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ASSESSMENT OF MOUSE EMBRYO VIABILITY BY ESTERASIC ACTIVITY DETECTION

EVALUAREA VIABILITĂŢII EMBRIONILOR DE ŞOARECE PE BAZA ACTIVITĂŢII ESTERAZICE

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In order to evaluate the esterasic activity within the viable embryos we used the Fluorescein diacetate (FDA) staining test. For staining was used a 0.5 mg/ml FDA stock solution. The embryos were recovered at 48 hours post coitus from superovulated Swiss mouse females. Before staining the embryos were microscopically evaluated by morphological criteria and classified in 4 quality codes. The two methods used for quality and viability assessment were correlated applying Pearson coefficient. The calculated value of the Pearson coefficient (r=1) showed a strong correlation between the two methods used and indicate FDA staining test and esterasic activity as a fast, easy and reliable method for embryo viability assessment.
Keywords: embryo, viability, FDA, esterasic activity

Introduction

The fluorescence staining allows high precision detection of the specific components within the living cells. Fluoresceine diacetate (FDA) is a nonfluorescent analog of fluoresceine which can easily penetrate the cellular membrane of the living cells and under cellular esterase activity is hydrolyzed to fluoresceine. The FDA metabolism depends on the esterasic activity and at the same time on the plasmatic membrane integrity. The Fluoresceine diacetate can be used together with other dye, Propidium iodide (Pi), which poses the capacity to penetrated the cellular membrane of the nonviable cells, binds inside the DNA molecules and show a red fluorescence while the cells are exposed to a fluorescence microscope. The use of the two dyes increases the accuracy of viable and nonviable cells identification (*Kroesen, 1992*).

The embryo viability assessment is an important step for the embryo transfer procedure. The morphological criteria evaluation is, at the moment, the most used method for embryo quality assessment, but the results and objectivity of the method are very much influenced by the operator experience. Fluorescence staining allows viable cells identification by enzymatic activity detection within the cells. The aim of the paper was to assess the mouse embryo viability by esterasic

activity using fluorescence staining methods (FDA staining test).

Materials and Methods

As biological material we used 27 days old, Swiss mice females, hormonal stimulated. The superovulation treatment was made administrating 5 UI/ml PMSG injection, followed at 48 hours by an other 5 UI/ml hCG injection. The embryo recovery was made 48 hours post coitus. The embryo manipulation was made in M2 media (cat. no. M7167). Following embryo recovery the embryos were tested for esterasic activity detection using FDA staining.

For staining with fluorochrome we used a 0.5 mg/ml FDA stock solution, diluted before staining by adding 50 µl FDA stock solution in 1 ml M2 media. For esterasic activity detection the embryos were introduce in the staining solution and incubated for 5 minutes, at 37°, 5% CO_2 and than visualized at 490-518 nm using a fluorescence microscope. The viable embryos manifesting esterasic activity within the blastomers showed a green fluorescence.

Results and Discussion

For this experiment we used 37 embryos. The developmental stages of the recovered embryos are presented in table 1.

Table 1

Developmental stages of the recovered embryos

Recovered embryos	2 cells		8 cells		Morula		Blastocyst	
n	n	%	n	%	N	%	n	%
37	3	8.10	16	43.24	10	27.02	8	21.62

From the 37 recovered embryos, 8.10% were in 2 cells stage, 43.24% were in 8 cell stage, 27.02% were in morula stage and 21,62% were in blastocyst stage.

The embryos recovered were first microscopically evaluated and group based on morphological criteria, in the next quality codes presented in table 2.

Table 2

Embryos classification based on morphological criteria

Embryos N	Very good Code 1		Good Code 2		Satisfying Code 3		Poor/Very poor Code 4	
	n	%	n	%	n	%	n	%
37	2	5.40^{a}	6	16.21^{ac}	11	29.72^{Aa}	18	48.64^{A}

Test χ^2, A-a $p \leq 0{,}001$; A-c $p \leq 0{,}05$; a-a $p > 0{,}05$

Based on morphological criteria 5.40% embryos were classified as code 1,, 16.21% were assessed as quality code 2, 29.72% were assessed as quality code 3 and most of the embryos (48.64%) were assessed as quality code 4.

After morphological evaluation the embryos were tested by esterasic activity detection using FDA staining test. The results obtained are presented in table 3.

Table 3

Embryos viability assessment using FDA staining test

Embryos	Viability			
	Viable		Nonviable	
N	N	%	N	%
37	24	64.8^{A}	13	35.2^{b}

Test χ^2, A-b $p \leq 0{,}01$

Following FDA staining 64.8% of the embryos were evaluated as viable (showed green fluorescence while were exposed to the fluorescence source), and 35.2% embryos were evaluated as nonviable (low number of blastomers showing green fluorescence). Within the proportion of viable and nonviable embryos were observed distinct significant differences ($p \leq 0.01$).

To establish a correlation between the two methods used for embryo quality and viability assessment (morphological criteria versus FDA staining) we applied the Pearson coefficient. The calculated value of the Pearson coefficient was $r = 1$, which indicates a perfect positive correlation between the two variables compared and indicates a strong correlation between the two methods.

In the pictures bellow are presented embryos evaluated by FDA staining test (Figure 1, 2, 3, 4).

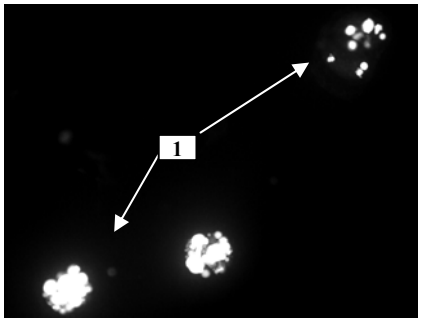

Figure 1. Fragmented embryos showing green fluorescence within the blastomers which presented esterasic activity (original)

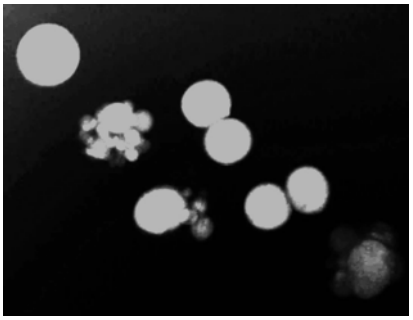

Figure 2. Viable embryos evaluated by FDA staining teste (original)

Fragmented embryos exposed to FDA staining showed green fluorescence within the blastomers which presented esterasic activity. After exposure to the fluorescence source the emission rates of fluorescence varied direct proportional with the intensity of the enzymatic activity within the blastomers. According to the specialty references the emission rate of the fluorescence are typically 10^8 s^{-1} , so that the fluorescence lifetime is near 10 nanoseconds. Many fluorophores displays subnanoseconds lifetime (*Lakowicz, 2006*).

Figure 3. Blastocyst and hatched blastocyst evaluated as viable by Fluorescein diacetat staining test (original)

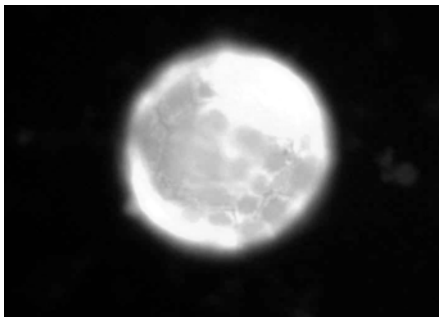

Figure 4. Viable blastocyst evaluated by Fluorescein diacetat staining test (original)

The nonviable embryos without enzymatic activity inside the cells lost their capacity to bind and transform the Fluorescein dictate to fluorescein and showed no fluorescence while were exposed to the fluorescence sours of the microscope.

Conclusions

1. Based on morphological criteria 5.40% embryos were classified as code 1, 16.21% were assessed as quality code 2, 29.72% were assessed as quality code 3 and most of the embryos (48.64%) were assessed as quality code 4.
2. Following FDA staining 64.8% of the embryos were evaluated as viable (showed green fluorescence while were exposed to the fluorescence source), and 35.2% embryos were evaluated as nonviable (low number of blastomers showing green fluorescence). The differences were distinct significant ($p \leq 0.01$).
3. The calculated value of the Pearson coefficient was $r = 1$ which indicates a strong positive correlation between the two variables compared and indicates a strong correlation between the two methods (morphological criteria and FDA staining test).
4. Esterasic activity detection by fluorescence staining is an easy, fast and objective method for embryo viability assessment.

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References

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