ESTABLISHING SOME MORPHOMETRIC PARAMETERS USABLE IN ESTIMATING THE QUALITY OF MOUSE EMBRYOS, IN DIFFERENT DEVELOPMENTAL STAGES

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Abstract

The aim of this paper was to establish some morphometric parameters that can be used in estimation of mouse embryos quality in different developmental stages. For experiments we used embryos recovered from mouse females superovulated with gonadotrope hormones (PMSG and hCG). Embryos recovery was performed at different time intervals after the vaginal plug was discovered. The morphometric parameters taken into consideration were: pellucid zone thickness, outer and inner diameter, and outer and inner perimeter. The embryos recovered were measured for establishing morphometric parameters and in vitro cultivated in KSOM media, supplemented with amino acids. The pellucid zone thickness at the embryos in morula stage (10.8±1.9 µm) was higher compared with the embryos in 2 cell stage (9.8±1.9 µm) and blastocyst stage (7.3±1.5 µm); the differences were very significant (p≤0,001, T test). The inner diameter at the embryos in blastocyst stage (111.3±5.8 µm) was larger comparative with the embryos in 2 cell stage (98.5±2.5 µm) and embryos in morula stage (99.4±3.1 µm); the differences were very significant (p≤0,001, T test). The outer diameter at the embryos in blastocyst stage (123.1±3.5 µm) was larger comparative with the embryos in 2 cell stage (116.5±3.3 µm) and embryos in morula stage (111.7±3.7 µm); the differences were very significant (p≤0,001, T test).

Key words: mouse embryos, morphometric parameters, quality

INTRODUCTION

The majority of the existent systems for mammalian embryo quality assessment are combining the information from morphological parameters like: developmental stage of the embryo, the fragmentation degree and the uniformity of the blastomeres [3]. The large number of embryos transferred necessary for obtaining a gestation indicate the lack of objectiveness and the absence of a standardized system for the evaluation of embryo [1, 2, 4]. The morphometric evaluation is offering valuable information in respect to embryo morphology increasing the objectiveness of embryo quality assessment. Also, morphometric evaluation of embryo quality is not traumatizing and does not affect embryo viability.

Presently, in order to perform morphometric measurements image analyze software can be used which can be very useful for this type of measurements. Besides the thickness of the pellucid zone, for performing the measurements we also taken into consideration other parameters like: inner and outer diameter, inner and outer perimeter.

MATERIAL AND METHOD

In vivo obtaining of the mouse embryos- steps:

- Females superovulation was performed according to the scheme presented in figure 1, and consisted from administration of 5 IU PMSG (Pregnant Mare Serum Gonadotropin), 0 day. At 48 hours from PMSG administration 5 IU of hCG (human Chorionic Gonadotropine) were administered, day 2 of the hormonal stimulation protocol. The animals were maintained in a light regime of 12 hours light and 12 hours dark (from 9:00 to 21:00). The hour15 marked the middle of light period.

- Mating of the females was realized after hCG administration, the females were mated with males in a ratio of 1:1 female: male. In the morning of the 3rd day, the vaginal plug was verified.

- Embryo recovery was performed at different time intervals from the observation of the vaginal plug.;

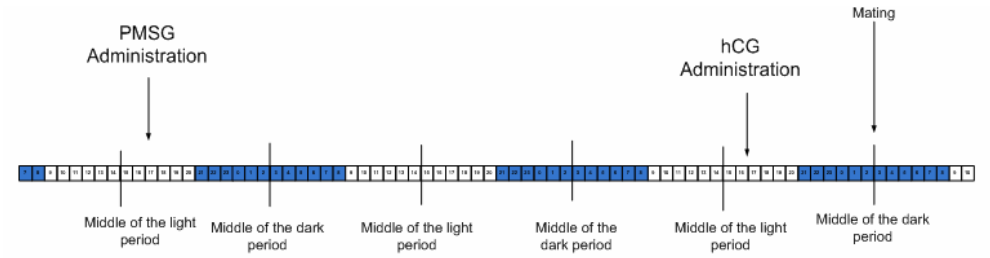


Figure 1. Superovulation inducing protocol for mouse females

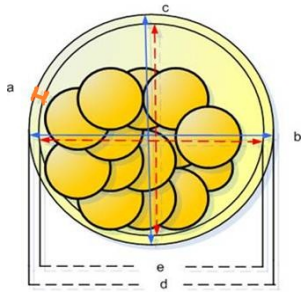
Performing the morphometric measurements:

For morphometric measurement we used viable mouse embryos.

The morphometric parameters taken into consideration were:

- Thickness of the pellucid zone;
- Inner diameter;
- Outer diameter;
- Inner perimeter;
- Outer perimeter;

In figure 2 there is a schematic representation of the performing of the morphometric measurements.



a – thickness of the pellucid zone;
b – outer diameter;
c – inner diameter;
d – outer perimeter;
e – inner perimeter;

Figure 2. Schematic representation of morphometric measurements

For morphometric measurements we used Quick Photo Micro 2.2 Software. This program is easy to use and can give information directly in Excel, by automatic calculation of the thickness of the pellucid zone, the diameters and perimeters of the embryos, by the simple marking of the interest surfaces. For each parameter of the embryo taken into consideration, we performed 10 measurements, and the value taken into consideration was the mean of this measurements.

Statistical analysis of the data

For statistic analyze of the data we used Minitab 15 software. In order to interpret the results obtained, we used Student test. T test is a difference significance testing procedure between two means. Theoretic t test can be used for whatever small lots, if the distribution for the two lots is normal and if the variance for the two lots is not significant different.

RESULTS AND DISCUSSIONS

For establishing the morphometric parameters usable in estimation of embryo quality, morphometric measurements were performed on viable embryos, in vivo obtained, in 2 cell, morula and blastocyst developmental stage according to the protocol described.

The embryos were measured for establishing the morphometric parameters and cultured in vitro on KSOM media supplemented with essential and nonessential amino acids.

The results obtained after performing the morphometric measurements are presented in table 1.

Table 1. Morphometric parameters for the embryos in vivo obtained

Specification	Developmental stage		
	2 cell	Morula	Blastocyst
Total number (N)	32	32	32
ZP thickness (µm)	9.8 ± 1.9^{a}	10.8 ± 1.9^{A}	7.3 ± 1.5^{aA}
Inner diameter (µm)	98.5 ± 2.5^{a}	99.4 ± 3.1^{a}	111.3 ± 5.8^{A}
Outer diameter (µm)	116.5 ± 3.3^{a}	111.7 ± 3.7^{a}	123.1 ± 3.5^{A}
Inner perimeter (µm)	303 ± 3.4^{A}	306.1 ± 6.5^{Ab}	321 ± 5.8^{a}
Outer perimeter (µm)	335.1 ± 3.76^{a}	344.5 ± 4.6^{a}	362.9 ± 3.15^{A}

Testul T A-a p≤0,001; A-b p≤0,05; A-c p≤0,01;a-a p>0,05

From table 1 it can be noticed that the thickness of the pellucid zone, at the embryos in two cell stage it was 9.8±1.9 µm, and for the embryos in morula stage 10.8±1.9 µm, the differences were very significant (p≤ 0.001 test T). For the embryos in blastocyst stage, the thickness of pellucid zone was 7.3±1.5 µm, smaller compared with 2cell stage embryos and embryos in morula stage, the differences were very significant (p≤ 0.001, test T).

For the inner diameter, it can be noticed that at 2 cell embryos it's value was 98.5±2.5 µm, and for embryos in morula stage it was 99.4±3.1 µm, the differences were not statistically assured (p>0.05). For the embryos in blastocyst stage the inner diameter was 111.3±5.8 µm, bigger than inner diameter of the embryos in 2 cell developmental stage and morula stage, the differences were very different (p≤0.001, T test).

At 2 cell embryos the outer diameter was 116.5±3,3 µm, for morula stage embryos it was 111,7±3,7µm the differences were not statistically assured (p>0.05, test T). in blastocyst stage the outer diameter was higher 123.1±5.8 µm, the differences between the 2 cells and morula compared with blastocyst stage embryos were very significant (p≤0.001, test T).

For embryos in two cell stage the inner perimeter was 303±3.4µm; in morula stage the embryos had an inner perimeter 306.1±6.5 µm. The differences between morula stage embryo and two cell stage embryos for inner perimeter were significantly different (p≤0.05, test T). In blastocyst stage the inner perimeter 321±5.8 µm, the differences were very significant compared with embryos in two cell and morula stage embryos (p≤0.001, test T).

For embryos in two cells the outer perimeter 335,1±3.76 µm, in morula stage, the embryos had an outer perimeter of 344.5±4.6 µm. the differences observed between morula stage embryos and two cell embryos were nor significant (p>0,05, test T). In blastocyst stage the outer perimeter was 362.94±3.15 µm, the differences compared with 2 cells embryos and morula stage embryos the differences were very significant (p≤0.001, test T).

CONCLUSIONS

1. **The thickness of the pellucid zone** at the embryos in 2 cell stage is 9.8±1.9 µm, at the embryos in morula stage it is 10.8±1.9 µm and at the embryos in blastocyst stage is 7.3±1.5 µm, the differences are very significant between all the developmental stages studied (p≤ 0,001).
2. **The inner diameter** at the embryos in blastocyst stage (111.3±5.8 µm) is higher compared with embryos in 2 cell stage (98.5±2.5 µm) and with embryos in morula stage (111.3±5.8 µm); the differences are very significant (p≤0.001). the differences observed between the inner diameter of the embryos in 2 cell stage and embryos in morula stage embryos are not significant (p>0,05)
3. **The outer diameter** at the embryos in blastocyst stage (123.1±3.5 µm) is higher

compared with the embryos in 2 cell stage (116.5±3.3µm) and with embryos in morula stage (111.7±3.7 µm), the differences are very significant ($p \leq 0.001$). The differences between the outer diameter of the embryos in 2 cell stage and morula stage are not significant ($p > 0.05$)

4. **The inner perimeter** at the embryos in blastocyst stage (321±5.8 µm), was higher compared with embryos in 2 cell stage (303±3.4µm) and morula stage embryos (306.1±6.5 µm), the differences are very significant ($p \leq 0.001$). The inner perimeter for the embryos in two cell stage is lower compared with morula stage embryos, the differences were significant ($p \leq 0.05$).

5. **The outer perimeter** at the embryos in blastocyst stage is bigger (362.94±3.15 µm), compared with embryos in two cells (335.1±3.76 µm) and morula stage (344.5±4.6 µm) the differences are very significant ($p \leq 0{,}001$). The outer perimeter of the embryos in two cells is smaller compared with embryos in morula stage, the differences are nor significant ($p > 0.05$).

6. From the morphometric parameters studied, the thickness of the pellucid zone and the inner diameter can be used for estimation of the quality of the mouse embryos in all developmental stages studied, the other morphometric parameters (inner and outer diameter and outer perimeter) can be used in estimation of the quality of the embryos in more advanced developmental stages.

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