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Differences in membrane properties between unfertilised and fertilised single rabbit oocytes demonstrated by electro-rotation. Comparison with cells from early embryos

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The apparent membrane capacity of tubular rabbit oocytes increases from 1.7–2.0 $\mu F/cm^2$ before fertilisation to 3.7–4.0 $\mu F/cm^2$ after fertilisation. The membrane conductivity measured on single cells was also increased by fertilisation from less than 1 mS/cm^2 to 14 mS/cm^2. Cells obtained from 2-, 4- or 8-cell embryos exhibited intermediate values of membrane capacity (2.3–2.8 $\mu F/cm^2$) and conductivity (5–22 mS/cm^2). The values quoted are those effective between 1 and 10 kHz, the frequency of the rotating field used. The large apparent capacities are probably due to the presence of structures such as microvilli which cause the actual membrane area to exceed the smooth sphere area. It must be assumed that these structures change in form or number on fertilisation, and that they persist in embryos, at least up to the 8-cell stage. No difference was apparent between cells fertilised in vitro or in vivo. Comparison of the above zona-free data with measurements on zona-complete oocytes indicate how fertilised and unfertilised rabbit eggs may be distinguished from one another, even in the presence of the zona pellucida.

An earlier paper [1] demonstrated how electric-field induced rotation [2] of individual mouse oocytes could be used to derive values for the membrane capacity and conductivity (C_m and G_m, the area-specific capacitance and conductance). In the case of mouse oocytes, C_m was found to be 1.2–1.3 $\mu F/cm^2$, but single results on hamster and human oocytes suggested C_m values 3-times higher [1]. If confirmed, such values would markedly exceed those of lecithin bilayers (up to 0.8 $\mu F/cm^2$ [3,4]) or the 1 $\mu F/cm^2$ typical of animal or plant cells [5,6]. A high apparent value for oocyte C_m is expected because electron micrographs show that the actual membrane area of the oocyte surface is extended by microvilli [7–9]. Measurements of the factor by which the microvilli increase the membrane area have been carried out on starfish eggs [10], where a factor of 2 was determined (which jumped to more than 3.3 at fertilisation, and then decreased again [10]). Electron-micrographic analyses of area are not yet available for mammalian eggs, but internal microelectrode measurements indicate high input capacitances consistent with large effective areas. In the case of rabbit ova matured in vivo and in vitro, apparent C_m values of 2.7 $\mu F/cm^2$ and 7.1 $\mu F/cm^2$ can be derived from the data of Ref. 11, by use of the cell radius measured here (54 μm, see Fig. 1 legend).

It was the aim of this work to see if changes in C_m measured by the non-invasive electro-rotation method could be used to distinguish between unfertilised and fertilised cells. Although it has been reported that there are differences in the rotation of unfertilised and of in vivo fertilised mouse oocytes [12], we have not been able to see differences between unfertilised and in vitro fertilised mouse eggs (Arnold, W.M., Schmutzler, R.K., Schmutzler, A.G., Van der Ven, H., Al-Hasani, S., Krebs, D. and Zimmermann, U. (1987), unpublished data). The interest in distinguishing between fertilised and unfertilised cells stems from the possible application to veterinary and to clinical in vitro fertilisation (IVF) programs. Rabbit eggs were chosen for this study not only because microelectrode results were available

Abbreviations: BSA, bovine serum albumen; f_c, 'characteristic frequency' of a rotation peak (that field frequency that gives fastest rotation).

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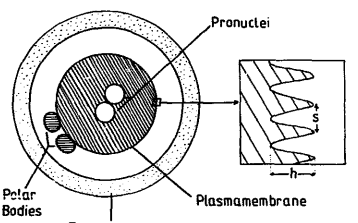

Fig. 1. The features of a recently fertilised rabbit egg that are referred to in the text, with a simplified representation of how the membrane area will be increased if membrane foldings or other structures such as microvilli are present. The radius of the fertilised oocytes in this work was 54.4±2.2 μm (n = 25), not significantly different from that of the unfertilised (53.9±1.8 μm, n = 24).

for comparison of electrophysiological data, but also because IVF work has shown them to be a good experimental model for human oocytes [14,15]. Tubular eggs were removed from donors 18 h after insemination and measured within 3 h. In vitro fertilised eggs were prepared by incubating super-ovulated eggs for 5 h with in vivo capacitated sperm, and then cultured for a further 5 h in Ham's F10 medium. The procedure is described fully in Refs. 15 and 16.

The rotation spectra of oocytes from rabbits were qualitatively similar to those from mice [1]. We report here only the field frequency giving fastest rotation (the characteristic frequency, f_c): this is often the most useful rotational parameter [2,17]. Rotation apparatus and media were as before [1], except that owing to the large radius of the rabbit eggs, the field strength was limited to 33 V/cm. It can be calculated (Eqn. 5 of Ref. 1) that even this field strength would have given rise to an induced membrane voltage of 190 mV at the f_c, rising to 270 mV at low frequencies. Use of frequencies below 1 kHz was avoided becasue of rapid deterioration (swelling and/or lysis) of the oocytes. Stability of oocytes in the low-ionic rotation medium was improved by inclusion of 6 mg/ml BSA.

Initial work with zona-intact oocytes (upper part of Fig. 2) showed that the f_c values of oocytes which had not been in contact with sperm (○, ●) were generally higher than those of fertilised cells. On the other hand, no difference could be seen between oocytes which had been treated in vitro with capacitated sperm (◇), and oocytes taken from mated does (□, ■). The work with zona-intact cells was difficult because of their slow rotation (see Fig. 2 legend) and tendency to stick to the chamber floor, which did not occur with zona-free cells (provided the floor was of polymethacrylate). Moreover, it can be seen from the lower part of Fig. 2 that the zona-free data do not exhibit so much scatter in their f_c values as the zona-complete ones. Further, if deductions about membrane properties are to be made, it is helpful to remove the zona [1]. For these reasons further work used zona-free cells.

As demonstrated in the lower part of Fig. 2, zona-free cells show a clear decrease in their f_c values after fertilisation, especially in media of higher conductivity. The two types of fertilised cells show no difference in f_c value. The dependence of f_c on medium conductivity was found to be accurately linear, as demonstrated by measuring single unfertilised cells at 3 or 4 conductivities (● in the lower part of Fig. 2). The existence of this linearity allows the application of the equation derived for the membrane-charging mechanism of electro-rota-

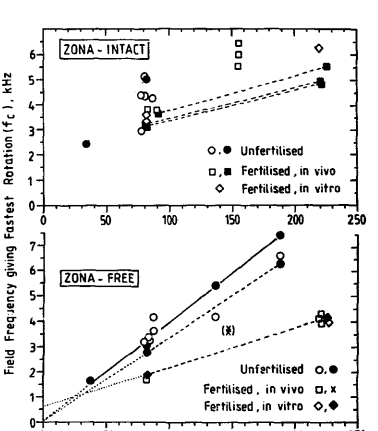

Fig. 2. Values of f_c of unfertilised and fertilised rabbit oocytes measured in media of a range of conductivities. Cells measured once only are shown as open symbols, whilst solid symbols connected by dashed lines represent a single cell measured at two different conductivities. In the case of the zona-complete cells, the influence of the zona does not allow interpretation of the data in terms of Eqn. 1. The solid line is the least-squares fit to data on one cell taken at four conductivities (correlation coefficient r = 0.999). The membrane parameters derived by use of Eqn. 1 are given in Table I. (Cells (×) were excluded because they were a different batch from other cells.) Typical rotation speeds, measured using 33 V/cm field strength at the f_c in medium of 80 μS/cm conductivity, were 19–50 deg./s for zona-complete cells, increasing to 100–140 deg./s for zona-free cells. The speeds increased slightly with medium conductivity, and showed no consistent difference between fertilised and unfertilised cells.

TABLE I

Values for membrane capacity (C_m) and conductivity (G_m) of fertilised and unfertilised oocytes and of cells from early embryos

Figures are derived from the zona-free data of Fig. 2, and from Fig. 3.

Type of cell: unfertilised, 'U'; fertilised, 'F'; or embryo, 'E'	Type of data: single-cell, 'SC'; or mean; also (number of points)	Figure number and symbol	Membrane parameters	
			C_m (μF/cm^2)	G_m (mS/cm^2)
U	SC (4)	2, ●	1.70	<1
U	SC (2)	2, ●	1.90	<1
F	SC (2)	2, ◆	3.97	15
F	Mean (7)	3, F	3.74	13
E (2-cell)	SC (2)	3, ●	2.59	2
E (4-cell)	SC (2)	3, ▲	2.27	13
E (4-cell)	SC (2)	3, ▲	2.32	5
E (4-cell)	SC (2)	3, ▼	2.75	10
E (4-cell)	Mean (9)	–	2.57	13
E (8-cell)	SC (3)	3, ■	2.56	22
E (8-cell)	SC (4)	3, ■	2.53	12
E (8-cell)	Mean (7)	–	2.61	12

The radii of the cells were: 2-cell, $a = 38.6$ μm;
4-cell, $a = 32.5 \pm 0.5$ μm (mean $\pm$ S.D., $n = 9$);
8-cell, $a = 23.4 \pm 0.8$ μm (two cells from one embryo)

tion, valid for medium conductivities much lower than that of the cell interior [2]:

$$f_c = \frac{G_m}{2\pi \cdot C_m} + \frac{\sigma}{\pi \cdot a \cdot C_m} + \frac{K_s}{\pi \cdot a^2 \cdot C_m} \qquad (1)$$

where a is the cell radius, and σ is the medium conductivity. K_s is the surface conductance, the effect of which can be ignored in the case of such large cells as oocytes [1] (because of the $1/a^2$ dependence). If a linear plot of f_c against σ is obtained, then the gradient is $1/(\pi \cdot a \cdot C_m)$ and the intercept $G_m/(2\pi \cdot C_m)$.

Application of this equation to the zona-free data of Fig. 2 gives the values for C_m and G_m given in Table I. Consistent values of C_m are obtained both from single-cell data and from mean values over all cells of a given type. It is clear that C_m is almost doubled during or shortly after fertilisation. The values for G_m are not so reproduceable as those for C_m, but do show a definite increase on fertilisation. Fig. 2 shows that no significant difference could be seen between cells fertilised in vitro or in vivo.

Early embryos could also be rotated, but adhesion of the zona to the chamber floor prevented useful measurements. However, individual embryonic cells rotated readily. (These cells were obtained by continuation of the pronase treatment used to remove the zona combined with trituration through a micropipette.) Cells separated from 2-cell, 4-cell and 8-cell embryos all had similar $f_c \cdot a$ values (normalisation using the cell radius corrects for the effect of differing cell radii, see Eqn. 1). Most cells were measured at two conductivities, those from 8-cell embryos (points ■ in Fig. 3) at 3 or 4 conductivities. The linearity of the dependence of $f_c \cdot a$ on σ confirms the applicability of Eqn. 1 to this system. The means of the radius-normalised data from unfertilised and fertilised cells (lines U and F in Fig. 3) lie significantly above and below the embryo cell data. Table I shows that indeed the C_m values of embryo cells lie between those of unfertilised and fertilised cells, but that the G_m values are approximately as high as those of the fertilised cells.

The difference in C_m between unfertilised and fertilised cells indicates that a large change in membrane structure occurs on fertilisation of rabbit oocytes. On the basis of a model such as given in the inset to Fig. 1, an increase in capacity would be produced if the spacing s were decreased (more structures per unit area), or if the height h were increased [10]. A second possibility would be the unfolding of stacked or folded membranes in which the repeat distance was originally so small that the conductivity between the stacks was low. The source of membrane required for the first possibility could be the cortical granules released in large numbers at fertilisation [7,9,10]. This is consistent with fertilisation-changes seen in electron micrographs of eggs from rat and hamster [18], and rabbit [19]. Ultrastruc-

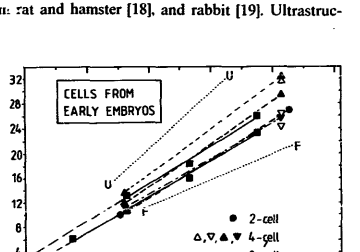

Fig. 3. Radius-normalised f_c values of cells isolated from early rabbit embryos (2-cell, 4-cell or 8-cell) measured in media of a range of conductivities. The normalisation enables direct comparison of cells of different radius (see Eqn. 1). Cells measured at a single conductivity are shown as open symbols. Solid symbols represent cells measured at two or more conductivities: the two continuous lines are the least-square fits to data from cells which were measured at three and four conductivities (correlation coefficients r of 0.996 and 0.997, respectively). The derived membrane parameters are given in Table I. The dotted lines identified by 'U' and by 'F' are the least-squares regressions of $f_c \cdot a$ against σ for all unfertilised-egg data ($r = 0.957$) and for all fertilised-egg data ($r = 0.995$), respectively (they allow comparison with the zona-free egg data of Fig. 2).

tural changes of mouse egg membranes upon fertilisation have also been documented [20].

One possible function for the microvilli may be that they act as a membrane reserve, ready to provide the additional bounding membrane that is required during the rapid division after fertilisation. (Each round of cell division at constant cytoplasmic volume demands an increase in membrane area by a factor of 1.26). Examination of Table I shows that the membrane capacity of cells from 2-cell embryos is indeed considerably less than that of fertilised egg cells, but in the next two rounds of cell division C_m stays close to 2.5 $\mu F/cm^2$, a value still much higher than the normal value close to 1 $\mu F/cm^2$ [5,6].

It must be assumed from the above that if conversion of microvilli into additional bounding membrane occurs, this is only during the first division. The persistence of a high C_m value into the 4- and 8-cell stages suggests some additional physiological role for the microvilli. One possibility is the provision of the membrane area necessary for the transport needs of a large, metabolically active cell. The increase in metabolic activity following fertilisation may be expected to give rise to an increase in the number of transport channels and therefore account for the higher values of G_m seen in fertilised and embryo cells compared to unfertilised cells.

Microelectrode measurements on unfertilised rabbit ova matured in vivo gave an input resistance of 10 MΩ [11], equivalent to a G_m of 0.27 mS/cm^2. This is consistent with the value of < 1 mS/cm^2 found here for unfertilised tubular cells. However, microelectrode measurements use a much lower range of frequencies than the 1–10 kHz used in these rotation measurements (the time constant measured [11] on mature ova of 10 ms is equivalent to use of 16 Hz measurement frequency). This means that C_m and G_m values measured by rotation are not necessarily comparable with those measured with microelectrodes, because the membrane may exhibit changes in properties between these frequency ranges. Such dispersive behaviour may be responsible for the α-dispersion of the impedance of certain tissues reviewed in Ref. 21. Calculation [1] indicates that a large increase in the rotational G_m over the low frequency value is expected. Causes of such a dispersion could be microvilli [1] or other membrane structures [21], or else the slow response of 'gating-charges' seen in excitable membranes [6] (and expected in other ion-transporting membranes).

We note that in-vivo fertilised mouse oocytes have been found to have lower f_c values than unfertilised [12], which was interpreted as a fertilisation-induced decrease in G_m. Another explanation would be the magnitude of C_m increase measured here, if it also occurred in mouse oocytes. However, the low C_m of mouse oocytes (1.2–1.3 $\mu F/cm^2$) compared to that of rabbits (Table I) suggests that the electrical effect of the microvilli of the two sorts of oocytes are very different. Further, the ultrastructural changes following artificial stimulation of rabbit oocytes are different from those seen in mouse oocytes [13]. It should also be mentioned that the mouse work [12] was carried out on zona-complete cells so that the change in f_c may have been caused by a change in the electrical properties of the zona rather than those of the membrane.

Application of the present results to re-implantation work will probably necessitate the use of intact cells. Any fertilisation-induced change in membrane properties will be partly masked due to electrical screening by the zona, which is more conductive than the low-ionic rotation media *. However, the properties of the rabbit zona appear to be rather variable, possibly because cumulus cells or parts of them remained attached to the zonae after isolation. The influence of the zona can be minimised by working in media of higher conductivity, which also has the advantage, in the rabbit system, of maximising the effect of a difference in C_m on the f_c (Fig. 2).

We conclude by pointing out that electro-rotation has enabled aspects of the fertilisation and ensuing division to be followed more extensively than previously. The results correlate well with the available microelectrode measurements. We know of no other C_m or G_m measurements on rabbit embryo cells. In some species the rotation technique can discriminate between unfertilised and fertilised cells in their intact state.

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* This conclusion is based on the same arguments as used for the mouse zonae [1], in view of their similar rotational behaviour. As with mouse cells, zona-complete rabbit cells exhibit higher f_c values than zona-free cells (Fig. 2), and isolated washed zonae give a co-field rotation peak (not shown).

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