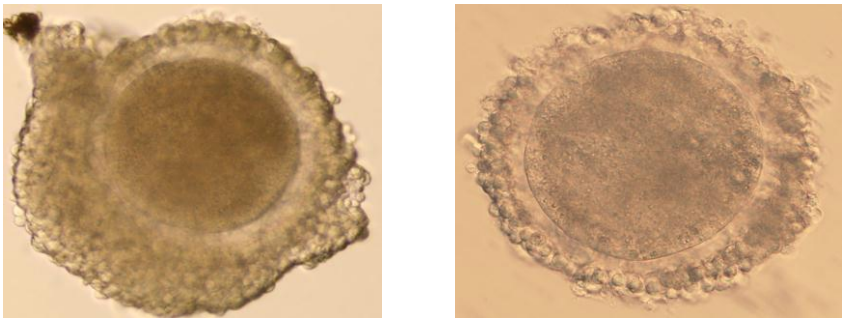

Fig. 1 Morphology of bovine oocytes (left) and caprine oocytes (right)

Effect of culture media on in vitro fertillization in bovine embryo production

The results revealed that IVF and IVC, developed from two cells to the blastocyst stage of SAGE media™, were better than that of TALP-IVF-SOF as determined by the embryo image analysis**.**

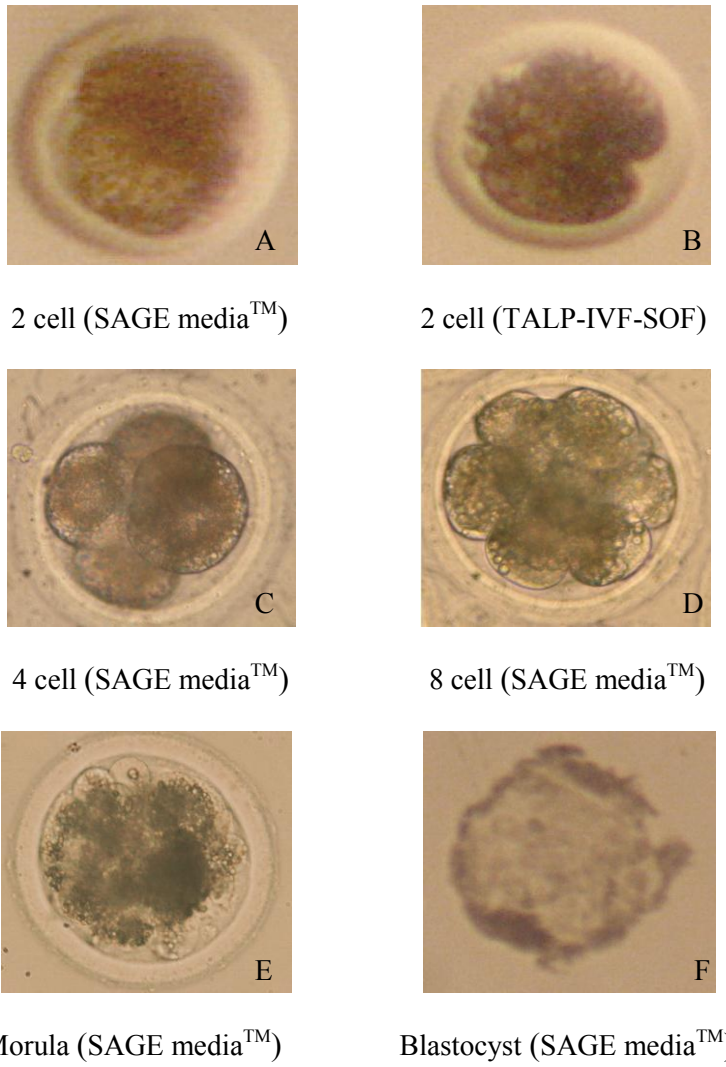

Fig. 2 (A-F) Morphology of bovine embryo during early development

CONCLUSION

The present study suggested that the number of cumulus cells is important for supporting embryo development and culture media can increase the cleavage.

ACKNOWLEDGEMENTS

The authors would like to express the most appreciation to the Thailand Research Fund Master, Research Grants: TRF-MAG Window II (MRG-WII535S012), and Research Group of Beef and Buffalo production, Department of Animal Science, Faculty of Agriculture, Khon Kaen University.

REFERENCES

Gandhi, A.P., Lane, M.L., Gardner, D.K. and Krisher, R.L. 2000. A single medium supports development of bovine embryos throughout maturation fertilization and culture. Human Reproduction, 15, 395-401.

Gordon. I. 1994. Laboratory production of cattle embryos. CAB International, Oxon, UK, 640.

Lonergan, P., Carolan, C. and Mermillod, P. 1994. Development of bovine embros in vitro following oocyte maturation under defined conditions. Reproduction Nutrition Development, 34, 329-339.

Nagano, M., Katagiri, S. and Takahashi, Y. 2006. Relationship between bovine oocyte morphology and in vitro development potential. Zygote, 14, 53-61.

Rose, T.A. and Bavister, B.D. 1992. Effect of oocyte maturation medium on in vitro development of in vitro fertilized bovine embryos. Molecular Reproduction Development, 31(1), 72-7.

Steel, R., Torrie, J. and Dickey, D. 1997. Principles and procedures of statistics: A biometrical approach. WCB/McGraw-Hill, a Division of the McGraw-Hill Companies, 666.

Yang, N.S., Lu, K.H. and Gordon, I. 1990. In vitro fertilization (IVF) and culture (IVC) bovine oocytes from stored ovaries. Theriogenology, 33(1), 352-362.