Comparison of media in cryopreservation of bovine oocytes and embryos

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Introduction

The aim of the present research was to test the survival rates, hatched rates after cryopresereved of embryos and oocytes comparison by a Cell freezing medium (BAMBANKER, L-TEC, Tokyo, Japan) and Ethylene Glycol(EG) used slow-rate freezing by programmable freezer or deep freezing with BICELL bio-freezing vessel(AS ONE,Osaka,Japan).

Materials and methods

Bovine ovaries were obtained at a local slaughterhouse, and the oocytes were aspirated from the follicle immediately after the slaughter of the animal. Collected cumulus oocyte complexes(COCs) were matured in 25mM Hepesbuffered TCM199(TCM199) supplemented with 5% calf serum(CS) and 0.02mg/mL of FSH for 20 hours(20 COCs/100μl).For embryos experiment of oocytes were fertilized with Holstein bull semen for 6 hours (5×10^6 sperm/ml).The presumptive zygotes were cultured in CR1aa medium supplemented with 5% CS for 9 days (fertilization = Day 0). The kinetics of embryo development were observed at 27,31 and 51h post insemination by stereomicroscope. The produced embryos were measured at 7,8 and 9 days post-IVF. Day 7~9, developed blastocysts were frozen for further study. In freezing, each control group blastocysts were washed and frozen by Dulbecco's phosphate-buffered saline(D-PBS) supplemented with 20% CS, 1.5M EG and 0.1M sucrose(SUC).Experiment group blastocysts were frozen by the cell freezing medium in programmable freezer and BICELL in deep freezing refrigerator. Frozen blastocysts of the slow-rate freezing method were thawed by allowing straws to stand in the air for 10sec, and warming them in a 30℃ water bath for 30 sec. Frozen embryos of deep freezing methods were thawed by allowing tubes to stand in the air for 10 sec, and warming them in a 38℃ water bath for 1 min. The thawed blastocysts were washed twice using 38°C D-PBS supplemented with 20% fetal calf serum(FCS). Subsequently, they were immersed in the same medium, held at 38°C for 10 min, and then each blastocysts was cultured in 20-μL droplets of TCM199 supplemented with 20% FCS and 0.1 mM ß-mercaptoethanol for 72 h. The re-expended

rates of blastocysts were determined 72 h after thawing. The procedure of oocytes experiment is maturation, frozen, thawing, fertilization, culture.

Results

In experiment 1, no significant difference was observed in survival rate between the control (1.5M EG) and a cell freezing medium group of blastocysts, but a minimum difference was observed in hatched rate after thawing. (table 1) In experiment 2, significant difference was observed in the survival rate between control and Cell freezing medium group. However, the hatched rate of the blastocysts was no result between control and cell freezing midium group. (table 2) In experiment 3, minimum difference was observed in survival rate between control and BAMBANKER group of oocytes, but no result in hatched rate.(table 3) In experiment 4,no result of cleavage rate and the hatched rate in deep freezing.(table 4)

(table 1)

Slow-rate freezing of survival rate/hatched rate (N/%)				
Freezing reagent	Blastocysts	24h	48h	72h
1.5M EG	17	17(100)/0	17(100)/2(11.8)	11(64.7)/ 2(11.8)
Freezing midium	18	16(88,9)/0	16(88,9)/0	12(66.7)/1(5.6)

(table 2)

Deep freezing of survival rate/hatched rate (N/%)				
Freezing reagent	blastocysts	24h	48h	72h
1.5M EG	17	12(70.5)[a]/0	12(70.5)[c]/0	12(70.5)[e]/0
Freezing midium	18	10(54.1)[b]/0	10(54.1)[d]/0	10(54.1)[f]/0

a-b,c-d,e-f($P<0.04$)

(table 3)

Slow-rate freezing of fertilized rate/hatched rate (N/%)				
Freezing reagent	oocytes	27h/7days	31h/8days	51h/9days
1.5M EG	76	0/0	0/0	2(2.6)/0
Freezing midium	48	0/0	0/0	4(8.3)/0

(table 4)

Deep freezing of fertilized rate/hatched rate (N/%)				
Freezing reagent	oocytes	27h/7days	31h/8days	51h/9days
1.5M EG	24	0/0	0/0	0/0
Freezing midium	13	0/0	0/0	0/0