

IN VITRO MATURATION OF BOVINE OOCYTES FOR IN VITRO FERTILIZATION

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Abstract

The objective of the present study was to assess the quality of various cultivation media used for the maturation of bovine oocytes that are prepared for IVF. Upon collection from slaughtered bovine ovaries and after morphological evaluation, a total number of 513 viable oocytes have been selected for cultivation, being divided into 3 batches, 171 oocytes / batch. The oocytes belonging to batch 1 were cultivated in TCM 199 NaHCO₃ + 10% FCS + FSH 20 µl/ml. The oocytes belonging to batch 2 were cultivated in TCM 199 NaHCO₃ + 10% FCS + HCG 2.3 x 10³ UI/ml + FSH 8 µl/ml + pyruvate 0.25 mM + 17β estradiol 1 µl/ml. The oocytes belonging to batch 3 were cultivated in TCM 199 NaHCO₃ + 10% FCS + 17β estradiol 1 µl/ml + FSH 20 µl/ml. The cultivation conditions, for all three batches, were: 24 hours at 39°C, 5% CO₂. Spermatozoa have been prepared using the Percoll method and IVF of the matured oocytes has been performed. Embryonic development has been assessed 72 hours and then up to 10 days after IVF. The results showed the superior quality of the oocytes belonging to batch 2 and matured using TCM 199 NaHCO₃ + 10% FCS + HCG 2.3x10³ UI/ml + FSH 8 µl/ml + pyruvate 0.25 mM + 17β estradiol 1 µl/ml, as their use for IVF yielded the highest number of viable embryos.

Key words: bovine, oocyte, in vitro fertilization, maturation, TCM 199, cultivation

Introduction

Embryo transfer technology has been used commercially over the past 2 decades [1,5]. The available statistics published by IETS show that the use of embryo transfer technology has increased rapidly during the 1980's and the early 1990's [3,8]. However, embryo production has stabilized over the past 5 years. Part of the reason for this plateau in the use of embryo transfer is the difficulty to harmonize this technology with the production goals of every herd [2,4,9]. The in vitro production of embryos (IVF) is an approach that may increase the efficiency of reproduction in a cow without compromising its production life. Combined with the technologies developed to control ovarian function, increased production of viable embryos through IVF of oocytes derived from growing follicles may prove a

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valuable method [6,7]. The objective of the present study was to assess the quality of various cultivation media used for the maturation of bovine oocytes that are prepared for IVF.

Material and Methods

The oocytes used in this study have been obtained from 104 slaughtered bovine ovaries, recovered in sterile saline with 100 UI penicillin/ml, at 35°C. The interval of time that passed from the recovery of the ovaries until processing them in the laboratory has not been longer than 2 hours.

The ovaries were washed with sterile saline in order to remove any trace of blood and the follicles between 1-8 mm were punctured using an 18G needle attached to a 10 ml syringe. The follicular fluid was aspirated, passed in a plastic Corning tube with maintenance medium represented by TCM 199 (Gibco) + heparin 2 UI/ml and then filtrated in order to concentrate the oocytes in a smaller amount of liquid. The oocytes have subsequently been passed in a Petri dish and evaluated using a stereomicroscope at a 20x magnification for counting and 60x for morphological evaluation. Only the adequate oocytes were selected, which presented homogenous cytoplasm and compact cumulus, with at least 3 layers of cells surrounding the oocyte (figure 1).

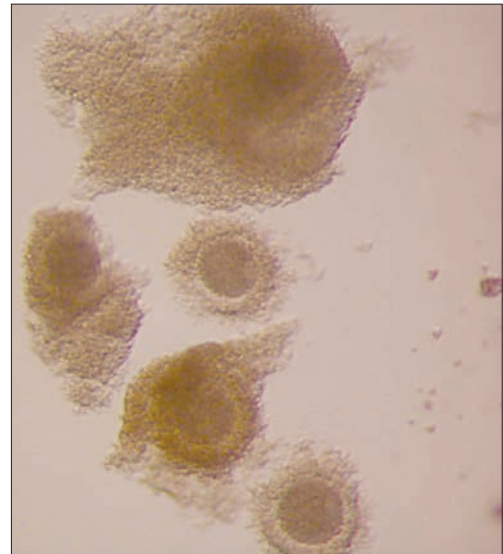


Fig. 1. Adequate oocytes, suitable for in vitro maturation

After morphological evaluation, a total number of 513 viable oocytes have been selected for cultivation, being divided into 3 batches, 171 oocytes/batch.

The oocytes belonging to batch 1 were cultivated in TCM 199 NaHCO_3 + 10% FCS + FSH (FSHp, Shering) 20 $\mu\text{l/ml}$.

The oocytes belonging to batch 2 were cultivated in TCM 199 NaHCO_3 + 10% FCS + HCG (APL) 2.3×10^3 UI/ml + FSH (Folltropin-V) 8 $\mu\text{l/ml}$ + pyruvate 0.25 mM + 17β estradiol 1 $\mu\text{l/ml}$.

The oocytes belonging to batch 3 were cultivated in TCM 199 NaHCO_3 + 10% FCS + 17β estradiol 1 $\mu\text{l/ml}$ + FSH (FSHp, Shering) 20 $\mu\text{l/ml}$.

The cultivation conditions, for all three batches, were: 24 hours at 39°C, 5% CO_2 .

The spermatozoa were thawed (5 s at room temperature and 30 s in water at 37°C) and added to a conical tube, on top of 2 ml of 90% Percoll solution and 2 ml of 45% Percoll solution. The mixture was centrifuged at 1000xg for 10 minutes, the spermatozoa were resuspended in 10 ml TALP and then centrifuged again at 200xg for 5 minutes.

The matured oocytes were passed into 4-wells culture dishes, in fertilization medium (FERT-TALP + 55 $\mu\text{g/ml}$ heparin) at a density of maximum 30 oocytes/well. The spermatozoa were added at a concentration of 1.3×10^6 spermatozoa / ml and finally the microdrops were covered with mineral oil (Sigma).

The dishes were vortexed at 18-20 hours and further incubated for 72 hours at 39°C, in 5% CO_2 . After this interval, the embryonic development was assessed recording the number of cells presented by every embryo as well as the presence of non-fertilized oocytes. The non-fertilized oocytes were discarded, the medium was changed and embryonic development was followed until day 10 after in vitro fertilization.

Results and Discussions

After evaluation of the embryonic development at 72 hours after IVF, the results obtained were as follows (table 1, chart 1 and figure 2):

Table 1. Results of the in vitro fertilization after 72 hours of culture

Batch number	Total number of oocytes	Non-fertilized oocytes	2-8 cells embryos
Batch 1	171	51 (29.82%)	120 (70.18%)
Batch 2	171	42 (24.56%)	129 (75.44%)
Batch 3	171	46 (26.90%)	125 (73.10%)

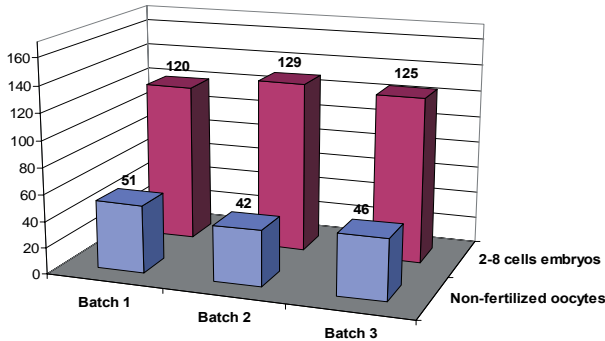


Chart 1. Results of the in vitro fertilization after 72 hours of culture

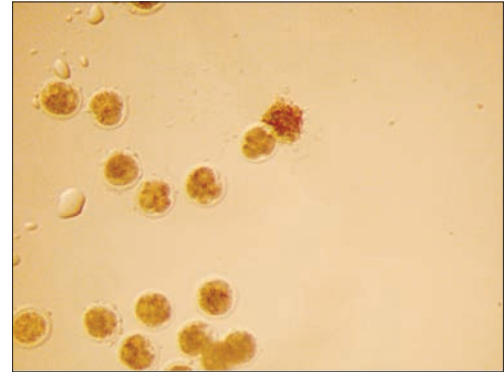


Fig. 2. Embryos obtained at 72 hours after IVF

The figures presented above show small differences between the three batches. The maturation medium used for batch 2 yielded the best results as it contained FSH, HCG, pyruvate and β -estradiol. All these hormones, together with the pyruvate offered the best conditions for oocyte maturation as well as its preparation for in vitro fertilization.

When embryonic development was assessed at 10 days of culture after the in vitro fertilization, the following results have been obtained (table 2, chart 2 and figure 3):

Table 2. Results of the in vitro fertilization after 10 days of culture

Batch number	Total number of embryos cultivated over 72 hours	Expanded blastocysts obtained
Batch 1	120	16 (13.33%)
Batch 2	129	21 (16.27%)
Batch 3	125	19 (15.20%)

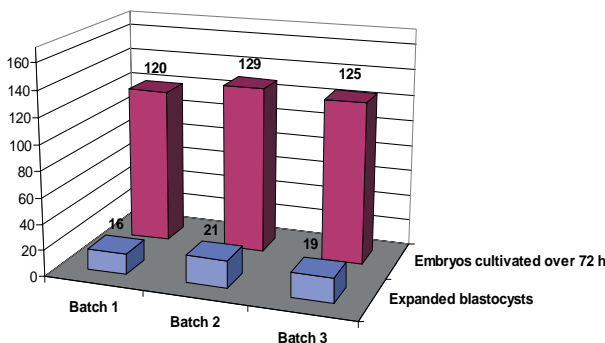


Chart 2. Results of the in vitro fertilization after 10 days of culture

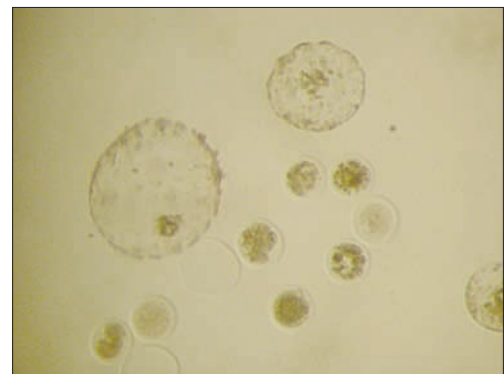


Fig. 3. Blastocysts obtained at 10 days after IVF

The figures shown above also show small differences between the three batches, but again, the highest number of expanded blastocysts has been obtained in batch number 2, proving the better quality of the oocytes matured using this maturation medium and subsequently the better quality of the embryos obtained from them.

Conclusions

After performing the research, the following conclusions have been drawn:

1. The evaluation of the best maturation conditions for oocytes has been performed, comparing various combinations of additives for the maturation medium.
2. At 72 hours after the fertilization, satisfactory results have been obtained for all three batches, even though the maturation medium used in batch number 2 yielded the best results, leading to the highest number of embryos obtained after IVF.
3. The results obtained at 10 days after the in vitro fertilization confirm the superior quality of the oocytes belonging to batch 2 and matured using TCM 199 NaHCO_3 + 10% FCS + HCG 2.3×10^3 UI/ml + FSH $8 \mu\text{l/ml}$ + pyruvate 0.25 mM + 17β estradiol $1 \mu\text{l/ml}$.
4. The supplementation of the maturation medium with gonadotropins and estradiol led to the increase of the percentage of fertilized oocytes, favoring the maturation of the cumulus cells and stimulating the metabolism of the oocyte.
5. We recommend the use of the medium containing TCM 199 NaHCO_3 + 10% FCS + HCG 2.3×10^3 UI/ml + FSH $8 \mu\text{l/ml}$ + pyruvate 0.25 mM + 17β estradiol $1 \mu\text{l/ml}$ for the in vitro maturation of bovine oocytes destined for in vitro fertilization.

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