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Production, Cryopreservation and Transfer of Bovine Embryos Cultured in Serum-Free Medium

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Summary

In vitro embryo culture techniques provide significant contributions not only for a basic research of fertilization and early embryogenesis, but also for a low cost mass production of bovine embryos for transfer, embryo diagnosis, nuclear cloning and the production of transgenic cows.

This presentation introduces newly developed serum-free media (IVD101 and IVMD101) that are effective for high yields of transferable embryos of excellent quality from in vitro-matured and fertilized oocytes. Both serum-free media are superior to a conventional serum-containing medium on the increased rates of blastocyst formation, post-thaw embryo viability, and pregnancy after transfer. Furthermore, reduced risks of calf mortality and large calf syndrome are also observed for the serum-free-derived embryos. Serum-derived embryos contain a large number of lipid droplets and immature mitochondria in their cytoplasm that may account for the lower production of transferable embryos and poor embryo quality.

Introduction

Serum supplementation into a culture medium and the co-culture of somatic cells are generally known to be beneficial for in vitro maturation (IVM) of oocytes and in vitro embryo culture (IVC). Serum provides nutrients, vitamins, growth factors, hormones, and anti-oxidative compounds, etc., for oocyte maturation and embryo development. However, the biological activities of sera

vary from lot to lot and there are potential risks for virus and mycoplasma infection. Even though somatic cell co-cultures are effective to overcome the developmental arrest of bovine preimplantation embryos in vitro (1,2), it is more complicated and time consuming to prepare somatic cell co-cultures. The uses of serum and co-culture systems have been considered to be potential risks of large offspring syndrome in livestock animals (3,4).

Survival rates of frozen in vitro-derived embryos, as measured either by post-thaw development in culture or by pregnancies following embryo transfer, have been lower than those for in vivo-derived embryos (5,6). Viability of frozen thawed in vitro-derived embryos could be affected by the culture conditions during the embryo development.

This study presents the development of novel serum-free culture systems, which assure superior production of transferable embryos with a higher survival rate after freezing and thawing, compared to the conventional culture system with a serum-containing medium.

Novel serum-free media for IVM and IVC

We have developed serum-free media (IVMD101 and IVD101) for in vitro maturation of bovine oocytes and embryo development (Table1, 7). IVMD101 medium is used for both in vitro maturation and embryo culture in the presence of somatic cells such as cumulus/granulosa cells under an atmospheric oxygen concentration (20%). IVD101 medium is designed for embryo culture in the absence of somatic cells under a low oxygen atmosphere (5%).

Comparative study of developmental and cryosurvival abilities of bovine embryos produced in either serum-free or serum-containing media

Developmental rates to the blastocyst stage of embryos cultured in either IVMD101 (36.5%) or IVD101 (37.1%) serum-free media were significantly higher than that in serum-containing medium (TCM199+5% calf serum (CS); 25.1%). Bovine transferable embryos (blastocysts) were frozen using the conventional slow freezing method in a 1.8M ethylene glycol solution. And then, the embryos were thawed in 35°C warm water, rehydrated in the culture medium (TCM199+5% CS),

Table 1. Composition of serum-free media (IVD 101, IVMD101) for in vitro maturation (IVM) and in vitro embryo culture (IVC)

Components	IVD101	IVMD101
Basal medium*	DM199	DM199
D-glucose (mM)	2.22	5.56
Sodium pyruvate (mM)	0.27	0.91
Sodium lactate (mM)	2.48	-
L-cystein (mM)	0.05	-
GSH (μM)	200	-
Taurine (mM)	5	5
Selenium (nM)	5	5
Insulin (μg/mL)	-	5
TGF-a (ng/mL)	-	10
Apotransferrin (μg/mL)	10	10
bFGF (ng/mL)	10	-
TGF-b1 (ng/mL)	1	-
TIMP-1 (μg/mL)	0.5	-
Aprotinin (μg/mL)	0.5	-
BSA (mg/mL)	1	1
HEPES (mM)	5	5
Gentamicin sulfate (μg/mL)	10	10

*Basal medium (DM199) does not contain glucose, Tween-80 and para-aminobenzoic acid from original components of TCM199.

and cultured on feeder layer of bovine granulosa cells at 38.5°C.The survival rates, based on hatching after 72hr of post-thaw culture, of blastocysts produced in IVMD101 (73.3%) and IVD101 (60.0%) serum-free media were superior to that of blastocysts produced in the serum-containing medium (48.1%). The morulae and blastocysts that developed in the serum-containing medium contained abundant lipid droplets in the cytoplasm, while those cultured in IVMD101 had many lysosome-like vesicles but fewer lipid droplets under an electron microscopic observation (8,9). This suggests that the presence of serum in the embryo culture medium may be the main cause of the abnormal accumulation of lipid droplets. As a result, bovine embryos with high lipid content may be more sensitive to

cryopreservation.

Pregnancy, birth rates and birth weights of calves from embryos produced in either serum-free or serum-supplemented media

A high incidence of excessive birth weights of calves, large calf size, dystocia, cesarean sections and calf mortality following non-surgical transfers of bovine in vitro-derived embryos has been reported by several investigators (10,11,12). In our study, the pregnancy rate of recipients receiving frozen serum-derived embryos was slightly higher than those receiving serum-derived embryos (39.6% vs 32.8%). Calf mortality for the serum-free-derived embryos was lower than that for the serum-derived embryos (4.9% vs13.6%). Average birth weights of calves from the serum-derived embryos were higher than those from the serum-free-derived embryos, while no significant difference was observed in the mean gestation of cows derived from the serum-free or serum-supplemented media. It is interesting that the birth weights of calves from the serum-derived embryos were more variable than those from the serum-free-derived embryos.

Conclusions

Newly developed serum-free media (IVMD101 and IVD101) are beneficial for an efficient production of good quality embryos for transfer. The serum-free media improved the rates of blastocyst formation and post-thaw embryo viability. In addition, calves from the embryos produced in the serum-free media showed the reduced risks of calf mortality and large calf syndrome.

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