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OBSERVATIONS REGARDING OOCYTES STORAGE POST MENDING FROM SLAUGHTER FEMALES

OBSERVAȚII PRIVIND PĂSTRAREA OVOCITELOR DUPĂ RECOLTARE DE LA FEMELE ABATORIZATE

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The oocytes viability must be taken as an important selection parameter for successful in vitro cultivation. The ovaries were collected from the slaughterhouse and maintained at 4°C for 7 days. Fallowing cumulus - oocytes complexes recovery the viability was tested by two staining methods. For the first experiment we used 27 cumulus - oocytes complexes, stained with Neutral red and for the second experiment we used 11 cumulus - oocytes complexes stained with Trypan blue. Fallowing staining with Neutral red 23 cumulus - oocytes complexes were assessed as viable (were stained in red – enzymatic activity within the cells) and for the Trypan blue staining 11 cumulus - oocytes complexes were assessed as viable (remained unstained – integers cellular membranes).
Key words: *pig, oocytes, viability. IVM*

Introduction

A higher interest persists in high scale production of swine embryos using IVM, IVF and IVP techniques.

Because of the fact that physiologically swine are similarly to humans, they became really important as potential xenograph donors and transgenic animals which are producing specific proteins. Tries to clone and to produce transgenic pigs by pronuclear microinjections requires the oocyte in vitro maturation, fertilization and embryo development. Alternatively are used with success ovaries recovered from slaughter houses.

In the last few years significant progressed have been made in IVM, IVF and in vitro cultivation of swine embryos. Although, is still needed the in vitro techniques improvement. It must been noted that, nowadays the mechanisms which regulate the in vitro maturation, oocytes fertilization and embryo development are not entirely understood. The success of high scale production of swine embryos by in vitro techniques has still many difficulties to confront.

The final conclusion is that, the first condition to obtain embryos is to use for in vitro maturation viable cumulus – oocytes complexes.

Materials and Methods

The ovaries were recovered from slaughter females and transported to the laboratory in 0.9% NaCl solution, supplemented with antibiotics and kept at 4°C for 7 days.

The follicular liquid with the cumulus – oocytes complexes (COCs) was taking with a 10 ml syringe. The oocytes recovered from the follicular liquid were divided in two groups. One group was composed from 27 cumulus-oocytes complexes and other group formed from 11 cumulus-oocytes complexes.

The both groups were washed in PBS (Phosfate Buffered Saline) and twice in maturation medium TCM 199 supplemented with 10% FCS (Fetal Calf Serum) and antibiotics.

The viability assessment of the cumulus-oocytes complex was made by Neutral red and Trypan blue staining test.

For the cumulus-oocytes complexes Neutral red staining we prepared a 1000 µl TCM 199 supplemented with 20% FCS +0.01g Neutral red. In the references literature is mentioned that Neutral red stains in red the viable cumulus-oocytes complexes.

For the cumulus-oocytes complexes Trypan blue staining we prepared a 1000 µl TCM 199 supplemented with 20% FCS +0.01g Trypan blue. In the references literature is mentioned that Trypan blue stains in blue the death cumulus-oocytes complexes.

The cumulus-oocytes complexes assessed as viable after the Trypan blue staining were in vitro maturated in TCM 199 medium supplemented with 20% FCS, in 3.5 cm Petri dishes. The temperature at which the oocyte maturation took place was 39°C, incubated under 5% CO2 in atmosphere, for 48 hours.

Results and Discussions

In table 1 are presented the viability rates of the cumulus-oocytes complex fallowing Nreutal red and Trypan blue staining.

Table 1

The results obtained after Neutral red and Trypan blue staining of cumulus-oocytes complex

No.	Oocytes number	Viability test	The viability of the cumulus-oocytes complex	
1	28 cumulus-oocytes complex	Neutral red	23	82.14%
2	11 cumulus-oocytes complex	Trypan blue	11	100%

Figure 1 a presents the cumulus-oocytes complex stained with Neutral red and figure 1 b presents the cumulus-oocytes complexes stained with Trypan blue.

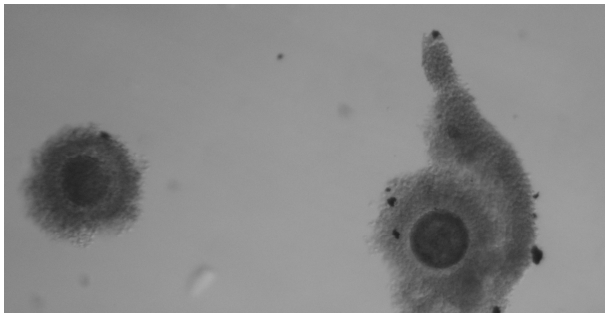

Figure 1 a. Cumulus-oocytes complex stained with Neutral red.

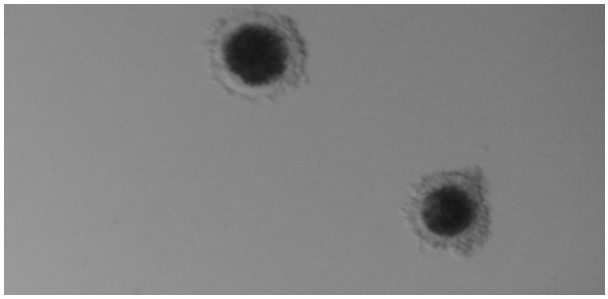

Figure 1 b. Cumulus-oocytes complexes stained with Trypan blue.

Analyzing the data's from table 1, we observed that the viable cumulus-oocytes complex presented an increased number of cumulus cells. For the Trypan blue staining we used only the cumulus-oocytes complex with increased number of cumular cells.

In table 2 we presented the results of in vitro maturation of cumulus-oocytes complex stained with Trypan blue.

Table 2

The results obtained after in vitro maturation of cumulus-oocytes complex after Trypan blue staining

No.	The number of cumulus - oocytes complex	Nr. Complexe ovocită-cumulus
1.	11	6 (54.54%)

Fallowing in vitro maturation of cumulus-oocytes complex we obtained 54.54% rate for the in vitro maturated oocytes in TCM 199 medium supplemented with 20% FCS.

Conclusions

1. The saws ovaries mended from the slaughter house and maintained at 4°C temperature can be kept a longer period of time without being affecting the viability of the cumulus-oocytes complex.

2. The Neutral red and Trypan blue can be used for assessing the swine cumulus-oocytes viability but only the oocytes evaluated with Trypan blue succeed through in vitro maturation

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