

Establishing some Correlations between Certain Morphometric Parameters and Embryo Quality

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Abstract

The aim of this paper was to establish some correlations between certain morphometric parameters and embryo quality. The morphometric parameters taken into consideration were: zona pellucida thickness, outer and inner diameter, and outer and inner perimeter. For experiments we used embryos recovered at 24 hours from mouse females superovulated with gonadotrope hormones (eCG and hCG). The embryos recovered were cultivated in KSOM media, supplemented with amino acids, and during the in vitro cultivation they were measured at different time intervals for establishing morphometric parameters. The data obtained were statistically analyzed using Minitab 15, using Fitted Line Plot regression that allows testing of the linear and polynomial regression of one variable. After statistical analyze of the data we found that the thickness of the zona pellucida can constitute a morphometric parameter that can be used as an indicator of subsequent development of the 2 cell embryos to morula and blastocyst stage respectively. The other morphometric parameters studied (outer and inner diameter, and outer and inner perimeter) cannot be used as indicators of the embryo development.

Keywords: embryo quality, morphometric parameters, mouse embryos

1. Introduction

Currently the morphological evaluation method is one of the most used methods for embryo quality assessment. This method has the advantage to be fast and noninvasive assuring a reduce manipulation of the embryos at low cost, but still holds the disadvantage to be subjective (the result is influenced by the surveyor experience) and does not offer information's related to cell membrane integrity nor identification of genetic and metabolic anomalies. The quality concept is difficult to define. Is considered that satisfying quality embryos are corresponding chronologically and morphologically to the

developmental stage and to the day of the embryo recovery [1].

Quality assessment following morphological criteria is one of the most modern methods for embryo evaluation.

One of the most important parameters used for morphological evaluation is the zona pelluciada thickness. The morphometric evaluation studies made on embryos indicated that among the morphometric parameters (zona pellucida thickness, outer and inner diameter, outer and inner perimeter of the embryos) the zona pellucida thickness and inner perimeter may be used for mouse embryo quality assessment in all developmental stages [2, 3, 4]. Zona pellucida is presenting as an amorphous shape dispose between the oocyte and the cumulus ooforus cells. A single morphological aspect that allows delimitation between species is the relative thickness of the zona pellucida [5].

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Between the zona pellucida thickness and the pregnancy rates has been observed the existence of a correlation. The embryos with a zona pelucida thickness higher of 15 µm have a lower implantation rate [6].

For zona pellucida thickness measurement can be used image analysis programs. These programs allow performing a series of analysis like; measurement of polar bodies angles, the pronucleus, the zona pellucida, and the cell diameter. Following analyzing the data's an embryo quality classification can be made.

The aim of this paper was to establish the existence of certain correlations between the main morphometric parameters and the embryo quality.

2. Materials and methods

For these experiments were used mouse embryos in vivo obtained by superovulation.

The superovulation was made as presented:


- 5 UI eCG (equine Chorionic Gonadotropin) administrated on day 0,
- 5 UI hCG (human Chorionic Gonadotropine) at 48 hours following eCG and following hCG administration the females were allowed to mate with the males,
- on the 3-ed day the vaginal plug was checked,
- on the 4-th day the embryo recovery was preformed .

The 2 cells embryos were measured for establishing the morphometric parameters and in vitro cultivated in KSOM media, supplemented with essential and nonessential amino acids. During in vitro cultivation at 48 and 72 hours interval morphomteric measurements were performed. Of the cultivated embryos for establishing correlations among the morphometric parameters and their quality were taken in consideration only embryos that reached the blastocyst stage at 72 hours. For performing the embryo morphomtery was used the Quick Photo Micro 2.2.program. This program is easy to use and offers information's directly in Microsoft Excell, by automatic calculation of zona pellucida thickness, embryo diameter and perimeter only by the simple mark of the areas of interest. For each parameter taken in to account were performed 10 measurements and the value taken in consideration represented the average of these measurements. For statistical interpretation was used the Minitab, 15. For testing the correlation we used the Fitted Line Plot regression, which allows the linear and polynomial regression (degree 2 and 3) testing.

3. Results and discussion

In table 1 are presented the results obtained after embryo recovery.

Table 1. Results obtained at embryo recovery from mouse superovulated females

| Superovulate females N | Vaginal plug females | | Recovered embryos | | | | | | | |
|---------------------------|----------------------|------|-------------------|---|--------|-------|-----------------|-------|-----------------|------|
| | | | Of which: | | Oocyte | | 2 cells embryos | | 4 cells embryos | |
| | n | % | N |  | n | % | n | % | n | % |
| 10 | 8 | 80.0 | 174 | 21.75 | 20 | 11.49 | 130 | 74.71 | 24 | 13.8 |

From table 1 can be observed that from the 10-th superovulated females only 8 (80.0%) were identified with vaginal plug, of which were recovered 174 embryos, representing 21.75 embryos/female. Of the 174 embryos collected, 20 (11.49%) were non-fertilized oocytes, 130 (74.71%) were two cell embryos and 24 (13.8%) were 4-cell embryos.

During in vitro cultivation of the 130 2-cell embryos only 32 reached the blastocyst stage, and the measurements were used to determine correlations. Correlation analysis for the zona pellucida thickness of the two cell stage embryos

that developed to morula stage showed a very strong, positive linear correlation. The inear regression equation was: morula = 1.524 + 0.9436 2 cells (S = 0.308064 R-Sq = 92.3% R-Sq(adj) = 92.0%) (Figure 1.a).

Correlation analysis for the pellucid zone thickness of the two cell stage embryos that developed to the blastocyst stage showed that there was a strong linear, positive correlation. The linear regression equation was: blastocyst = 0.9771 + 0.6468 x 2 cells (S = 0.554719 R-Sq = 63.4% R-Sq(adj) = 62.2%) (figure 1.b).

Correlation analysis for the pellucid zone thickness of morula stage embryos that developed to the blastocyst stage showed that there was a moderate, positive, linear correlation. The linear

regression equation was: blastocyst = 0.875 + 0.5983 morula (S = 0.632947 R-Sq = 52.3% R-Sq(adj) = 50,8%) (Figure 1.c).

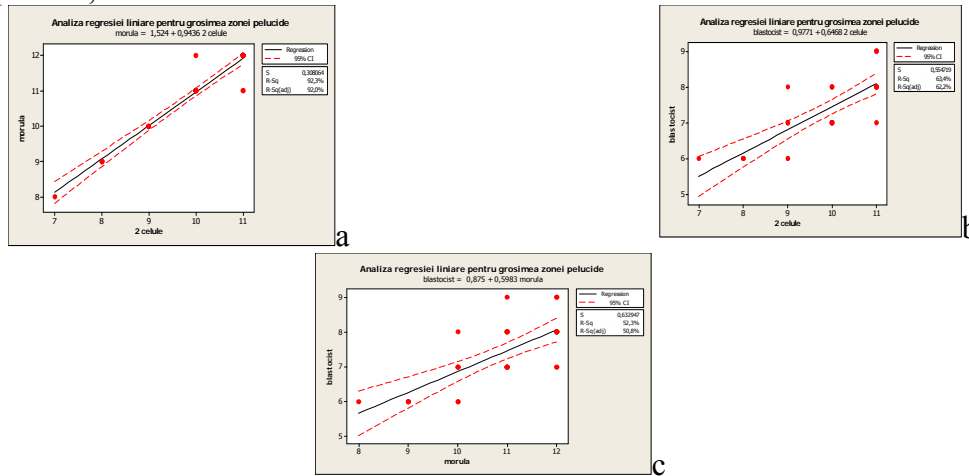


Figure 1. Graphical representation of linear regression for the thickness of the zona pellucida

Correlation analysis for the inner diameter of two cell stage embryos that developed to morula stage showed that there is a very weak negative, linear correlation. The regression line equation was: morula = 685.4 – 11.74 2 cells + 0.05876 2 cells**2 (S = 3.21894 R-Sq = 4.2% R-Sq(adj) = 0.0%) (figure 2.a).

blastocyst = 97.37 + 0.1418 2 cells (S = 5.94709 R-Sq = 0.4% R-Sq(adj) = 0.0%) (figure 2.b).

Correlation Analysis for the inner diameter of two cell stage embryos that developed to morula stage showed that there is a very weak, negative linear correlation. The regression line equation was:

blastocyst = 153.0 – 0.4191 morula (S = 5.80266 R-Sq = 5.2% R-Sq(adj) = 2.0%) (figure 2. c).

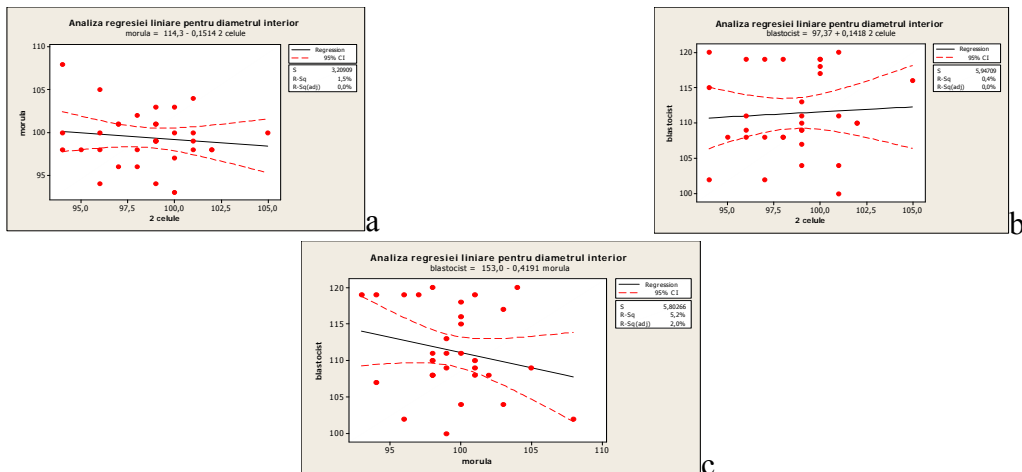


Figure 2. Graphical representation of linear regression for the thickness of the inner diameter

Correlation Analysis for the outer diameter of two cell stage embryos that developed to morula stage showed no correlation between (figure 3.a.). Correlation analysis for the outer diameter of two cell stage embryos that developed to the blastocyst stage showed a very weak, positive, linear

correlation, the regression line equation was: blastocyst = 103.9 + 0.1651 x 2 cells (S = 3.54804 R-Sq = 2.4% R-Sq (adj) = 0.0%) (figure 3.b).

Correlation analysis for the outer diameter of morula stage embryos that developed to the blastocyst stage showed a very weak, negative

linear correlation the regression line equation was:
 blastocyst = 134.5 – 0.1008 morula (S = 3.57105

R-Sq = 1.1% R-Sq(adj) = 0.0%) (figure 3. c).

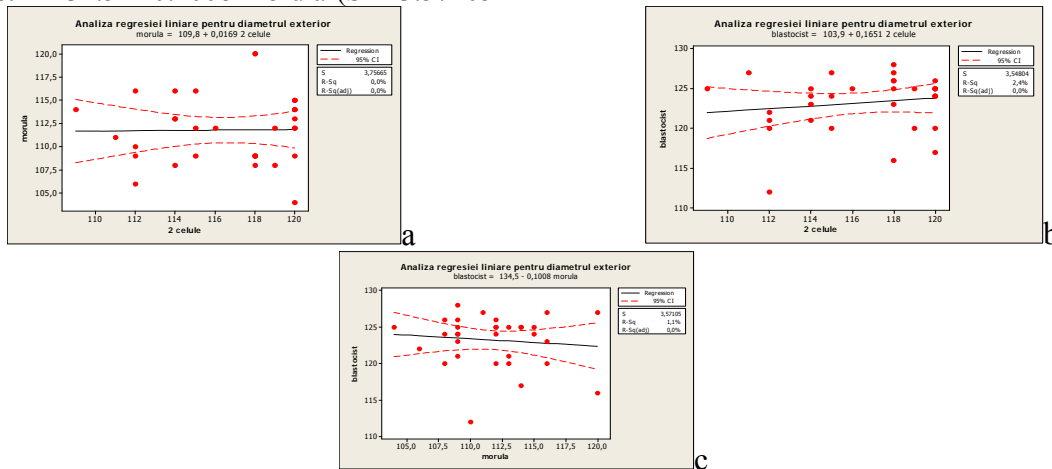


Figure 3. Graphical representation of linear regression for the outer diameter

Correlation analysis for the inner perimeter of two cell embryos that developed to morula stage showed a positive very weak correlation between them, the regression line equation was: morula = 189.2 + 0.3859 x 2 cells (S = 6.47525 R-Sq = 4.1% R-Sq(adj) = 0,9%) (figure 4.a).

(S = 5.88691 R-Sq = 0.4% R-Sq(adj) = 0.0%) (figure 4. b).

Correlation analysis for the inner perimeter of the two cell stage embryos that developed to the blastocyst stage showed that there was a negative, very weak, linear correlation, the regression line equation was: blastocyst = 352.4 – 0.1024 2 cells

Correlation analysis for the inner perimeter of the embryos in morula stage that developed to the blastocyst showed a very weak, linear, positive correlation. The regression line equation was: blastocyst = 291.7 + 0.0969 x morula (S = 5.86283 R-Sq = 1.2% R-Sq(adj) = 0,0%) (figure 4.c).

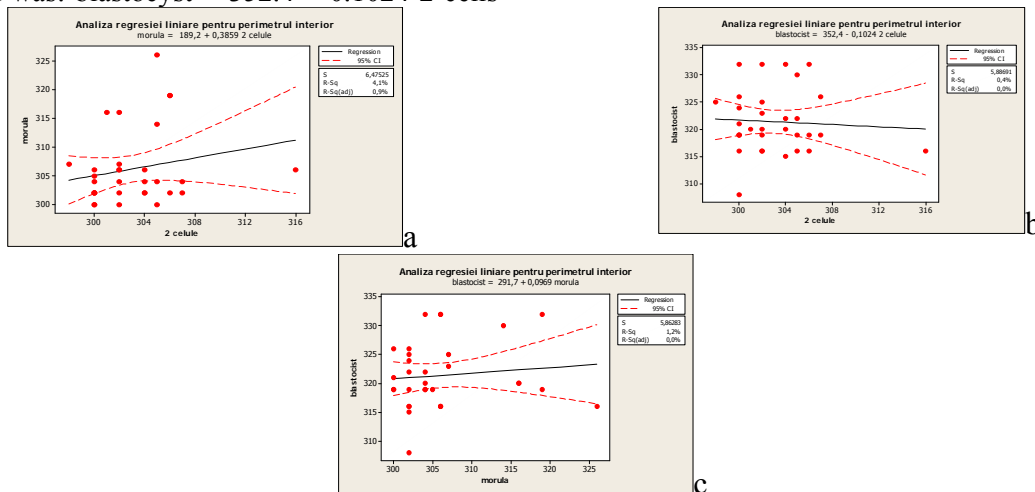


Figure 4. Graphical representation of linear regression for the inner perimeter

Correlation analysis for the outer perimeter of the two cell stage embryos that developed to morula stage showed that there was a weak correlation between them, the regression polynomial equation was: morula = - 26283 + 243.1 2 cells – 0.738 2 celule**2 + 0.000744 x 2 cells**3 (S = 26.2844 R-Sq = 8.0% R-Sq(adj) = 0,0%) (figure 5.a). Correlation analysis for the outer perimeter of the

two cell stage embryos that developed to the blastocyst stage showed a weak negative, moderate correlation between them, the polynomial regression equation was: blastocyst = - 58336 + 524.0 2 cells – 1.555 x 2 cells**2 + 0.001535 x 2 cells**3 (S = 16.6631 R-Sq = 21.0% R-Sq(adj) = 12.5%) (figure 5.b). Correlation analysis for the outer perimeter of the morula

stage embryo that developed to the blastocyst stage showed a very weak correlation, the polynomial regression equation was: blastocyst = -16948 + 149.8 morula - 0.4308 morula**2 +

0.000412 morula**3 (S = 18.6184 R-Sq = 1.4% R-Sq(adj) = 0.0%) (figure 5.c).

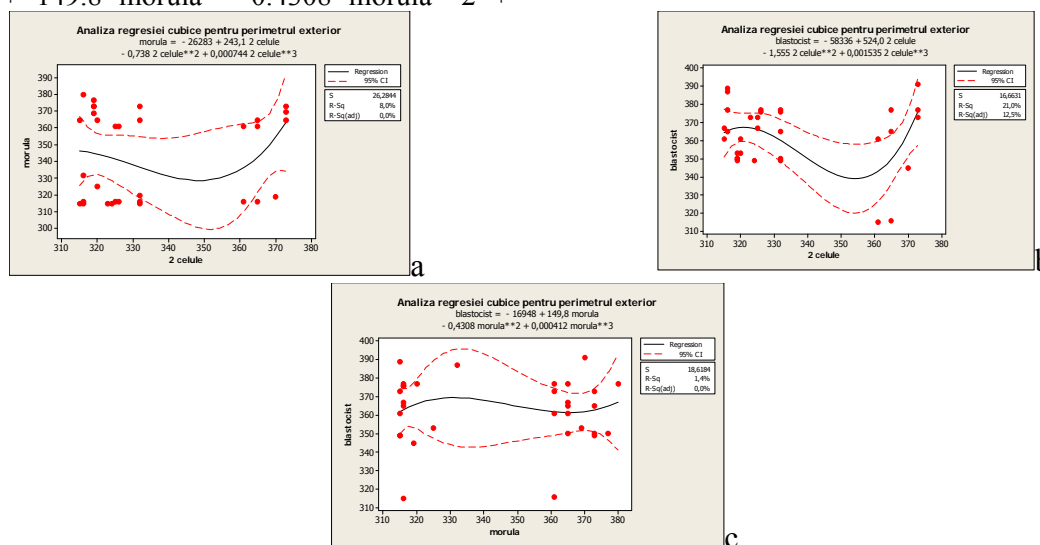


Figure 5. Graphical representation of linear regression for the outer perimeter

4. Conclusions

1. Between the pellucid zone thickness of the two cell stage embryos that developed to morula stage, there is a very strong, linear and positive correlation, (morula = 1.524 + 0.9436 x 2 cells).
2. Between the pellucid zone thickness of the 2 cell stage embryos that developed to the blastocyst stage there is a strong linear correlation (blastocyst = 0.9771 + 0.6468 x 2 cells).
3. Between the pellucid zone thickness of the morula stage embryo that developed to the blastocyst stage there is a moderate, linear, positive correlation (blastocyst = 0.875 + 0.5983 x morula).
4. The pellucid zone thickness represents a morphometric parameter that can be used as an embryo quality indicator apart of the embryo developmental stage. The rest of the morphometric parameters under study (inner and outer diameter, inner and outer perimeter) can not be used as indicators of the embryo quality.

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