Ann. Biol. anim. Bioch. Biophys., 1978, **18** (2 B), 529-533.

Observations on follicular lactate concentrations and the influence of granulosa cells on oocyte maturation in the rat (including data on second polar body formation)

by G. H. ZEILMAKER

Department of Endocrinology, Growth and Reproduction, Erasmus University, Postbus 1738, *Rotterdam, The Netherlands*

Summary. High concentrations of lactate were detected in preovulatory follicles of the rat ovary before as well as after the time of the expected LH surge (27 mM). Explanted oocytes obtained from pre-puberal rats and surrounded by cumulus cells matured in thet presence of 20 mM lactate. Lactate production by the follicle under the influence of LH can not be regarded as a factor initiating maturation.

In order to test whether availability of oxygen might induce oocyte maturation whole preovulatory follicles were incubated *in vitro*. LH induced disappearence of the germinal vesicle in most cases. A small slit in the follicle wall, presumably allowing access of oxygen induced maturation in a limited number of cases.

The presence of rat granulosa cells in oocyte cultures had no discernable effect on germinal vesicle breakdown. It is concluded that there is no indication for the presence of a maturation-inhibiting factor produced by granulosa cells.

Moreover some data on the formation of a second polar body 24-32 hrs after incubation *in vitro* of ovarian oocytes are reported.

Much attention has been paid to the question :What changes occur in the follicular environment after exposure to LH, particularly related to queries about the nature of the signal for the completion of the first meiotic division in the oocyte ? Since it is well-known that oocytes from mammalian species can complete maturation *in vitro* without exogenous hormones it appears that that effect of the LH surge on maturation is not a direct one but an effect on the environment of the oocyte. The same can be said with regard to the luteinizing potency of granulosa cells *in vitro* which is acquired before the LH surge (Channing, 1970), although critical data with respect to the exact relationship with the LH surge are not yet available.

In the case of mouse oocyte maturation it was observed that pyruvate and oxaloacetate were of critical importance for maturation *in vitro* (Biggers *et al.*, 1967). A lack of energy substrate should therefore be considered in this particular case as a condition which suppresses maturation. Rat oocytes mature in media containing lactate and to a certain extent even in media without energy substrate (Zeilmaker and Verhamme, 1974). Lactate is formed in the medium of explanted follicles after addi-

tion of LH (Hillensjö, 1976), presumably by an increase in glycolytic activity in the follicle. Since lactate is a substrate for rat oocytes and also mouse oocytes, provided enough NAD is present, it could be that a rise in follicular lactate concentration is instrumental in the induction of oocyte maturation.

In order to investigate this matter further the follicular lactate concentrations were determined at 11 : 00, 16 : 00 and 19 : 00 hrs during the day of pro-œstrus, i.e. prior to during and after germinal vesicle breakdown in the oocyte (Zeilmaker and Verhamme, 1977).

Ovaries were dissected from prooestrous rats and placed on solid CO_2. After thawing to 0 °C follicles were dissected and homogenized in cold HCl. Lactate was determined spectrophotometrically and the lactate concentration in the follicles was calculated. The results are shown in table 1 and illustrate that before and after onset of maturation the environment of the oocyte contains very high lactate concentrations. These lactate concentrations support maturation *in vitro*, but apparently not *in vivo* prior to LH exposure. Based on the result of a model experiment (Zeilmaker *et al.*, 1972) in which maturation of oocytes *in vitro* depended on the aerobic generation of ATP, we proposed that in the follicle the oxygen tension might be too low for this process to occur.

TABLE 1

Lactate concentrations in serum and preovulatory follicles before and after initiation of oocyte maturation

	No. of animals	Lactate concentration (mM) ± SEM
Serum 11-12 a.m.	6	5,68 ± 0,49
Serum 4-5 p.m.	5	4,92 ± 0,38
Serum 7-8 p.m.	9	5,25 ± 0,34
Follicles 11-12 a.m.	7	23,3 ± 2,5 (1)
Follicles 4-5 p.m.	7	29,1 ± 3,75 (1)
Follicles 7-8 p.m.	8	23,9 ± 1,85 (1)

(1) Based on follicle volume of 0,392 mm^3, not significantly different in T-test.

In vitro maturation of rat oocytes surrounded by cumulus cells (1)

	No. of oocytes incubated (2)	No. with first polar body	No. with germinal vesicle	No. without germinal vesicle
Basic salt solution				
+ 10 mM lactate	62	22	12	28
+ 20 mM lactate	215	77	69	60

(1) Isolated from ovaries of 30 days old rats.
(2) No. of cells alive after 3 hrs of incubation.
(Data from Zeilmaker and Verhamme, 1977)

We have tested this possibility *in vitro* with whole follicles in which oocyte maturation does occur only after LH exposure, and have attempted to induce a change in redox potential allowing the oocyte to generate ATP necessary for maturation.

In table 2 it can be seen that the original observation of Tsafriri *et al.* (1972) can be duplicated : LH causes germinal vesicle breakdown within 6-7 hrs. Also when the follicle is penetrated with a fine injection needle the nucleus disappears in a significant number of cases. If one assumes that a lack of activity of the cytochrome oxidase system suppresses maturation, the activation of this system by LH through increased permeability of the follicle wall or by allowing access of oxygen through an artificial opening might be a reasonable explanation. It is also shown that the oocyte cumulus complex matures very well once free in the same medium.

TABLE 2

Culture of follicles (6-7 hrs) and oocyte maturation
(isolation at pro-œstrus 10 a.m.)

	No. foll. + GV/total	p. 100	No. foll. — GV/total	p. 100
Control cultures	83/98	(84)	3/98	(3)
Follicles + LH (5 μg/ml).....	0/35	(0)	25/35	(71)
Follicles + incision	63/95	(66)	21/95	(22)
Oocytes + cumulus same follicles...	0/20	(0)	18/20	(90)

Another explanation for the lack of maturation has attracted much attention. It was shown by Tsafriri and Channing (1975) that porcine oocytes mature *in vitro* very well once isolated from the follicle but that the presence of granulosa cells from large follicles would inhibit germinal vesicle breakdown. These data led to the postulation and subsequent isolation of a maturation-inhibiting factor produced by the granulosa cells of the pig.

We have investigated the possible existence of such a factor in the rat. Oocytes and granulosa cells were obtained from 30-day old prepuberal rats and incubated in Brinster's medium containing 20 mM lactate. The 10^7/ml granulosa cells attached to the bottom of the dish and spread over the surface after 24 hrs, which is an indication of their viability. Oocyte maturation was not appreciably affected by these cells (table 3)

TABLE 3

Rat oocytes in 20 mM lactate, 30 days old

	#	+ pb	+ GV	— GV
+ cumulus	215	77	69	60
— cumulus	66	22	19	27
— cumulus and 10^7 granulosa cells/ml	73	17	15	41

These date do not exclude the possibility that a specific follicular substance may be present which suppresses oocyte maturation in the rat but at least under the present conditions one cannot find an indication for its presence. It may be recalled that Nekola and Smith (1974) also failed to show an influence of follicle cells *in vitro* on maturation of mouse oocytes (polar body formation).

It seems likely that a change in the follicular environment induced by LH of yet unknown nature, but possibly activating the cytochrome oxidase system, is instrumental in maturation induction. One may question if this change also affects the steroid-synthesing activity of the granulosa cells or, to the contrary, whether oocyte maturation is dependent on changes in the function of the granulosa cells. It may be recalled however that the LH threshold in the rat for maturation induction is lower than that for luteinization (Vermeiden and Zeilmaker, 1974) and that steroid synthesis inhibitors do not inhibit oocyte maturation (Tsafriri *et al.*, 1976).

Another aspect of oocyte maturation concerns the formation of the second polar body in the rat. Under normal conditions this structure is formed following sperm penetration but under some circumstances completion of the second meiotic division in the rat can be induced by application of a cold shock *in vitro* or *in vivo* and by some forms of anaesthesia (Thibault, 1949 ; Austin and Braden. 1954). Explantation of tubal oocytes, which are in the metaphase of the second meiotic division, leads to the formation of the second polar body within 1 hr (Zeilmaker and Verhamme, 1974). This process was not dependent on the activity of the cytochrome oxidase system since KCN and uncoupling agents did not inhibit it.

Interesting is our recent observation (Zeilmaker and Verhamme, 1976) that ovarian oocytes, in which the first polar body is abstricted after 8-10 hrs, form a second polar body after 24-31 hrs of culture. Contrary to the rather synchronous formation of the first polar body (in 34 cultures PBI the first polar body was formed after 8 hrs. and 20 min., SE 20 min.), the second polar body appeared at an unpredictable time between 24 and 31 hrs after explantation.

It is well known that the first polar body always rapidly degenerates *in vivo* and often also *in vitro*. In such cases there is no doubt about the origin of this second polar body, which might have been supposed to originate by division of the first polar body. This was particularly clear in 37 oocytes with a first polar body formed *in vitro* ; 16 bodies in these oocytes had degenerated after 21 hrs of culture and 13 subsequently formed a second polar body, 4 of which were sectioned and found to contain chromatin in both polar bodies.

27e *Congrès international des Sciences physiologiques, Symposium « Germ and somatic cell interaction » Paris*, 21-23 *juillet* 1977.

Résumé. De fortes concentrations en lactate sont identifiables dans les follicules préovulatoires de rattes aussi bien avant qu'après le moment probable de la décharge de LH-(27 mM). Des ovocytes explantés de rattes prépubères et entourés de leur cumulus reprennent leur méïose en présence de 20 mM de lactate. La production de lactate sous l'influence de LH ne peut pas être regardée comme le facteur d'initiation de la maturation nucléaire.

De manière à reconnaître si la disponibilité en oxygène peut être cause de la maturation, des follicules entiers sont incubés. La LH induit la reprise de la méïose (GVBD) dans

la plupart des cas. La présence d'une petite fente dans la paroi folliculaire est accompagnée d'une reprise de la méïose dans quelques cas, peut-être en permettant l'apport d'oxygène.

La présence de cellules de la granulosa avec les ovocytes en culture n'a pas d'effet discernable sur la GVBD. Il n'y a donc pas d'indication de la présence d'un facteur inhibiteur de la reprise de la méïose par les cellules de la granulosa.

Quelques données sur la formation du 2e globule polaire, 24-32 h après le début de l'incubation sont mentionnées.

References

AUSTIN C. R., BRADEN A. W. H., 1954. Induction and inhibition of the second polar division in the rat egg and subsequent fertilization. *Austr. J. biol. Sci.*, **7**, 195-209.

BIGGERS J. D., WHITTINGHAM D. G., DONAHUE R. P., 1967. The pattern of energy metabolism in the mouse oocyte and zygote. *Proc. nat. Acad. Sci (USA)*, **58**, 560-567.

CHANNING C. P., 1970. Influences of the *in vivo* and *in vitro* hormonal environment upon luteinization of granulosa cells in tissue culture. *Recent Progr. Horm. Res.*, **26**, 589-622.

HILLENSJO T., 1976. Oocyte maturation and glycolysis in isolate preovulatory follicles of PMS-injected immature rats. *Acta endocr.*, **82**, 809-830.

NEKOLA M. V., SMITH D. M., 1974. Oocyte maturation and follicle cell viability in vitro. *Europ. J. Obstet. Gyn. Reprod. Biol.*, **4**, S-125-S-131.

THIBAULT C., 1949. L'œuf des mammifères, son développement parthénogénétique. *Ann. Sci. nat. zool.*, **11**, 133-219.

TSAFRIRI A., LINDNER H. R., ZOR U., LAMPRECHT S. A., 1972. *In vitro* induction of meiotic division of meiotic division in follicle-enclosed rat oocytes by LH, cyclic AMP and prostaglandin E2. *J. Reprod. Fertil.*, **31**, 39-50.

TSAFRIRI A., CHANNING C. P., 1975. An inhibitory influence of granulosa cells and follicular fluid upon porcine oocyte meiosis *in vitro*. *Endocrinology*, **96**, 922-927.

TSAFRIRI A., LIEBERMAN M. E., AHRÉN K., LINDNER H. R., 1976. Dissociation between LH-induced aerobic glycolysis and oocyte maturation in cultured graafian follicles of the rat. *Acta endocr.*, **81**, 362-366.

VERMEIDEN J., ZEILMAKER G. H., 1974. Relationship between maturation division, ovulation and luteinization in the female rat. *Endocrinology*, **95**, 341-351.

ZEILMAKER G. H., HULSMANN W. C., WENSINCK F., VERHAMME C., 1972. Oxygen-triggered mouse oocyte maturation *in vivo* and lactate utilization by mouse oocytes and zygotes. *J. Reprod. Fertil.*, **29**, 151-152.

ZEILMAKER G. H., VERHAMME C. M. P. M., 1974. Observations on rat oocyte maturation *in vitro* : morphology and energy requirements. *Biol. Reprod.*, **11**, 145-152.

ZEILMAKER G. H., VERHAMME C. M. P. M., 1976. Second polar body formation in rat ovarian oocytes matured *in vitro*. *Europ. J. Obst. Gyn. Repr. Biol.*, **6**, 35-38.

ZEILMAKER G. H., VERHAMME C. M. P. M., 1977. Lactate concentrations in preovulatory follicles of pro-œstrous rats, before and after onset of oocyte maturation. *Acta endocr*, **86**, 380-383.