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Short Contribution

Surface area/volume ratio and growth equation of the human early embryo

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ABSTRACT **A study of the S/V ratio and growth equation of 49 oocytes and 120 human embryos was carried out. The S/V ratio of the internal and external limits of the zona remains unchanged during successive cleavages. The mean blastomere increases 22% its S/V ratio after each division. The mean blastomere growth equation for the 2-cell to 8-cell stages follows the expression $y = 1.271\ x^{1.021}$ (y= longest and x= smallest diameter), but the results obtained for the whole embryo do not coincide with those resulting from the application of the growth equation.**

KEY WORDS: *human oocyte and embryo, ooplasm, zona pellucida, blastomeres, surface area/volume ratio, growth equation*

In mammalia, a variety of techniques have been developed to evaluate the viability of preimplantation embryos (Butler and Biggers, 1989; Casey *et al.*, 1989). However, none of these methods, most of which are based on morphological evaluation, have been universally accepted by the commercial embryo transplant industry (Shea, 1981; Youngs *et al.*, 1987). In humans, under current practices, evaluation of oocyte or embryo quality is based on the subjective visual appraisal of morphological characteristics (Testart *et al.*, 1983; Humeau, 1985; Plachot *et al.*, 1987).

In a previous study in humans, Goyanes *et al.* (1990) used morphometry in terms of volume, coefficient of form, coefficient of variation and coefficient of diversity during early development stages. This was one of the first attempts to achieve a quantitative, and thus objective, categorization of human oocytes and embryos (Arnold *et al.*, 1987; Fuhr *et al.*, 1987). The knowledge of the dimensions of oocytes and embryos as well as their evolution throughout fertilization and embryo cleavage could make it possible to establish the standard patterns of our species and criteria for decisions concerning *in vitro* fertilization.

The surface area to volume (S/V) ratio and the growth equation have not been established yet. Thus, some reports emphasize the importance of the S/V ratio of oocytes and embryos (Schneider, 1986). Brower and Schultz (1982) correlated surface area to volume ratio with uptake of nutrients necessary for oocyte growth. Other workers related the ability of embryos to survive freezing and thawing with blastomere dimensions and consequently with the ratio of surface area to volume (Schmidt *et al.*, 1987). Lehtonen (1980) calculated the surface area to volume ratio and its increase in each division during early stages.

Ever since Huxley (1932) first drew attention to the biological significance of relative size and shape of living organisms through their evolution, diverse morphological, physiological and ecological traits have been studied by the growth equation. Furthermore, this mathematical tool has served to understand size changes as well as cellular differentiation and growth processes in animals and plants (Gould, 1966; Pagel and Harvey, 1989).

This present study attempts to analyze some of the interactions between size and shape involved in the development of the *in vitro* cleavage stages of human embryos. Here, we present our results concerning the surface area/volume ratio and the growth equation of embryos in an attempt to establish the standard dimensions of normal oocytes and embryos in humans.

Table I presents the surface area/volume ratio of the structures which constitute the metaphase II oocyte and the early stages of the human embryo.

Following fertilization, changes in some of the quantitative dimensions were detected. Ooplasm external limits at the Metaphase II oocyte stage show a lower S/V ratio than at the pronuclear stage ($p<0.05$). The mean blastomere increased (22%) its surface area/volume ratio after each cleavage due to decrease in blastomere volume.

With respect to the inner limit of the zona, which corresponds to ooplasm plus perivitelline space, the S/V ratio statistically increased through fertilization ($p<0.05$). But once the pronuclear stage was reached, this ratio remained unchanged during successive cleavages up to the 8-cell stage ($p>0.05$).

We also calculated the S/V ratio of the whole oocyte and embryos (zona pellucida external limit) for the stages prior to and following fertilization. A very slight change was detected during fertilization. Differences were not observed after fertilization or during early developmental stages ($p>0.05$).

Table II shows the S/V ratio of the structures constituting the 2-

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TABLE I

SURFACE AREA/VOLUME (μ^{-1})±SE OF THE HUMAN OOCYTES AND EMBRYONIC STAGES

	Metaphase II		Pronuclear stage		2-cell		3-cell		4-cell		5-cell		8-cell	
	N	S/V	N	S/V	N	S/V	N	S/V	N	S/V	N	S/V	N	S/V
Ooplasm	49	0.045 ± 0.0003	18	0.046± 0.0005										
Mean blastomere					15	0.065 ± 0.001	14	0.074 ± 0.002	25	0.082 ± 0.001	10	0.104 ± 0.005	12	0.191 ± 0.005
Zona pellucida Inner limit	14	0.040 ± 0.001	10	0.042± 0.0003	15	0.043 ± 0.001	14	0.041 ± 0.001	25	0.041 ± 0.001	10	0.042 ± 0.001	12	0.041 ± 0.001
Zona pellucida external limit	49	0.033 ± 0.001	18	0.035± 0.001	15	0.032 ± 0.001	14	0.032 ± 0.001	25	0.033 ± 0.002	7	0.034 ± 0.001	11	0.034 ± 0.001

cell to 5-cell embryos which successfully implanted and progressed to birth. The S/V ratio of the mean blastomere increased 21% after each cleavage. However, no changes in the S/V ratio are produced at the inner and the external limits of the zona ($p>0.05$). When we compared all the morphologically normal embryos (Table I) with the successfully implanted embryos (Table II), no differences were detected in the S/V ratio of the mean blastomere or between inner and external limits of the zona of the two groups ($p>0.05$).

We also calculated the growth equation for the embryos and their mean blastomere. The measurements of the longest (Y) and smallest (X) diameter were attained by photographing 2-cell to 8-cell embryos (Table III). These data were converted to logarithms and plotted. The mean blastomere measurements resulted in a nearly rectilinear line (Fig. 1). The least-squares (regression line) for these points resulted in an allometric equation: $\log y = \log b + (a \cdot \log x)$. In their exponential form $y = a\ x^b$. In our case $y = 1.271\ x^{1.021}$. However, applying this procedure to the whole embryo (zona pellucida external limit) the plotted data showed an expanded, random distribution (Fig. 2), thus suggesting no significant changes in shape or size throughout cleavages.

Oocytes and embryos are three-dimensional and morphologically changing organisms. In this respect, morphometry-stereology provides a variety of methods which, when applied to fertilization and early embryonic development, make possible a quantitative and objective appraisal. Goyanes *et al.* (1990) emphasized the importance of quantifying size and shape in terms of volume, coefficient of form, coefficient of variation and coefficient of diversity so as to characterize human oocytes and embryos in an *in vitro* fertilization programme. In a further step, we calculate here the surface area to volume ratio and the growth equation throughout fertilization and the first three cleavages.

The S/V ratio gives geometric feature of cells, thus allowing a direct characterization of some of their physiological properties (Miyamoto *et al.*, 1988). In humans, we found that ooplasm at Metaphase II shows a S/V ratio of 0,045 μm^{-1} which increased 1% across fertilization. This increase would correspond to the loss of 10% ooplasm volume noted by Goyanes *et al.* (1990). Coincidentally, Abramczuk and Sawicki (1974) found that fertilization in the mouse causes a 12% reduction in ooplasm volume, perhaps secondary to the polar body extrusion and the release of cortical granules into the perivitelline space.

On the other hand, the 22% increase in the S/V ratio of the mean blastomere throughout each of the successive cleavages is a consequence not only of the 28.5% volume decrease in each cleavage but also of the progressive elongation of the blastomere (coefficient of form 0.96 to 0.8) (Goyanes *et al.*, 1990). Both events induce a relative increase in the blastomere surface area with respect to its volume. In this way, the calculated S/V ratio increases 65% from the 2-cell to 8-cell stages. In the mouse, the ratio of surface area to volume increases 25-30% in each division (Lehtonen, 1980).

Some reports describe the behavior of blastomere cleavage during the early development stages in terms of inter- and intracellular events, such as the blastomere contact area, the movement of the blastomeres towards a more stable equilibrium configuration, as well as the consequences of these events on compaction and cell

TABLE II

SURFACE AREA/VOLUME (μ^{-1}±SE) OF THE HUMAN EMBRYOS WHICH IMPLANTED AND PROGRESSED TO BIRTH

	2-cell		4-cell		5-cell	
	N	S/V	N	S/V	N	S/V
Mean blastomere	5	0.065 ± 0.003	14	0.084 ± 0.002	7	0.1036± 0.005
Zona pellucida inner limit	5	0.044 ± 0.001	14	0.042 ± 0.001	7	0.044 ± 0.001
Zona pellucida external limit	5	0.032 ± 0.001	14	0.032 ± 0.001	7	0.033 ± 0.001

TABLE III

THE LARGEST (Y) AND SMALLEST (X) DIAMETER (μ±SE) OF THE MEAN BLASTOMERES FROM 2-CELL TO 8-CELL HUMAN EMBRYOS

	2-cell		3-cell		4-cell		5-cell		8-cell	
	N		N		N		N		N	
Y	22	97.04 ± 1.28	14	84.51 ±1.33	22	73.66 ± 0.94	3	67.53± 1.37	12	51.17± 1.51
X		68.97 ± 1.27		62.42 ±1.14		58.63 ± 0.84		49.2 ± 1.85		40.71± 1.06

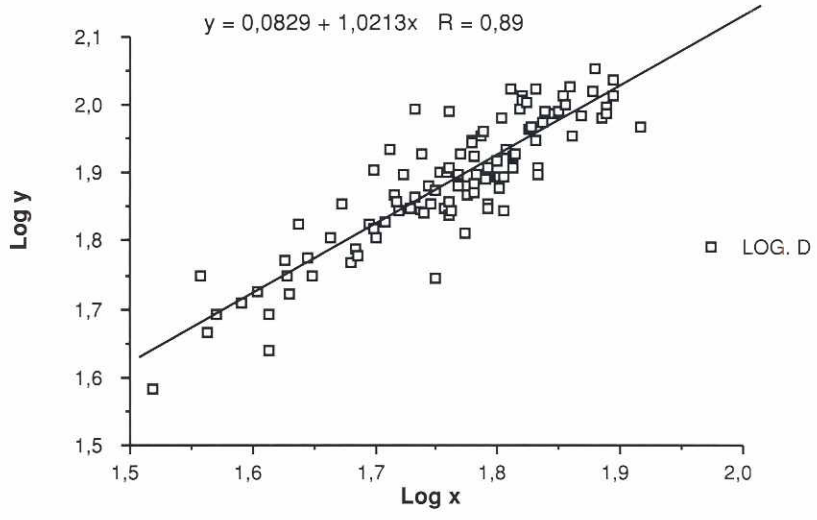

Fig. 1. Growth equation of the mean blastomeres from 2-cell to 8-cell human embryos.

differentiation (Ducibella and Anderson, 1975; Graham and Lehtonen, 1979; Handyside *et al.*, 1987). The S/V ratio would provide a dynamic and quantitative view of the blastomere contact areas of the embryo so as to better understand the magnitude of cell interactions and their movements across cell divisions. Since cleavages occur within the dimensionally unchanging zona envelope, the S/V ratio would also reflect the influences of physical forces acting on cell flattening and cell contacts.

On the other hand, our measurements of the whole embryo (external limits of the zona) indicate that the S/V ratio shows minimal variations during fertilization, remaining unchanged afterwards. This result agrees with a previous report (Goyanes *et al.* 1990) indicating that the volume and shape of the zona pellucida remain unchanged during fertilization and the first three cleavages. Although this conclusion seems generally sound for oocytes and embryos incubated throughout 30-48 hours, Chan (1987) reported that the thickness of the zona of hamster oocytes slightly increased the first hours after insemination. Possibly, this event could be due to the process of adaptation of the envelopes to the physiochemical characteristics of the culture medium.

When we compared the average S/V ratios of all the examined embryos with those successfully implanted and progressed to birth, no differences were detected. According to our data of the standard deviation, and excluding the blastomeres, the physiological variability of the S/V ratios of the embryo structures would be less than 3%. This results also points to the S/V ratio as a nearly constant value for each one of the embryo structures.

Brower and Schultz (1982) considered that oocytes are large spherical cells with a minimal S/V ratio and emphasized the importance of this relation for the uptake of nutrients. Likewise, some reports refer to the ability of the embryo to survive freezing in relation to the stage of development, blastomere size and S/V ratio (Friedler *et al.*, 1987; Schmidt *et al.*, 1987). Furthermore, Schmidt *et al.* (1987) pointed out that «differences in blastomere dimen-

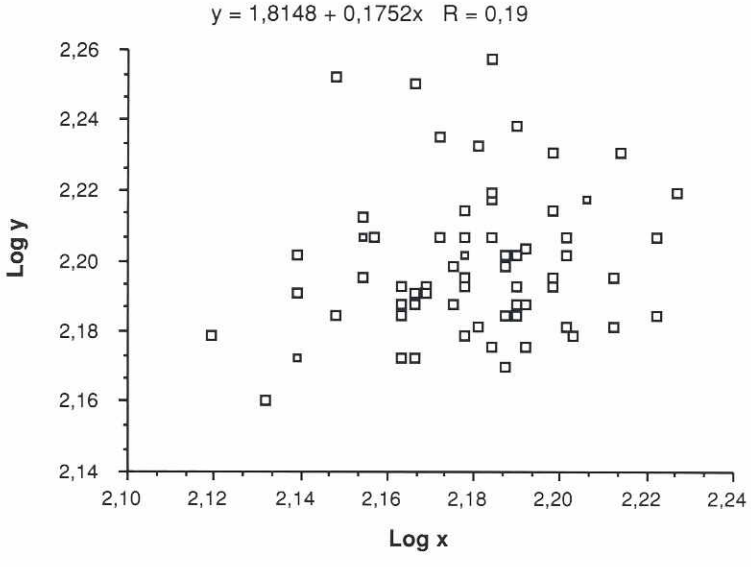

Fig. 2. Unchanged dimensions of the zona pellucida external limit, from 2-cell to 8-cell human embryos. *These values do not correspond to the results of applying the growth equation (R= 0,19).*

sions could affect dehydration during cooling or rehydration during thawing. Theoretically, the larger embryos might be expected to be more sensitive to freezing and thawing due to the differences in ratio of surface area to volume».

Let us now take a look at the growth equation, which has been widely used for whole organisms, organs, and cells (Lindstedt and Calder, 1981; Banse, 1982; Pagel and Harvey, 1989; Houck *et al.*, 1990). Efforts have been directed towards mathematically describing the correlated changes in size and shape shown by living beings through development (Gould, 1966). In a broader sense an allometric magnitude determines the differences in the proportions related to changes in absolute magnitude of each structure. Fishel *et al.* (1985) came near to this concept when, in an equation, they related parameters such as time after insemination, cell stage and cell number at a given time. The growth parameter calculated by Fishel *et al.* (1985) attempted to indicate the rate of cleavage and embryo viability. However, in their study these authors found that blastulation and hatching resulted from both rapid and slow cleavage, and that the rate was independent of subsequent viability. These results suggest that this parameter would not be a good indicator of embryo prognosis, although it would define some characteristics of embryo development.

From our results we observe that the mean blastomere growth equation from the 2-cell to 8-cell stages shows a positive allometric behavior (allometric coefficient, 1.021), thus showing that the rate of decrease is greater for the longest diameter than for the smallest one. These results quantify the progressive change in blastomere size and shape through the first three cleavages. This tendency towards a progressive elongation would produce a troublesome thermodynamic equilibrium and molecular interchange problems at the level of the cell envelope. So we judge the positive allometry and negative growth tendency of blastomeres to be a transitional and reversible period at this time when the zona remains unchanged. However, when we applied these procedures to the whole embryo (zona pellucida external limit), we found no changes in size or shape through its early development, thus not corresponding to the results obtained from applying the growth equation.

Experimental Procedures

Oocyte recovery and IVF

Oocytes and embryos resulted from an *in vitro* fertilization program, where patients were suppressed by administration of LH-RH analogs from day 22 of the previous cycle and then stimulated with FSH and HMG, choosing doses for every individual patient related to E_2 and ultrasonography results (usual long protocol). Luteinization was done by the administration of HCG 5000 iu in a single dose. Oocyte recovery was made by transvaginal follicular puncture.

After recovery, oocytes were incubated for 2-5 h in 1 ml Menezo's B_2 medium at 37°C in a humidified atmosphere 95% air-5% CO_2. Motile spermatozoa were recovered by the swim-up procedure. Around 10^5 spermatozoa were layered over each oocyte and incubation was performed by 36-70 h into the Menezo's B_2 medium.

S/V ratio and Growth equation

Forty-nine living oocytes and 120 embryos from a human *in vitro* fertilization program at different developmental stages were directly photographed. Real measurements from the micrographs enlarged at 300x were estimated by conversion related to a calibrator. Perimeters were determined by a curvimeter. The area of the oocyte and embryo structures on micrographs was obtained by the point-counting and planimetry procedure (Weibel and Bolender, 1973; Baak and Oort, 1983). The number of micrographs analyzed was determined using Williams' progressive mean technique with a ±5% confidence limit.

S/V ratio was estimated by Miyamoto *et al.*, (1988):

$S/V=4\ P/\pi A$, where P: mean profile perimeter
A: mean profile area

Growth equation ($y= a\ x^b$) was calculated following Huxley's (1932) procedure (Gould, 1966; Pagel and Harvey, 1989), where **y** and **x** are the longest diameter and smallest diameter respectively of 72 embryos at different developmental stages. Thus, taking logarithms on both diameters and performing a linear regression analysis, the values of **a** and **b** were obtained.

Statistical analysis employed the chi-square test of homogeneity. Pooled data were examined using analysis of variance. The difference between the two group means was tested by Student's *t* test.

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