# The Using of Morphometric Parameters in Establishing the Viability of Mouse Embryos

**Ada Cean, Nicolae Păcală, Alexandra Ivan, Ioan Bencsik, Dorel Dronca, Gabi Dumitrescu, Oana Ciobotaru**

*Banat's University of Agricultural Sciences and Veterinary Medicine, Faculty of Animal Sciences and Biotechnologies 300645 Timisoara, Calea Aradului 119, Romania*

**Abstract**

The aim of this paper was to investigate if morphometric parameters can be used in establishing the viability of the mouse embryos. For the experiments, we used mouse mature oocytes and embryos in vivo obtained. The morphometric parameters taken into consideration were: pellucid zone thickness, outer and inner diameter, and outer and inner perimeter and for oocytes and zygotes the cellular mass diameter was also measured. The oocytes were measured immediately after recovery then they were in vitro fertilized. After 4-6 hours after fecundation the oocytes that manifested the extrusion of the second polar body (zygotes) were measured, and at 24 hours after fecundation the unfertilized oocytes were also measured. The embryos were obtained from mouse females superovulated with gonadotrope hormones (eCG and hCG). For the experiments we used embryos in different developmental stages (2, 4 and 8 cells, morula and blastocyst). After recovery the embryos were morphologically analyzed and divided in viable (quality code 1, 2 and 3) and nonviable embryos (quality code 4) (IETS Manual, 1989) and they were measured for establishing the morphometric parameters value. The data obtained were statistically analyzed using Minitab 15, and T test. For the oocytes it was noticed that the pellucid zone thickness is registering a slightly increase if the oocyte is fertilized, without significantly difference from recovery, but if the oocyte is not fertilized the pellucid zone thickness decrease from 8.3±1.5 µm to 8.0±1.5 µm. For the embryos in early developmental stages only the thickness of the pellucid zone can be an indication of the viability. For the embryos in morula stage the thickness of the pellucid zone and inner diameter can be used as indicator of viability. For the embryos in blastocyst stage the thickness of the pellucid zone, the inner and outer diameter can be used as a viability indicator.

**Keywords**: morphometric measurements, mouse embryos, mouse oocytes, viability.

## 1. Introduction

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, 3]. Because of these factors quality scoring of embryos is difficult, since the majority of the existent systems for scoring embryo quality are based on information obtained from morphological parameters like the developmental stage of the embryo, the degree of fragmentation, the uniformity of the embryo cells [1,3]. Morphometric evaluation is offering valuable information in respect to embryo morphology increasing the objectiveness of embryo quality assessment. Morphologic evaluation of the quality of the embryos lacks precision, is highly subjective and necessitates trained personal. This suggests the need for a more standardized method for embryo quality [4,5,6]. Morphometric measurements offer valuable information regarding embryo morphology increasing the objectivity of embryo quality scoring. The aim of this paper was to investigate if morphometric

* Corresponding author: Ada Cean, Tel 0256 277 100, Fax 0256 277 110, Email telea_ada@animalsci-tm.ro

parameters can be used in establishing the viability of the mouse embryos.

## 2. Materials and methods

**Obtaining the oocytes and mouse embryos:**
For morphometric measurements we used oocytes and embryos in vivo obtained from superovulated females. The superovulatory treatment was performed as follows: in 0 day, 5IU of eCG were administrated; at 48 hours after eCG administration 5IU of hCG were administered. For mature oocytes obtaining the recovery was performed by oviduct flushing, at 13 hours after hCG administration. For obtaining in vivo mouse embryos, after hCG administration the females were put with the males, and the next day the vaginal plug was verified. Embryo recovery was performed at different time intervals.

**Morphometric measurements**
For embryo morphometric masurements we used Quick Photo Micro 2.2.

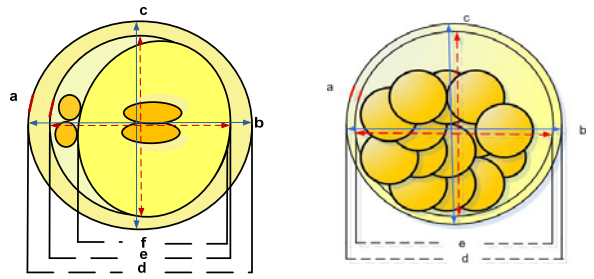


**Figure 1.** Schematic representation of morphometric measurements performed on embryos and oocytes

This program is easy to use and can generate information directly in Excell, by directly calculating the thickness of the pellucid zone of the diameters and perimeters of the embryos by the simple marking the interest surfaces.
For each parameter of the embryo that was taken into consideration, 10 morphometric measurements were performed, and the value taken into consideration was the mean. Morphometric masurements were performed as described in figure 1.
For oocytes morphometric masurements were performed immediately after recovery, at 4-6 hours after in vitro fecundation at the oocytes at which the second polar body was noticed (zigotes, viable) and at 24 hours after fecundation (on unviable oocytes). All the embryos recovered were morphometric measured and divided into viable and unviable. Viable embryos were considered the embryos that fitted in 1, 2, and 3 quality codes as described in IETS Manual (1989) [7]. The unviable embryos were considered the embryos that were quality code 4.
**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**.**

## 3. Results and discussion

Results obtained after morphometric measurements on oocytes are presented in table 1.

**Table 1**. Morphometric parameters of the viable and unviable zygotes

| Specification | Oocytes immediately after recovery | Zygotes at 4-6 hours after fecundation (viable embryos) | Unfertilized oocytes at 24 hours after fecundation (unviable embryos) |
|---|---|---|---|
| Thickness ZP (µm) | 8.3±1.5$^{a}$ | 8.5±0.9$^{Aa}$ | 8.0±1.5$^{ac}$ |
| Inner diameter (µm) | 99.5± 9.6$^{a}$ | 94.4± 4.6 $^{A}$ | 99.4± 3.1$^{a}$ |
| Outer diameter (µm) | 118.2± 6.5$^{a}$ | 111.9±5.1$^{A}$ | 118.1± 10.4 $^{a}$ |
| Inner perimeter (µm) | 296.7± 12.5$^{a}$ | 294.2± 11.3$^{a}$ | 304.0± 13.9$^{A}$ |
| Outer perimeter (µm) | 323.5±20.5$^{a}$ | 328.6± 20.9 $^{a}$ | 335.7± 21.6 $^{A}$ |
| Cell mas diameter (µm) | 74.4±3.7$^{a}$ | 82.0±2.7 $^{A}$ | 74.3±2.8$^{a}$ |

*T Test A-a p≤0.001; A-b p≤0.05; A-c p≤0.01;a-a p>0.05*

For the thickness of pellucid zone at the oocytes measured at recovery we found a value of 8.3±1.5 µm, at 4-6 hours from fecundation, at the zygotes that the second polar body was noticed, the thickness of pellucid zone was 8.5±0.9µm. For the oocytes that were not fecundated, at 24 hours after in vitro fecundation, the thickness of pellucid zone was 8.0±1.5 µm. It can be noticed that the

thickness of pellucid zone at zygotes (8.5±0.9µm) is higher comparative with the thickness of pellucid zone at oocytes measured at recovery (8.3±1.5µm), the differences were statistically insignificant (p>0.05, T test). The thickness of pellucid zone oocytes that were not fecundated, at 24 hours after in vitro fecundation (8.0±1.5µm) is lower compared with the thickness of pellucid zone of the oocytes measured at recovery (8.3±1.5 µm), but the differences were statistically insignificant (p>0,05, T test). The thickness of pellucid zone at the oocytes that were not fecundated, at 24 hours after in vitro fecundation is significantly lower compared with the thickness of pellucid zone at zygotes at 4-6 hours from fecundation (p≤0,001, T test).

Inner diameter at the oocytes measured immediately after recovery was 99.5± 9.6 µm, for the zygotes it was 94.4±4.6 µm, and for unfertilized oocytes it was 99.4±3.1 µm. statistic analyze of the results showed that between the inner diameter of the oocytes at recovery and the oocytes at 24 hours after fecundation the differences observed are nor significant (p>0.05, T test). The differences observed between the dimensions registered for the inner diameter immediate after recovery (99.5±9.6µm) and zygotes at 4-6 hours after fecundation (94.4±4.6 µm) are statistically significant (p≤0,001, T test). Also the differences observed between the inner diameter at the unfertilized oocytes (99.4±3.1 µm) and the inner diameter of the zygotes (94.4±4.6 µm) are very significant (p≤0,001, T test).

For the outer diameter at the oocyte at recovery, the value was 111.9±5.1 µm, and for unfertilized oocytes it was 118.1± 10.4 µm. The statistical analyze of the results showed that between the outer diameter of the oocytes at recovery and the outer diameter of the oocytes at 24 hours from fecundation the differences are not significant (p>0,05, T test). The differences observed between the dimensions registered for the outer diameter of the oocytes at recovery (118.2± 6.5 µm) and zygotes at 4-6 hours form fecundation (111.9±5.1µm) are very (p≤0.001, T test). Also, the differences observed between the outer diameter of the oocytes unfertilized (118.1± 10.4 µm) and the diameter of the zygotes (94.4±4.6) are very significant (p≤0.001, T test).

For the inner perimeter at the oocytes measured immediately after recovery there were determined a value of 296.7± 12.5 µm. At 4-6 hours from fecundation, at the zygotes the inner perimeter was 294.2± 11.3µm, for the unfertilized oocytes the inner perimeter 304.0± 13.9 µm. It can be noticed that inner perimeter of the unfertilized oocytes is bigger compared with the inner perimeter of the oocytes at recovery (296.7± 12.5µm) and of the zygotes (294.2± 11.3µm), (p≤0.001, T test). Between the inner perimeter dimensions at oocytes immediately after recovery and at zygotes are not statistically different (p>0.05, T test).

For the outer diameter, at the oocytes at recovery it's value was 323.5±20.5 µm, for zygotes it was 328.6± 20.9 µm, and for unfertilized oocytes 335.7± 21.6 µm. Statistical analyze of the results showed that the differences between the dimensions observed for the outer perimeter showed that there are no significant differences for this parameter between oocytes at recovery, zygotes and unfertilized oocytes (p≤0.05, T test). The differences observed between the outer perimeter of the zygotes are significant (p≤0.001, T test).

For the cellular mass at the oocytes measured immediately after recovery its value was 74.4±3.7 µm, lower compared with the zygotes (82.0±2.7 µm). For the unfertilized oocytes the diameter of the cellular mass was 74.3±2.8 µm. The statistical analyze of the results showed that between the diameter of the cell mass at the oocytes at recovery and unfertilized oocytes there are no significant differences (p>0.05, T test). The differences observed between the diameter of the cell mass at recovery (74.4±3.7 µm) and the diameter of cell mass at zygotes (82.0±2.7 µm) are very significant (p≤0,001, T test). Also, the differences observed for the diameter of the cell mass at the unfertilized oocytes (74.3±2.8 µm) and the zygotes (82.0±2.7 µm) are very significant (p≤0.001, T test).

After the analysis of the morphometric measurements for mouse oocytes it can be noticed that the thickness of the pellucid zone registers a slight increase after the appearance of the second polar body, without it being significantly different compared with the thickness of the pellucid zone at recovery. If the fecundation dose not happens, the thickness of the pellucid zone drops from 8.3±1.5 µm at 8.0±1.5 µm. The inner diameter dose not differ significantly if the oocyte is not fertilized, but, after the appearance of the second polar body it manifest a significant drop from

99,5±9,6µm to 94,4±4,6 µm. Also, at the viable oocytes there was noticed a drop of the exterior diameter. For the outer and inner perimeter it can be noticed an increase of the perimeter at unfertilized oocyte do to the aparence of the degenerative processes installing. The diameter of the cell mass stays mainly unchanged for the unfertilized oocytes (74.3±2.8 µm) and the oocytes at recovery (74.4±3.7 µm), but at viable oocytes there can be noticed a significant increase of the diameter of the cell mass (82.0±2.8 µm).

In table two we presented the results obtained at morphometric measurements performed on viable (quality code 1, 2 and 3) and unviable (quality code 4) in different developmental stages.

**Table 2.** Morphological parameters at viable (quality code 1, 2 and 3) and unviable (quality code 4) embryos

| Developmental stage | Quality | Morphometric parameter | | |
|---|---|---|---|---|
| | | Pellucid zone thickness (µm) | Inner diameter (µm) | Outer diameter (µm) |
| 2 cells | Viable | 9.2± 1.3$^{A}$ | 98.0±2.1$^{a}$ | 116.6±3.0$^{a}$ |
| | Unviable | 8.0± 1.5$^{c}$ | 98.0± 2.1$^{a}$ | 117.8±3.5$^{a}$ |
| Morula | Viable | 10.8± 1.1$^{a}$ | 98.7±3.1$^{a}$ | 118.6±6.0$^{a}$ |
| | Unviable | 8.6±1.1$^{A}$ | 105.8± 6.8$^{A}$ | 116.5±3.7$^{a}$ |
| Blastocyst | Viable | 7.3± 1.0$^{a}$ | 111.9±5.3$^{a}$ | 120.8±6.1$^{a}$ |
| | Unviable | 9.1±1.0$^{A}$ | 106.0±4.4$^{A}$ | 111.7±7.9$^{A}$ |

*Testul T A-a p≤0.001; A-b p≤0.05; A-c p≤0.01;a-a p>0.05*

From table 2 it can be noticed that for the thickness of the pellucid zone at viable 2 cells embryos it was registered a value of 9.2±1.3µm. The thickness of the pellucid zone at unviable 2 cells embryos registered a lower value (8.0±1.5) compared with 2 cell embryo evaluated as viable, the differences are statistically significant (p≤0.05, T test). At the viable embryos in morula stage the thickness of the pellucid zone was higher (10.8±1.1 µm) compared with unviable embryos in morula stage (8.6±1.1 µm), the differences were very significant (p≤0.001, T test). For the embryos in blastocyst stage the thickness of the pellucid zone was 7.3±1.0 µm, and for the unviable embryos in blastocyst stage the thickness of the pellucid zone was 9.1±1.0 µm, the differences observed are very significant (p≤0.001, T test).

For the inner diameter, at the viable and unviable embryos, at 2 cell stage embryos, we measured the same value (98.0± 2.1µm). For the unviable embryos in morula stage the inner diameter was higher (105.8±6.8 µm), compared with the inner diameter for the embryos in morula stage (98.7±3.1µm), the differences were very significantly statistically (p≤0.001, T test). The inner diameter for the unviable embryos in blastocyst stage was 106.0±4.4µm, lower than the inner diameter of the viable embryos in blastocyst stage (111.9±5.3µm), the differences are significant (p≤0.001, T test).

For the outer diameter at unviable embryos in 2 cell stage its value was 117.8±3.5 µm, higher compared with viable embryos in 2 cell stage (116.6±3.0 µm) the differences were not significant (p>0,05, T test). The outer diameter at the unviable embryos in morula stage was 116.5±3.7µm, compared with viable embryos in morula stage (118.6±6.0 µm) there were no significant differences (p>0.05, T test). At unviable embryos in blastocyst stage the outer diameter wad 111.7±7.9 µm, compared with viable embryos in blastocyst stage (120.8±6.1µm) the differences were very significant (p≤0.001, T test).

From the analysis of table 2 data it can be seen that the thickness of the pellucid zone for the unviable embryos in 2 cell and morula stage is lower, compared with the thickness of the pellucid zone of the embryos appreciated as viable, in the same developmental stage, the differences are statistically assured. For the exterior and interior diameter at the embryos in 2 cell and morula stage there were no significant differences registered regardless of the viability of the embryos. So, from the studied parameters for the 2 cell embryos and morula embryos only the thickness of the pellucid zone can be a viability indicator.

For the embryos appreciated as unviable (code 4) in blastocyst stage is bigger, compared with the thickness of the pellucid zone at the viable embryos in blastocyst stage, the differences were statistically assured. Inner and outer diameter at the unviable embryos in blastocyst stage is lower

compared with viable embryos in blastocyst stage, the differences were statistically assured.

After the analysis of the data in table 1 and 2 regarding the morphometrical parameters of the embryos we can state that there are significant differences between viable and unviable embryos that can be used to assess the viability of the embryo, as follows:

- Viable zygotes (quality code 1, 2 and 3) have the thickness of the pellucid zone of 8.5±0.9 µm and the diameter of the cellular 82.0±2.7µm;
- 2 cell embryos that have the thickness of pellucid zone lower than 9.2±1.3 µm are unviable;
- Embryos that in morula stage have the thickness of pellucid zone lower than 10.8 µm and a higher inner diameter than 98.7±3.1µm are unviable;
- Embryos that in blastocyst stage have the thickness of pellucid zone higher 7.3±1.0 µm, inner diameter lower than 111.9±5.3 µm and an outer diameter lower than 120.8±6.1 µm are unviable.

## 4. Conclusions

1. The thickness of the pellucid zone at fecundated oocytes has a mean value of 8.5±0.9 µm and is significantly higher compared with the thickness of the pellucid zone at unfertilized oocytes (8.0±1.5) ($p \leq 0{,}001$, T test);
2. The thickness of the pellucid zone is significantly lower at unviable 2 cell embryos (8.0± 1.5µm) compared with the thickness of the pellucid zone at viable 2 cell embryos (9.2± 1.3µm) ($p \leq 0.05$, T test);
3. The thickness of the pellucid zone at unviable embryos in morula stage (8.6±1.1µm) is lower compared with the thickness of the pellucid zone at viable embryos in morula stage (10.8±1.1µm), ($p \leq 0.001$, T test).
4. The thickness of the pellucid zone of the unviable embryos in blastocyst stage (9.1±1.0µm) is higher compared with the thickness of the pellucid zone at viable embryos in blastocyst stage (7.3± 1.0, µm), the differences are very significant ($p \leq 0.001$, T test).
5. The inner and outer diameter at unviable embryos in blastocyst stage (106.0±4.4 µm and 111.7±7.9 µm respectively) is lower compared with the viable embryos in blastocyst (111.9±5.3 µm and 120.8±6.1 µm respectively), the differences are statistically assured.

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