Effect of Culture Media on Mare Oocytes Maturation

Hussam ARYAN*, Ioan GROZA, Iancu MORAR, Liviu BOGDAN, Anamaria PETREAN, Omar MARDENLI

¹Department of Veterinary Obstetrics, Gynecology and Reproduction, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania,

* Corresponding author: hussamaryan@yahoo.com

Bulletin UASVM Veterinary Medicine 73(1) / 2016,
Print ISSN 1843-5270; Electronic ISSN 1843-5378
DOI:10.15835/buasvmcn-vm: 11610

Abstract

432 mare oocytes were collected from ovaries of slaughtered animals and cultured in three mediums supplemented with hormones (FSH, LH) and nutritive substances and were cultivated for 24 hours in three media, the highest cultivation rate in three media was obtained in TCM-199 medium 53.33% (80 oocytes), followed by SOF medium, maturation rate was 39.65% (58 oocytes), but the less maturation rate (34.54%) was observed in the medium composed of TCM+ 5% SOF + 5% FCS + 1% glutamine + 1% A.A + 500 µl antibiotic). Oocytes were selected and classified in two quality categories (mature, degenerated).

Keyword: Degenerated, maturation, medium, oocyte, recovery.

INTRODUCTION

Oocytes recovery and cultivation media were selected according to the species, stage of follicular development, recovery technique and storage duration (Groza *et al.*, 2004). Cultivation systems were diversified rapidly, based on consecrate elements, besides ensuring their supplements (hormones, vitamins, protein) as increasing the potential for development after fertilization. These supplements are: epidermal growth factor, growth hormone, bovine serum albumin (BSA), synthetic oviduct fluid (SOF) etc. (Squires, 2005). We believe that mare oocytes in vitro fertilization protocol, ensuring complete maturation by cultivation in different complex mediums are key points for the success of this biotechnology.

The aim of this paper is to evaluate media and solutions used in oocyte maturation for improving their original contributions and correlation morphological study with the degree of maturation and selection for fertilization.

MATERIAL AND METHOD

Research was conducted on a number of 432 mare oocytes collected from ovaries of slaughtered

animals in abattoir Cetina (Baia Mare), Romania. After examining on the stereo- and inverted microscope, recovered oocytes were identified, selected and classified into two quality categories based on morphological characteristics. Only cultivable oocytes were used for *in vitro* maturation due to their ability to continue their development and precure „*in vitro*” fertilization (Fig. 1).

Before being transferred to the maturation medium, selected oocytes were subjected to the washing process in medium collection by repeatedly aspiration (three times). Last pass was done in the maturation medium to remove impurities and to avoid contamination. Selection of media used for maturation was performed bearing in mind necessities of protein, vitamins and hormones to initiate and ensure the development potential of mare oocytes to metaphase II of meiotic division (appearance first polar body).

Using a fine pipette with a diameter of 50 µl, 5-6 oocytes were placed in medium. The prepared plates were introduced to the incubator for 24 hours at a temperature 38.2 - 38.5 °C and 5.3% CO₂. Depending on the morphological characteristics

observed after cultivation for 24 hours and stereo- and inverted microscope examination.

RESULTS AND DISCUSSION

Mare oocyte *in vitro* cultivation which was performed in medium for 24 hours in three culture medium supplemented originally, aimed for completion of maturation and preparation for in vitro fertilization protocol. 432 mare oocytes were cultured. Cultivation process in three culture media as follows :

150 oocytes were cultured in medium TCM -199 supplemented with 10 % FCS + 25 mM HEPES + 1 mM sodium pyruvate + 1% glutamine + 1% AA + 10 µg / ml of FSH + 2 µg / ml of LH + 500 ul antibiotic;

137 oocytes were cultured in medium consisting of TCM -199 supplemented with 5 % SOF + 5% FCS + 1% glutamine + 1% AA + 500 ul antibiotic;

145 oocytes were cultured in medium consisting of SOF supplemented with 10 % FCS + 1% glutamine + 1% AA+ 500 ul antibiotic.

Fig. 1 Recoverd oocytes used for in vitro maturation

Tab.1 Composition of media used for mare oocyte *in vitro* maturation

Media	Composition
TCM-199	TCM-199 + 10% FCS + 25mM HEPES + 1mM sodium Piruvate + 1% glutamine + 1% A.A + 10 µg/ml FSH +2 µg/ml LH +500 µl antibiotic
SOF	SOF + 10% FCS + 1% glutamine + 1% A.A + 500 µl antibiotic
TCM + SOF	TCM+ 5% SOF + 5% FCS + 1% glutamine + 1% A.A + 500 µl antibiotic

Fig. 2 Mature Oocyte

Fig. 3 Degenerated Oocyte

After stereo- and inverted microscope examination, mare oocytes were classified into two quality categories:

Mature oocytes: presenting expanded cumulus to five layers of cellule around the oocyte, the presence of the first polar body, homogeneous cytoplasm, integrity pellucida membrane and uniform space perivitelin;

Degenerate oocytes: presenting unexpanded cumulus or partial denudation, granular cytoplasm, retracted and space perivitelin ununiform.

The obtained results after mare oocytes in vitro cultivation and classification of quality are presented in the following table (Table 2):

Comparing results of maturation for 24 h in three media, it highlights those obtained in TCM-199 medium supplemented with 10% FCS + 25 mM HEPES + 1 mM sodium pyruvate + 1% glutamine + 1% AA + 10 µg / ml FSH + 2 µg/ ml LH + 500 µl antibiotic, where the maturation rate was 53.33% (80 oocytes), followed by SOF medium

supplemented with 10% FCS + 1% glutamine + 1% AA + 500 µl antibiotic, maturation rate was 39.65% (58 oocytes), but the less maturation rate 34.54% (47 oocytes) was observed in the medium composed of TCM+ 5% SOF + 5% FCS + 1% glutamine + 1% A.A +500 µl antibiotic (Fig.4).

CONCLUSIONS

432 mare oocytes were cultivated for 24 hours in three media cultivation;

The highest cultivation rate in three mediums was obtained in TCM-199 medium supplemented with 10% FCS + 25 mM HEPES + 1 mM sodium pyruvate + 1% glutamine + 1% AA + 10 µg / ml FSH + 2 µg/ ml LH + 500 µl antibiotic 53.33% (80 oocytes), followed by SOF medium supplemented with 10% FCS + 1% glutamine + 1% AA + 500 µl antibiotic, maturation rate was 39.65% (58 oocytes), but the less maturation rate 34.54% (47 oocytes) was observed in the medium composed

Tab. 2 Mare oocytes quality classification after 24 hours of maturation

<i>In vitro</i> maturation media	Period of IVM	Cultivated oocytes number	Mature Oocytes	Degenerated oocytes
TCM + 10% FCS + 25mM HEPES + 1mM sodium pyruvate + 1% glutamine + 1% A.A + 10 µg/ml FSH + 2 µg/ml LH + 500 µl antibiotic	24 h	150	80 53.33%	70 46.67%
SOF + 10% FCS + 1% glutamine + 1% A.A + 500 µl antibiotic	24 h	145	58 39.65%	87 60.35%
TCM+ 5% SOF + 5% FCS + 1% glutamine + 1% A.A + 500 µl antibiotic	24 h	137	47 34.54%	90 65.46%

Fig.4. Mature oocytes after in vitro cultivation for 24 h

of TCM+ 5% SOF + 5% FCS + 1% glutamine + 1% A.A + 500 µl antibiotic;

Oocytes were selected and classified in two quality categories (mature, degenerated);

We recommend that the extension of the cultivation period from 24 to 28 or 30 hours is necessary to achieve the degree of maturation before in vitro fertilization, and use media supplemented with hormones (FSH and LH), amino acids and synthetic oviduct fluid (SOF).

REFERENCES

1. Groza I, Hârceaga L., Moise D., Bogdan L., Morar I., Sotoc O., Ciupe simona. (1996). Cercetări privind fecundarea "in vitro" a ovulelor de vacă. Simpozion – Lucrări Ştiinţifice de Medicină Veterinară, Timişoara, p.271;
2. Squires, E.L.(2005). Integration of future biotechnologies into the equine industry. Anim. Reprod. Sci. 89, 187–198;