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Concentration of cyclic AMP during the maturation of pig oocytes *in vivo* and *in vitro*

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Intracellular concentrations of cyclic AMP (cAMP) were measured in pig oocytes maturing *in vivo* or *in vitro*. Maturation *in vivo* was induced with 500 iu hCG administered to gilts treated with pregnant mares' serum gonadotrophin (PMSG). Although PMSG did not affect cAMP concentrations (basal values, 1.69 ± 0.28 fmol per oocyte), hCG induced a transient rise (8.86 ± 1.15 fmol per oocyte 12 h after hCG injection). Similarly, the cAMP concentration rose in oocytes maturing *in vitro* if the oocytes (surrounded or not by cumulus cells) were co-cultured with the follicle wall in the presence of LH. The same increase in cAMP was obtained when denuded oocytes were co-cultured with mural granulosa cells. Theca cells exhibited only a moderate activity, while cumulus cells were totally ineffective. Granulosa cells exposed to LH lost their stimulating influence after 24 h of culture. In the presence of FSH, cAMP production by the oocyte was unaffected by any type of follicle cell. The role of cAMP in the control of oocyte maturation was investigated using dibutyryl cAMP. The presence of dibutyryl cAMP prevented the resumption of meiosis in a dose-dependent manner, but when it was present during the first 12 h of culture only, meiotic progression was accelerated (0 versus 47% of oocytes had germinal vesicles in groups treated with dibutyryl cAMP and control groups, respectively, after 24 h of culture). The results demonstrate that: (i) cAMP concentrations increase transiently in oocytes before the resumption of meiosis; (ii) increased concentrations of cAMP depend on the stimulation of oocyte adenylyl cyclase, possibly by a soluble factor produced by follicle cells exposed to LH; (iii) the increase in cAMP is probably confined to the first 10–20 h of maturation owing to the progressive reduction of the stimulating influence of LH-treated somatic cells; and (iv) a high concentration of cAMP throughout maturation maintains meiotic arrest and a transient increase may facilitate meiosis.

Introduction

Shortly after commencing the first meiotic division, the oocyte is arrested at the early prophase stage at about the time of birth. During the subsequent growth phase, the oocyte cannot resume meiosis, but after completing growth, approximately in coincidence with antrum formation, the oocyte acquires the competence to progress through the meiotic cycle if removed from the follicle. However, physiologically the condition of meiotic arrest is maintained and there is compelling evidence that the somatic cells of the follicle are responsible for the inhibition of nuclear maturation (for review, see Wassarman, 1988). The nature of the signal used by somatic cells to maintain meiotic arrest and how this signal reaches the oocyte is still a matter of discussion.

Some factors produced by granulosa cells such as the oocyte-maturation inhibitor (Tsafriri and Channing, 1975) are considered to accumulate in follicular fluid and maintain meiotic arrest. Other models are based on the production of an inhibitory factor in the somatic compartment, and its subsequent transfer to the oocyte selectively through the gap junctions between the oocyte and the foot processes of corona radiata cells. According to this hypothesis of an inhibitory factor, control of meiotic progression could depend either on the production of an inhibitor or on its transfer to the oocyte.

After the gonadotrophin surge, meiosis is resumed. The sequence of events triggered by LH is still far from clear. While it is generally accepted that LH acts primarily on the somatic compartment of the follicle, as no LH receptors have been found on the oolemma, it is still unclear how these cells react to LH to induce the resumption of meiosis. One possibility is that the cells become uncoupled from the oocyte, leaving it free to resume meiosis. In this context, it is hypothesized that as LH uncouples somatic cells from the oocyte, the flow of cyclic AMP (cAMP) (a proposed inhibitory signal) to the oocyte is interrupted (Dekel and Beers, 1978). This model has some experimental support, but an overall analysis of results does not allow the precise identification of a general mechanism in different species. The disruption of cell–cell communication in the cumulus–oocyte complex has been shown in several species to occur after nuclear maturation has started (Moor *et al.*, 1980; Eppig, 1982; Motlik *et al.*, 1986) and intracellular

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concentrations of cAMP do not always correlate with the stage of nuclear arrest or progression (Crosby *et al.*, 1985; Racowsky, 1985). In addition, some recent studies have shown an increase, rather than a decrease, in cAMP during the maturation both of pig and rabbit oocytes (Mattioli *et al.*, 1992; Yoshimura *et al.*, 1992).

The present experiments were designed to study the changes in cAMP concentrations during the maturation of pig oocytes. Since the approach *in vitro* may not always reproduce the physiological conditions under which the oocyte matures, in the first part of the paper the concentrations of cAMP throughout maturation were measured *in vivo*. In the second part, experiments *in vitro* were then undertaken to investigate the interaction between the somatic and germinal compartments of the follicle involved in the modulation of cAMP concentrations in the oocyte. We found that cAMP concentrations in the oocyte increased transiently *in vivo* as a result of hCG stimulation. Experiments *in vitro* showed that cAMP is actively produced by the oocyte under the stimulation of LH-treated granulosa cells and these observations suggest that the transient rise in the concentrations of the nucleotide may play a positive role in the resumption of meiotic maturation.

Materials and Methods

Oocyte maturation in vivo

Prepubertal Large White gilts, averaging 80 kg body weight, were treated with 1250 iu pregnant mares' serum gonadotrophin (PMSG) (Folligon: Intervet-Italia, Milan) and oocyte maturation was induced by the successive injection of 500 iu hCG (Corulon: Intervet-Italia) 60 h later. Groups of 4–5 animals each were ovariectomized under sodium pentobarbital anaesthesia (20–30 mg kg^{-1} body weight, i.v.) before PMSG injection, 6, 24, 48 and 60 h after PMSG injection, and 6, 12 and 24 h after hCG administration. Immediately after ovariectomy, the single follicles were isolated in dissection medium (PBS and 0.4% BSA; Sigma Chemical Co., St Louis, MO) supplemented with the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX; Sigma; 0.2 mol l^{-1}). Oocytes were then collected and treated for cAMP determination. They were mechanically denuded by using a finely drawn pipette and then incubated in a 100°C bath for 10 min to eliminate any enzymatic activity, according to Davis *et al.* (1986).

Oocyte maturation in vitro

For each experiment *in vitro*, a pool of healthy follicles 5–7 mm in diameter obtained from the ovaries of slaughtered prepubertal gilts (unless otherwise specified) were selected on the basis of their translucent appearance, good vascularization and the compactness of granulosa layer and cumulus mass. For one specific experiment, follicles were also isolated from the ovaries of Large White gilts pretreated 60 h earlier with 1250 iu PMSG to stimulate follicular growth.

The following preparations were used:

(1) Follicular oocytes: cumulus-enclosed oocytes were maintained in connection with the everted follicle wall as described by Mattioli *et al.* (1989). Groups of seven everted follicles with their respective oocytes were co-cultured at 39°C in 2 ml of TCM 199 (Sigma Chemical Co.) supplemented with 10% fetal calf serum (Gibco Laboratories, Grand Island NY) and 70 mg Kanamycin l^{-1} (Sigma Chemical Co.) (maturation medium) in 35 mm dishes (Nuclon, Naperville IL) kept under constant agitation in a humidified atmosphere of 5% CO_2 and 95% air. Under these culture conditions, functional cumulus–oocyte coupling is maintained throughout maturation (Mattioli *et al.*, 1988).

(2) Cumulus-enclosed oocytes: groups of seven oocytes maintained in their cumulus masses were cultured in 2 ml of maturation medium.

(3) Denuded oocytes: groups of seven oocytes mechanically denuded of cumulus and corona cells by repetitive aspiration through a small bore pipette were cultured in 2 ml of maturation medium.

After the given culture times the oocytes were collected for the determination of their cAMP content as described for matured oocytes *in vivo*. Owing to the abundant somatic tissues present in many of the cultures, some oocytes allocated to the different experimental groups could not be found. This accounts for the variable number of oocytes that appear in the different experimental groups within the same experiment.

With these preparations the following aspects were investigated.

Concentrations of cAMP during maturation in vitro *with or without LH.* To investigate whether the changes in the concentration of cAMP during the maturation *in vitro* were similar to those recorded *in vivo*, groups of denuded oocytes, cumulus oocytes and follicular oocytes were matured with or without LH (1 μg USDA-pLH-B1 ml^{-1}) and samples were collected at the beginning of the culture or after 3, 6, 12, 24 and 44 h of culture.

Co-culture experiments. To investigate whether the increase in cAMP in the oocyte was produced by the oocyte itself or was the result of the transfer of the nucleotide from cumulus–corona cells, denuded oocytes or cumulus oocytes were co-cultured with everted follicles (seven per dish) with or without LH. To remove any possible influence of adenylyl cyclase present on the remnants of the foot processes of corona cells, many of which remain embedded in the zona pellucida after denuding the oocyte, zona-free oocytes were also prepared by treating them with 0.1% pronase (Boehringer-Mannheim, Mannheim) to remove the zona pellucida before co-culture. As previous experiments showed that cAMP reaches maximum concentration after 3 h of culture, the oocytes in these experiments were cultured for only 3 h and then processed for cAMP determination.

Influence of mural granulosa cells, cumulus cells and thecal cells on the concentrations of cAMP in the oocyte. To define which compartment of the follicle is involved in the stimulation of cAMP production in the oocyte, healthy follicles were everted and the granulosa cells gently removed with the aid of fine watchmaker forceps. The cells were then dispersed by repetitive aspiration through the tip of a P200 Gilson pipette and counted by phase contrast microscopy with the aid of a Thoma haemocytometer. Denuded oocytes were then cultured with

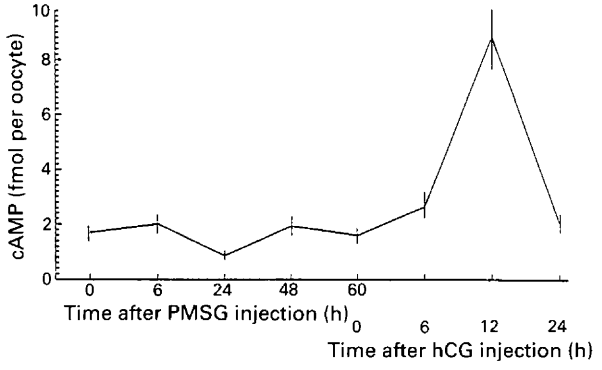

Fig. 1. Cyclic AMP (cAMP) concentrations in pig oocytes throughout the follicular growth induced by pregnant mares' serum gonadotrophin (PMSG) and hCG-triggered oocyte maturation (vertical bars are means ± SEM).

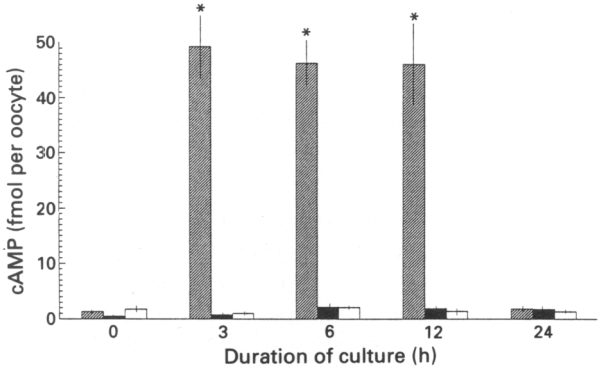

Fig. 2. Cyclic AMP (cAMP) concentrations in (▨) follicle oocytes, (■) cumulus oocytes and (□) denuded oocytes during the first 24 h of maturation *in vitro* in the presence of 1 μg LH ml^{-1}. *Concentrations significantly different from that at time zero ($P < 0.01$, Student's t test).

granulosa cells (the equivalent of seven follicles per dish, about 8×10^6 cells ml^{-1}), with cumulus cells (8×10^6 cells ml^{-1} obtained by repetitive aspiration of cumulus oocytes through the tip of a P200 Gilson pipette), or with seven everted follicles from which mural granulosa cells were removed with the aid of fine watchmaker forceps. Control incubations were carried out with intact everted follicles. In these experiments, the cell preparations were stimulated with LH and with FSH (1 μg USDA-pFSH-B1 ml^{-1}).

The influence of the maturational status of the follicles was assessed by isolating granulosa cells (8×10^6 cells ml^{-1}) from large (6–7 mm in diameter), medium (4–5 mm) or small (2–3 mm) follicles from slaughtered gilts or from large follicles isolated from PMSG-primed gilts and incubating them with denuded oocytes in the presence of LH. Denuded oocytes were obtained from medium–large follicles. Preliminary experiments showed that oocytes isolated from follicles of different sizes have the same ability to produce cAMP when cultured with everted follicles in the presence of LH.

Transient increase in cAMP. As the increase in concentration of cAMP in the oocyte was temporary, experiments were conducted to assess whether this could depend on: (i) decreasing ability of LH-treated follicle cells to stimulate the adenylyl cyclase of the oocyte; or (ii) decreasing ability of the oocyte to respond to the stimulation exerted by LH-treated somatic cells. Since cAMP concentration returned to basal value after 24 h of culture, freshly collected granulosa cells or granulosa cells cultured for 24 h with LH were incubated for 3 h, always in the presence of LH, with freshly collected denuded oocytes or with denuded oocytes that had already been incubated for 24 h with LH-treated granulosa cells. The oocytes were finally processed for cAMP determination.

Effect of dibutyryl cAMP on meiotic maturation

The final series of experiments investigated the influence of cAMP on oocyte maturation. In a preliminary experiment, follicular oocytes were cultured with increasing concentrations of dibutyryl cAMP (db-cAMP, Sigma), to assess any dose-dependent influence on meiotic progression. In a second experiment, groups of 42 follicular oocytes were exposed to dibutyryl-cAMP (1 mmol l^{-1}) for various periods at different phases of maturation.

At the end of each experiment, the oocytes were fixed in acetic alcohol (1:3, v:v) for 48 h and then stained with 1% lacmoid (Sigma) to evaluate their nuclear status at × 400 by phase contrast microscopy.

Radioimmunoassay for cAMP

Intracellular cAMP was measured using a highly specific immunoassay purchased from Amersham International, Amersham, Bucks. The samples (100 μl) corresponding to individual oocytes, were treated with 5 μl of an acetylating mixture (acetic anhydride:triethylamine 1:2, v:v) to increase the sensitivity of the assay (Harper and Brooker, 1975). This parameter was further increased by diluting the antiserum to achieve a final sensitivity (expressed as the amount of unlabelled hormone that causes a 10% inhibition of tracer antiserum binding B:Bo) of 0.57 fmol per tube. The assay has negligible crossreactivity with other nucleotides and in particular with IBMX (< 0.00001%). The intra-assay and interassay precision expressed as the coefficient of variations were 5.6 and 7.1%, respectively (20 determinations). Concentrations of cAMP are expressed as fmol per oocyte.

Statistical analysis

Data are expressed as means ± SEM. The concentrations of cAMP were compared by the Student's t test. χ^2 analysis was used to compare the percentages of oocytes undergoing maturation. Values of $P < 0.01$ were considered significant.

Results

Concentrations of cAMP throughout oocyte maturation in vivo

The content of cAMP in oocytes recovered from follicles of prepubertal gilts was 1.69 ± 0.28 fmol per oocyte (mean ± SEM

Table 1. Cyclic AMP (cAMP) concentrations in cumulus-enclosed, denuded or zona-free oocytes cultured with or without everted follicles in the presence or absence of LH

Treatment	cAMP (fmol per oocyte)						
	Cumulus-enclosed oocytes	Cumulus enclosed oocytes + everted follicles	Denuded oocytes	Denuded oocytes + everted follicles	Zona-free oocytes	Zona-free oocytes + everted follicles	Follicular oocytes
− LH	0.73 ± 0.11^{a} (44)	1.27 ± 0.47^{a} (48)	1.02 ± 0.51^{a} (45)	1.28 ± 0.61^{a} (42)	1.18 ± 0.21^{a} (32)	1.27 ± 0.35^{a} (29)	1.57 ± 0.85^{a} (39)
+ LH	0.87 ± 0.19^{a} (40)	64.59 ± 17.74^{b} (39)	1.52 ± 0.43^{a} (57)	80.73 ± 6.24^{b} (54)	0.97 ± 0.18^{a} (25)	76.83 ± 9.18^{b} (23)	82.31 ± 14.62^{b} (36)

Values are means ± SEM.
Values with different superscripts are significantly different ($P < 0.01$, Student's t test).
Numbers in parentheses indicate the number of oocytes tested.

Table 2. Cyclic AMP (cAMP) concentrations recorded in denuded oocytes after culture for 3 h with mural granulosa cells, theca shells or cumulus cells in the presence of LH or FSH

Treatment	cAMP (fmol per oocyte)		
	Mural granulosa	Theca	Cumulus
− LH	1.62 ± 0.33^{a} (45)	2.06 ± 0.28^{a} (38)	1.07 ± 0.12^{a} (32)
+ LH	52.33 ± 3.21^{c} (35)	34.12 ± 3.53^{b} (38)	1.77 ± 0.35^{a} (33)
+ FSH	1.34 ± 0.12^{a} (38)	2.42 ± 0.39^{a} (32)	2.01 ± 0.52^{a} (28)

Values are means ± SEM.
Values with different superscripts are significantly different ($P < 0.01$, Student's t test).
Numbers in parentheses indicate number of oocytes tested.

Table 3. Cyclic AMP (cAMP) concentrations in freshly collected oocytes or preincubated oocytes (24 h with LH-treated granulosa cells) cultured in the presence of LH for 3 h with freshly collected or preincubated (24 h with LH) granulosa cells

Granulosa cells	cAMP (fmol per oocyte)	
	Fresh oocytes	Preincubated oocytes
Fresh	78.66 ± 10.38^{a} (48)	58.66 ± 14.56^{a} (48)
Preincubated	2.80 ± 0.81^{b} (36)	1.81 ± 0.31^{b} (40)

Values are means ± SEM.
Values with different superscripts are significantly different ($P < 0.01$, Student's t test).
Numbers in parentheses indicate number of replicates.

of 30 oocytes pooled from four gilts). PMSG treatment did not modify these values (Fig. 1). By contrast, a marked and well-defined rise in cAMP was recorded after hCG injection. The increase was already detectable after 6 h and reached values significantly higher than before either PMSG or hCG were administered (8.86 ± 1.15 fmol per oocyte, $n = 36$; five gilts, $t = 6$, $P < 0.01$) 12 h after hCG injection. The intracellular concentration of the cyclic nucleotide was again low 24 h after hCG injection.

Concentrations of cAMP throughout oocyte maturation in vitro

The mean content of cAMP in oocytes maturing *in vitro* was 1.33 ± 0.35 fmol per oocyte ($n = 28$) at the beginning of the culture. In the presence of LH, a sharp rise in cAMP of up to 49.39 ± 5.62 fmol per oocyte ($n = 21$) was recorded in follicle oocytes after 3 h of culture (Fig. 2). The amount of cAMP then progressively reduced and after 24 h of culture it was again at basal values (1.85 ± 0.77 fmol per oocyte, $n = 24$) and remained at this value until the end of maturation.

There was no significant change in the amount of cAMP in follicular oocytes cultured without LH. Cumulus oocytes and denuded oocytes showed constant low amounts throughout maturation, regardless of the presence (Fig. 2) or absence of LH in the tissue culture medium.

Co-culture experiments

Cumulus oocytes, denuded oocytes or zona-free oocytes co-cultured with everted follicles exhibited a marked increase in cAMP, similar to that recorded in follicular oocytes, when LH was added to the culture medium (Table 1).

The influence of the different types of somatic cells – mural granulosa, theca or cumulus cells – on cAMP production by the oocyte is shown (Table 2). The amount of cAMP was significantly higher in denuded oocytes cultured with LH-stimulated granulosa cells (52.33 ± 3.21 fmol per oocyte, $n = 35$), whereas cumulus cells exposed to LH could not increase the concentration of cAMP in denuded oocytes (1.77 ± 0.35, $n = 33$; see Table 2). In addition, LH-stimulated theca cells increased the amount of cAMP in the oocytes (34.12 ± 3.53 fmol per oocyte, $n = 38$; Table 2). Exposure of mural granulosa cells, thecal cells, or cumulus cells to FSH did not influence the content of cAMP in denuded oocytes.

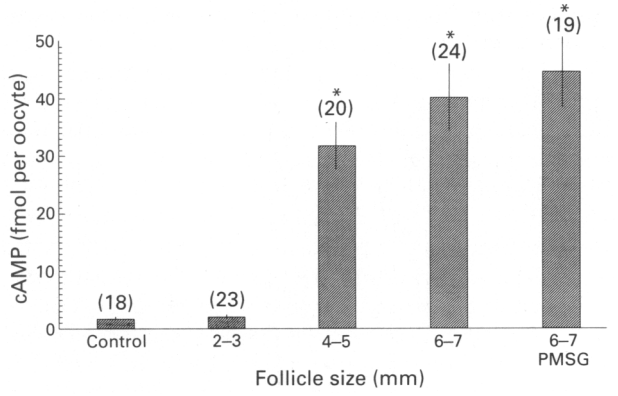

Fig. 3. Cyclic AMP (cAMP) concentrations in denuded oocytes cultured for 3 h without granulosa cells (control) or with granulosa cells (8×10^6 cells ml^{-1}) isolated from follicles of different size obtained from prepubertal gilts and from gilts primed with pregnant mares' serum gonadotrophin (PMSG). The numbers of replicates are given in parentheses. *Concentrations significantly different from the control ($P < 0.01$, Student's t test).

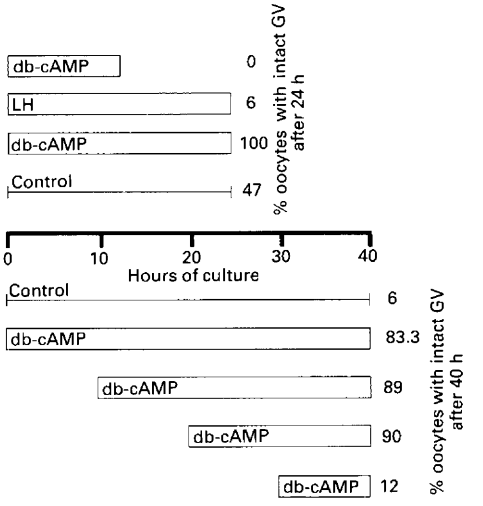

Fig. 4. Effect of dibutyryl cAMP (db-cAMP) (1 mmol l^{-1}) during various periods on the meiotic maturation of follicular oocytes evaluated after 24 and 40 h of culture. GV: germinal vesicle.

Transiency

Granulosa cells completely lost their ability to stimulate cAMP accumulation in denuded oocytes after 24 h of culture with LH (2.80 ± 0.81 fmol per oocyte, $n = 36$) (Table 3). By contrast, denuded oocytes exposed to LH-stimulated granulosa cells for 24 h could still respond to freshly collected and LH-stimulated granulosa cells (58.66 ± 14.56 fmol per oocyte, $n = 48$).

Influence of follicle size on the stimulation of cAMP production by the oocyte

Granulosa cells harvested from large follicles independently of PMSG priming could stimulate cAMP accumulation in the oocyte. This influence progressively decreased as the diameter of the follicle was reduced. Granulosa cells isolated from follicles with a diameter of 2–3 mm could not stimulate cAMP production by the oocyte (Fig. 3).

Effect of cAMP on nuclear maturation in vitro

The addition of dibutyryl cAMP inhibited meiotic resumption in a dose-dependent fashion. Nine per cent (3 of 33), 55% (15 of 27), 83.3% (29 of 35) and 100% (12 of 12) of the oocytes had an intact germinal vesicle after 40 h of culture in the presence of 0.2, 0.5, 1 and 5 mmol dibutyryl cAMP l^{-1}, respectively. The percentage of oocytes with an intact germinal vesicle after 40 h of culture in the absence of any inhibitor was 6% (Fig. 4). The addition of the membrane-permeable nucleotide after 10 or 20 h of culture was as effective in inhibiting meiotic resumption as it was at the beginning of culture (Fig. 4). Oocytes treated with dibutyryl cAMP from 30 h displayed a normal meiotic progression and the percentage of oocytes with a germinal vesicle at the end of the culture (40 h) was similar to that in controls (12% versus 6%). By contrast, treatment with dibutyryl-cAMP during the first 12 h of culture markedly accelerated meiotic progression while after 24 h of culture the percentage of oocytes that had undergone germinal vesicle breakdown was significantly higher than in controls (100% versus 53%, $\chi^2 = 61.4$, $P < 0.01$) and similar to that recorded in LH-treated oocytes (Fig. 4).

Discussion

The results of the experiments demonstrate that pig oocytes induced to mature *in vivo* show a transient increase in the intracellular concentration of cAMP. The concentration returns to basal values at about the time of germinal vesicle breakdown. The patterns of cAMP changes are similar throughout the maturation of follicular oocytes *in vitro*, even if the temporary increase in cAMP is slightly accelerated and of greater amplitude. The reason for this difference is presently unknown. Taken together, these data do not support the hypothesis that a reduction in cAMP concentration within the oocyte is involved in triggering the resumption of meiosis. Apart from this increase, the concentration of the nucleotide 24 h after hCG administration (at the time of germinal vesicle breakdown) is similar to that recorded before hCG injection, when the oocyte is still in a condition of stable meiotic arrest.

Although these findings do not fit the model proposed for small rodent oocytes, in which cAMP is considered the maturation inhibitor, they are consistent with observations made in rabbits (Yoshimura *et al.*, 1992) and in sheep (Moor and Heslop, 1981), in which an increase in cAMP has been observed within the oocyte at the time of the resumption of meiosis. More generally, these results agree with the findings of others who failed to observe a relationship between cAMP concentration and meiotic arrest in sheep and pig oocytes (Crosby *et al.*, 1985; Racowsky, 1985).

The experiments *in vitro* demonstrate that the increase in cAMP could be obtained only when the oocyte, whether enclosed in the cumulus cells or denuded, was maintained in the presence of the follicle wall stimulated with LH. First of all, this observation demonstrates that pig oocytes can synthesize

cAMP. Moreover, since the zona pellucida of denuded oocytes prevents any direct contact with the everted follicle, cAMP production is likely to be stimulated by a soluble factor that originates mainly from mural granulosa cells. As also shown by Crosby *et al.* (1985) for sheep oocytes, this influence is exerted directly on the oocyte and not via the cumulus–corona radiata cells.

Moreover, the experiments *in vitro* demonstrate a functional difference between the cells of the somatic compartment of the follicle. The concentrations of cAMP in the oocyte are clearly influenced by LH-stimulated mural granulosa cells, while they are totally unaffected by cumulus cells. Functional differences between mural granulosa cells and cumulus cells have clearly been shown by Zoller and Weisz (1978) and Peng *et al.* (1991). The absence of response of cAMP production in cumulus oocytes or in denuded oocytes co-cultured with cumulus cells may depend on the extremely low concentration of LH receptors on cumulus cells compared with mural cells (Lawrence *et al.*, 1980). Alternatively, a specific LH transduction system resulting in the production of the previously mentioned stimulating factor may be operative only in mural granulosa cells. Theca shell preparations exhibited an intermediate influence that cannot be attributed to granulosa cell contamination as indicated by preliminary histological examinations.

Both LH and FSH are involved in the regulation of granulosa cell function and their influence sometimes overlaps so that it may be difficult to establish the specific functions of each gonadotrophin. However, in this case the stimulating factor, the chemical nature of which is under investigation, was produced by granulosa cells as a specific response to LH, while FSH did not have any effect. The influence exerted by everted follicles was shown to clearly depend on the maturational status of the follicle. Large follicles collected after PMSG stimulation or isolated from follicles of unknown background, but with a diameter of 6–7 mm, exhibited a similar marked stimulating effect on the production of cAMP by the oocyte, while this influence was lower in medium-size follicles and virtually absent in follicles with a diameter of less than 3 mm. The acquisition of an adequate receptor concentration by the somatic cells or the development of the proper 'mechanism' for the production of the stimulating factor may account for these differences. If we assume that the rise in cAMP represents an intracellular signal of the LH peak, this arrangement might provide a local system that addresses the stimulating influence of the gonadotrophin surge only to oocytes contained in large follicles.

In view of the comparison between oocyte maturation *in vivo* and *in vitro*, it becomes clear that cumulus oocytes cultured under conditions in which oocytes are often matured *in vitro* do not experience the increase in cAMP observed during maturation *in vivo*. Only in the presence of the follicular somatic compartment, derived from medium–large follicles, can the oocyte undergo the same modifications that occur *in vivo*.

The increase in the concentration of cAMP is confined to the first hours of maturation both *in vivo* and *in vitro*. This transient increase in cAMP seems to strictly depend on the progressive desensitization of granulosa cells to LH. This is likely to happen as a result of the downregulation of LH receptors caused by the peak concentration of LH required to trigger oocyte maturation. By contrast, the responsiveness of the oocytes to LH-stimulated follicle cells is maintained unaltered throughout the culture. The presence of high cAMP concentrations during the first part of maturation only is particularly interesting when the physiological significance of this nucleotide is considered. Experiments *in vitro* with dibutyryl cAMP showed that when the concentrations of the nucleotide are high throughout culture, germinal vesicle breakdown is almost totally prevented. This inhibitory influence seems to be exerted shortly before germinal vesicle breakdown, as dibutyryl cAMP added after 20 h of culture could still prevent the resumption of meiosis almost completely. However, from the data *in vivo* and *in vitro* it is clear that these treatment schedules reproduce neither the situation *in vivo* nor *in vitro*. By contrast, when we tried to mimic the LH-induced increase in cAMP by adding the analogue during the first 12 h of culture, a significant acceleration of meiotic progression was achieved. Although much more experimental support is required to validate the hypothesis, on the basis of the results shown here cAMP may be an intracellular signal involved in the complex mechanism leading to the resumption of meiosis.

The transient increase in the concentration of cAMP may also exert a positive influence on the cytoplasmic maturation. In fact, Mattioli *et al.* (1991) have shown that the addition of LH to the culture medium increases the percentage of oocytes matured *in vitro* that can sustain the formation of male pronucleus after fertilization. The analysis of cumulus–oocyte coupling suggests that this effect of LH depends on its stimulation of the intercellular communication between cumulus cells and the oocyte. The increased concentration of cAMP described in this paper is likely to be the LH-induced factor responsible for this increased heterologous coupling. This nucleotide is known to increase the number and the permeability of gap junctions (Loewenstein, 1981) and evidence has been provided (Racowsky, 1985) that forskolin-treated pig oocytes display a transient increase in the degree of intercellular coupling with cumulus cells that parallels the increase in the intracellular concentration of cAMP.

A profound influence of the transient cAMP rise on different aspects of oocyte maturation may therefore be hypothesized. Further studies are required to characterize and define these influences.

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