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Original Article

Effect of various concentrations of Minimal Essential Medium vitamins (MEM vitamins) on development of sheep oocytes during in-vitro maturation

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Abstract

Background: Improvements in culture media formulations have led to an increase in the ability of sheep embryo in culture throughout the preimplantation period.

Objective: This study was carried out to evaluate the effects of various concentrations of MEM vitamins during in vitro maturation of sheep oocytes and subsequent embryo development.

Materials and Methods: Sheep ovaries were collected from a slaughterhouse and transported to the laboratory. Oocytes were matured in SOF medium supplemented with, eCG, hCG and EGF in various concentrations of MEM vitamins (control, 0.5, 1 and 1.5×) for 24h. The cumulus oocyte compelex (COCs) were co-incubated with epididymal spermatozoa of post mortem rams in synthetic oviduct fluid fertilization (SOFF) medium with 10% heat inactivated estrous sheep serum for 18h. Embryos were cultured in synthetic oviduct fluid culture 1 (SOFC1) medium for 48h followed by cultured in synthetic oviduct fluid culture 2 (SOFC2) medium for six days.

Results: Addition of 0.5 and 1 × MEM vitamins significantly increased ($P< 0.05$) overall blastocyst development (21.62% and 22.33%; respectively) compared with 1.5×MEM vitamins (15.59%), but there was no difference between control, 0.5 and 1×MEM vitamins in the percentage of embryos successfully developing to the blastocyst stage (19.50%, 21.62% and 22.33% respectively).

Conclusion: It seems that addition of 1.5× of MEM vitamins has detrimental effect on blastocyst rate.

Key words: *Embryo development, IVM, MEM vitamins, Sheep, SOF.*

This article was extracted from M.Sc. thesis.

Introduction

Using in vitro production of embryos allows us to obtain a large number of offsprings from live or slaughtered animals (1) for research, genetic improvement or commercial purposes (2). In sheep, techniques for in vitro fertilization and in vitro culture of embryos (IVC) seems to be under control. However in most domestic species In-vitro maturation (IVM) of oocytes needs to be perfected (1).

During IVM, oocytes undergo a series of cytoplasmic changes before the resumption of nuclear maturation, leading to variable competence of the resulting embryos (3). The alteration of basic maturation conditions can significantly affect oocyte competence as reflected by the morula and blastocyst yield after in vitro fertilization (IVF) (3).

Consistently successful and reliable oocyte maturation (both cytoplasmic and nuclear maturation) would dramatically improve the efficiency of preimplantation embryonic development as well as fetal development (4). Previous reports have indicated that the addition of water-soluble vitamins to the culture medium improved hatched blastocyst and enhanced development of rabbit and hamster embryos to the blastocyst stage (5-7).

However, the presence of Minimal Essential Medium vitamins (MEM vitamins) to semi-defined maturation medium enhanced subsequent developmental ability of caprine oocytes (8). In addition, supplementation of MEM vitamins during IVM enhanced subsequent parthenogenetic development in porcine oocytes (9).

While, the presence of low concentrations of MEM vitamins during IVM improved

development of procine nuclear transfer embryos (10). The addition of myo-inositol compared to MEM vitamins containing myo-inositol in SOF medium is more beneficial for subsequent sheep embryonic development (11).

The addition of 1×MEM vitamins in semi defined maturation medium (SDMM) containing BSA-V and BSA-FFA as compared to addition of 1×MEM vitamins in SDMM containing BSA-05471 and controls is more beneficial for subsequent blastocyst development (12).

The MEM vitamins used for IVM contained B-complex including thiamin hydrochloride, riboflavin, pyridoxal hydrochloride, folic acid, D-calcium pantothenate, myoinositol, nicotinamide and choline chloride which function as coenzymes for energy production and synthesis of organic molecules. However, on the basis of our knowledge the benefical effects of various concentrations of MEM vitamins during maturation of sheep oocytes and subsequent in vitro embryo development have not been reported.

The objective of this study was to determine if supplementation of various concentrations of MEM vitamins in SOF maturation media (without protein supplements) could improve the maturation of sheep oocytes and their subsequent in vitro embryo development.

Materials and methods

Study design

Prospective experimental study.

Chemicals

Unless mentioned otherwise, growth factors and other chemicals were purchased from Sigma (USA, St. Louis, MO), plastics from Falcon (UK, Becton Dickinson, Lincole, NJ), and hormones (eCG and hCG) from Intervet Schering-Plough Animal Health (Netherlands).

All the media were incubated at 39^{o}C under humidified atmosphere of 5% CO_2 in air for 1 h prior to use.

Oocyte collection

Sheep ovaries were transported from the local abattoir to laboratory in 0.9% saline at 30-35^{O}C. They were washed three times with warmed 0.9% saline. COCs were aspireated from 2 to 6 mm follicles using an 21- gauge needle attached to a 10 ml syringe. COCs were collected in SOF HEPES medium supplemented with 14.24 mg/ml heparin and 50 μg/ml gentamicin. In this medium, 20 mM $NaHCO_3$ was replaced with 20 mM HEPES, pH 7.4, and was supplemented with MEM amino acids and 4 mg/ml BSA.

In-vitro maturation

COCs with at least three layers of nonexpanded cumulus and homogeneous cytoplasm were selected and washed three times in SOF HEPES and once in SOF maturation medium supplemented with, 0.23 mmol/L sodium pyruvate, 50 ng/ml epidermal growth factor (EGF), 10 IU/ml equine chorionic gonadotrophin (eCG) and 10 IU/ml human chorionic gonadotrophin (hCG) and penicillin/streptomycin (100 U/ml penicillin, 100 μg/ml streptomycin) (13).

The COCs were matured in 4 treatment groups: (I) SOF maturation medium (control), (II) SOF maturation medium supplemented with 0.5×MEM vitamins, (III) SOF maturation medium supplemented with 1×MEM vitamins and (IV) SOF maturation medium supplemented with 1.5×MEM vitamins. Oocytes were matured in groups of 10 per 50 μl droplets in culture dishes under 10 ml mineral oil. Maturation proceeded for 22 to 24h at 39^{O}C and 5% CO2 in air with almost 100% humidity.

In-vitro fertilization

At the end of the maturation period, oocytes were briefly placed in SOF maturation medium supplemented with 500 IU/ml hyaluronidase, followed by gentle pipeting to dissociate surrounding cumulus cells and washed three times in fertilization medium. Oocytes (10 to 15) were placed into a 100 μl drop to which sperm cells were added 5 min later. An epididymal spermatozoa was

collected from post mortem rams and kept at room temperature for up to 2 h, then washed in SOF, and centrifuged twice at 200×g for 5 min.

The medium used was SOF, as originally described by Tervit *et al* (14), enriched with 10% heat inactivated estrous sheep serum. Sperms were counted by a hemacytometer and checked for acceptable motility (i.e., at least 80% progressively motile); then oocytes were inseminated by addition of sperm to result in a final concentration of 1.0×10^6 motile sperm per milliliter. Sperm and oocytes were incubated under mineral oil at 39°C under humidified atmosphere of 5% CO_2 for 18 h.

In-vitro culture

Embryo culture took place in modifid SOF under mineral oil in a humidified atomosphere of 5% CO_2 and 5% O_2 and 90% N_2 at 39°C. Between 15 and 18 h after insemination, presumptive zygotes were denuded of surrounding cumulus cells by repeated pipetting in SOF HEPES and subsequently washed three times in SOF HEPES before being transferred to the culture droplets (50 µl) in groups of 10 to 15 embryos.

Cleavage was assessed after 48 h of culture, and the number of embryos developing to the morula and blastocyst stages were assessed on day 8. To prevent toxic accumulation of ammonium as a result of amino acid degradation, SOF medium was replaced every 48 h. In this study, we used a sequential culture system.

The first (SOFC1) medium contained 0.8% crystallized BSA, MEM nonessential amino acids, 1mM glutamine, 1.5 mM glucose, and 10 µM EDTA for the first 48 h. Then, the medium was replaced by the second system (SOFC2) containing 0.8% crystallized BSA, MEM nonessential amino acids, MEM essential amino acids, 3 mM glucose, and 1 mM glutamine for the remaining 6 days of culture.

Statistical analysis

Each experiment was replicated at least 6 times and COCs were randomly allocated into each treatment group. The data were analyzed by one-way ANOVA using the SPSS program. A percentage morula data were subjected to $\sqrt{x}$ transformation before analysis. Duncan's multiple range test was used to test the differences between treatments. $P<0.05$ indicated as a significant difference.

Results

In the present study, there were no differences between treatments in the percentage of embryos successfully developing to the cleavage stage, expressed as a percentage of total inseminated oocytes (Table I). There were no differences in development to morula of cleaved embryos (Table I). No significant difference was also observed in blastocyst rates between 0.5×, 1×MEM vitamins and control groups (21.62%, 22.33% and 19.50%; respectively). The presence of 1.5×MEM vitamins significantly decreased the percentage of blastocyst ($p<0.05$) as compared with addition of 0.5 and 1×MEM vitamins (Table I).

Table I. The effect of different concentrations of MEM vitamins on the in vitro maturation and development of sheep oocytes.

Treatments	Oocytes (n)	Cleavage (%)	Morula (%)*	Blastocyst (%)*
SOF	246	58.58±6.77[a] (144)	36.74±5.25[a] (53)	19.50±1.16[ab] (28)
SOF + 0.5×MEM	250	60.03±4.86[a] (150)	34.88±3.05[a] (52)	21.62±1.02[a] (32)
SOF + 1 × MEM	251	67.07±5.71[a] (168)	39.08±5.02[a] (65)	22.33±1.11[a] (37)
SOF + 1.5 × MEM	254	70.44±4.19[a] (179)	33.21±5.23[a] (59)	15.56±2.0[b] (28)

Note: Data expressed as mean±SEM of six replicates. SOFM= synthetic oviduct fluid medium (control); SOF+0.5×MEM=SOF maturation medium supplemented with 0.5×MEM vitamins; SOF+1×MEM=SOF maturation medium supplemented with 1×MEM vitamins; SOF+1.5×MEM=SOF maturation medium supplemented with 1.5×MEM vitamins.

* Morula and blastocysts per cleaved oocytes.

[a,b] Different letters indicate statistical difference within each column ($p<0.05$).

Discussion

Synthetic oviductal fluid (SOF) medium, which was originally based upon the biochemical and physiological analysis of sheep oviductal fluid (14), has been used in the IVM of bovine (3,16), goat (8), bitch oocytes (17) and in the in vitro culture of sheep embryos (14, 18). In the present study, the effect of various conecntrations of water-soluble MEM vitamins in the in-vitro maturation of sheep oocytes was evaluated. Water-soluble vitamins and quasi vitamins may play a significant role in oocytes metabolism.

The addition of 0.5 and 1×MEM vitamins to maturation medium had no significant effect ($p>0.05$) on enhancing sheep embryo development in comparison to control groups, although there was a numerical increase. The development of embryos to the blastocyst stage was improved by the addition of 0.5 and 1×MEM vitamins to SOF medium compared with using of 1.5×MEM vitamins.

The benefical effects of low concentration of MEM vitamins have been observed during in vtiro maturation of porcine oocytes (9, 10) but, addition of vitamins to IVC medium did not promote continued parthenogenic development to blastocyst stage in pig embryo (9).

Koo *et al* (19) showed that using 1× or more of MEM vitamins was ineffective or had a detrimental effect on IVC of porcine embryos, while Bormann *et al* (8) reported a beneficial effect in IVM of caprine oocytes when 1×MEM was used. Such a difference between goat and pig oocytes in their response to various concentrations of water-soluble MEM vitamins is unclear. But, the effect of vitamin concentrations appears to be species-dependent (10).

A study on bovine zygotes indicated that the addition of vitamins during IVF had no effect or tended to decrease the frequency of embryos attaining the blastocyst stage (20). A numerical reduction in bovine morula percentage was also detected by Takahashi and First (20, 21).

Inositol is an affector of structure and function of membranes. This vitamin may play a role in the phosphatidylinositol second messenger system (22). Inositol may play an essential role in the formation of pronuclei during in vitro fertilization (6). In an in vitro culture study with sheep embryos Gardner *et al* showed that the addition of vitamins significantly increased blastocyst metabolism as determined by glucose uptake and lactate production per cell. They concluded that vitamins facilitated changes in relative activities of specific enzymes involved in energy metabolism, leading to an increase in glucose utilization (18). It has also been reported that the presence of amino acids and vitamins in culture medium reduce the elevation in glycolytic activity, prevent any drop in oxidative capacity and maintain viability at levels of in vivo developed blastocysts (23).

Results from the present study indicated that 0.5 and 1×MEM vitamins as compared to 1.5× had a positive effect during maturation resulting in an increased development of blastocyst from cleaved embryos. The MEM vitamins solution contained 6 vitamins and 2 quasivitamins. Therefore, the effect of vitamins in a specific combination (MEM) rather than the effect of individual vitamins in various concentrations was determined.

A significant reduction in blastocyst percentage (1.5×) indicating a detrimental effect on ovine blastocyst development may be due to the high concentration of any of the vitamins in MEM. It is possible that, some of the vitamins, which had no effect in MEM mixture at low concentrations, exerted a negative effect at higher concentrations. Further research is obviously needed to determine the effect of individual vitamins in the various concentrations and combination in the in vitro maturation and subsequent ovine embryo development.

Conclusion

In conclusion, the results of the present study indicate that the addition of 1.5×MEM

vitamins may have detrimental effect on ovine blastocyst rate.

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