Induction of meiotic maturation of follicle-enclosed oocytes of rabbits by a transient increase followed by an abrupt decrease in cyclic AMP concentration

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Summary. The involvement of cyclic adenosine monophosphate (cAMP) in mammalian oocyte maturation was assessed using cultures of rabbit cumulus–oocyte complexes and perfused rabbit ovaries. Rabbit cumulus–oocyte complexes were cultured in Brackett's medium with or without forskolin at 10^{-4} , 10^{-5} or 10^{-6} mol l⁻¹ for 3–6 h. At 3 or 4 h spontaneous meiotic maturation was significantly ($P < 0.05$) inhibited by forskolin at 10^{-4} mol l⁻¹. With prolonged incubation, spontaneous maturation progressed despite exposure to forskolin. In the second experiment ovaries were perfused for 12 h with forskolin (10^{-4} , 10^{-5} or 10^{-6} mol l⁻¹) or medium alone. Neither ovulation nor degeneration of follicular oocytes occurred in any perfused ovary. The percentage of follicular oocytes achieving germinal vesicle breakdown was significantly ($P < 0.001$) increased in response to forskolin in a dose-related manner. In an additional experiment, ovaries were perfused with forskolin at 10^{-4} mol l⁻¹. A significant increase in the cAMP content in the follicle was observed within 30 min, but the ability to produce cAMP in response to forskolin decreased as the duration of perfusion was increased. Intraoocyte cAMP increased significantly within 30 min and reached its maximum 2 h after exposure to forskolin. Thereafter, cAMP levels in the oocytes decreased abruptly. This drop in intraoocyte cAMP concentration was followed by the resumption of meiosis. The alterations of intraoocyte cAMP contents following exposure to hCG *in vivo* paralleled those observed in the ovaries perfused with forskolin. These data suggest that a transient, but not continuous, increase in cAMP concentration after the gonadotrophin surge may be required to initiate oocyte maturation.

Keywords: oocyte maturation; cAMP; forskolin; gonadotrophin; rabbit

Introduction

The apparent antagonism between the stimulation by luteinizing hormone (LH) and the inhibition by modulators of cAMP levels in the female gamete is a paradox because a predominant effect of LH is to increase cAMP concentration in preovulatory follicles (Tsafiriri *et al.*, 1972; Marsh, 1976). Membrane-permeable cAMP derivatives or phosphodiesterase inhibitors have been shown to inhibit spontaneous oocyte maturation in mammals (Cho *et al.*, 1974; Magnusson & Hillensjö, 1977; Dekel & Beers, 1978). Oocyte cAMP originates in the cumulus cells and is transferred to the oocyte via the heterologous gap junctions that exist between the two types of cell. Gonadotrophin stimulation that ultimately terminates cumulus–oocyte communication has been proposed to initiate resumption of meiosis by interrupting the direct transfer of cAMP to the oocyte (Dekel &

Beers, 1980). However, a reduction in the intercellular coupling by LH stimulation could not be the trigger to resume meiosis because initiation of meiotic maturation preceded LH-induced termination of the communication (Moor *et al.*, 1981; Eppig, 1982). Increasing the cAMP of the cumulus cell did not result in a detectable rise in oocyte cAMP (Schultz *et al.*, 1983a). A model for the inhibition of meiosis in mice proposed recently that a putative inhibitor of cumulus cell origin is activated by increased cAMP concentration and subsequently passed to the oocyte through the gap junction where it acts to suppress germinal vesicle breakdown (Eppig *et al.*, 1983; Freter & Schultz, 1984).

Forskolin, a cardioactive diterpene isolated from the roots of *Coelus forskohli*, activates adenylate cyclase in broken cell preparations as well as in intact tissues and increases intracellular cAMP concentrations (Seamon & Daly, 1981; Seamon *et al.*, 1981). This diterpene has recently been reported to inhibit the spontaneous maturation of denuded or cumulus-enclosed oocytes in mammals (Schultz *et al.*, 1983b; Dekel *et al.*, 1984; Racowsky, 1984). However, forskolin has been shown to induce meiotic maturation of follicle-enclosed oocytes in rats (Dekel & Sherizly, 1983). Thus, further studies are needed to clarify the role of cAMP in the physiological regulation of mammalian oocytes. The *in vitro* perfused rabbit ovary is an extracorporeal model that allows the intrafollicular environment of the oocyte to be controlled for study (Yoshimura *et al.*, 1985, 1986; Holmes *et al.*, 1986). In this study the ovary preparation perfused *in vitro* was used to examine the effect of forskolin on the meiotic resumption of rabbit oocytes in relation to cAMP accumulation.

Materials and Methods

Animals

Sexually mature Japanese white rabbits were used in all experiments. Animals were isolated for a minimum of 3 weeks before the experimental procedure and were caged individually under controlled light and temperature with free access to Purina Rabbit Chow and water. Rabbits weighing at least 3.5 kg were anaesthetized with intravenous sodium pentobarbital (32 mg kg^{-1} body weight), anticoagulated with heparin sulfate ($120 \text{ units kg}^{-1}$), and then subjected to laparotomy. Mature ovaries of normal appearance were used in the experiments.

Culture of isolated oocytes

A total of 36 ovaries was used in the experiment of *in vitro* maturation of cumulus-enclosed oocytes. Ovaries were removed and placed in Brackett's medium (Brackett & Oliphant, 1975) under a dissecting microscope. Follicles greater than 1.0 mm in diameter were punctured using a 26-gauge needle and the contents of the follicle were aspirated. Cumulus-oocyte complexes of uniform size were selected and rinsed three times in fresh medium. Cumulus-enclosed oocytes were then cultured in Brackett's medium with or without different concentrations of forskolin (Calbiochem Behring, La Jolla, CA) at 37°C and 100% humidity using 5% CO_2 in air for 3–6 h. A stock solution of forskolin was prepared in 95% ethanol. The concentration of ethanol did not exceed $5 \mu\text{l ml}^{-1}$, and an equivalent volume of ethanol was added to the control medium. The maturity of individual oocytes was examined at 3, 4 or 6 h of culture after isolation.

Perfusion system

Each ovarian artery was cannulated *in situ*, and the ovary, the ovarian artery, vein and supportive adipose tissue were then removed from the rabbit and immediately placed in a perfusion chamber. Ovaries were perfused with tissue culture medium 199 (M.A. Bioproducts, Walkerville, MD) supplemented with insulin, heparin sulfate, streptomycin and penicillin. The perfusion technique and surgical procedure, including arterial cannulation and ovarian removal, were previously described in detail (Lambertsen *et al.*, 1976; Yoshimura *et al.*, 1987). The perfusate of one ovary contained forskolin dissolved in 95% ethanol to yield a final concentration of 10^{-4} , 10^{-5} , or $10^{-6} \text{ mol l}^{-1}$. The contralateral ovary served as a control and was perfused in the presence of a corresponding concentration of ethanol (less than 0.2% in the perfusate). Both ovaries were perfused simultaneously for 12 h. Six rabbits were used for each concentration of forskolin. Ovaries were observed periodically for evidence of follicle growth and rupture. At the end of perfusion, mature follicles that were greater than 1.5 mm in diameter were punctured and aspirated to recover follicular oocytes for microscopic evaluation. Perfusate samples (3 ml) were withdrawn at 0, 1, 2, 4, 6, 8 and 12 h after the onset of perfusion.

Examinations of oocytes

After culture and perfusion of isolated oocytes the oocytes were fixed and stained with 1% lacmoid in 45% acetic acid for microscopy. Oocytes were assessed for stage of maturity and signs of degeneration as described previously (Yoshimura *et al.*, 1990). The degree of oocyte maturity was expressed as the percentage of oocytes that had achieved germinal vesicle breakdown. Oocytes were also assessed for any degenerative change such as vacuolation, cytolysis, necrosis, cytoplasmic flocculation, karyorrhexis or disintegration.

Cyclic AMP alterations in the follicle and oocyte

In the perfused rabbit ovaries, intrafollicular cAMP accumulated to its maximum 1 h after exposure to forskolin. Ovaries were perfused with or without forskolin at a concentration of 10^{-3} to 10^{-7} mol l^{-1} for 1 h to determine the effect of forskolin on cAMP formation in the follicle. Cyclic AMP was measured in mature follicles greater than 1.5 mm in diameter after 1 h of perfusion. The effect of forskolin on the intrafollicular cAMP concentration was examined using a total of 48 ovaries. In the subsequent experiment, the time-dependent accumulation of cAMP in the follicles and oocytes was determined in response to forskolin treatment using a total of 268 ovaries. Increases in the concentrations of cAMP in follicles and oocytes induced by forskolin at 10^{-4} mol l^{-1} were examined at the onset of perfusion and at 0.5, 1, 2, 4, 6, 8, and 12 h after the onset of perfusion. The alteration of cAMP content in the follicles derived from forskolin-treated ovaries was also compared with that in ovaries perfused with 50 iu of human chorionic gonadotrophin (hCG). In the final experiment using a total of 92 rabbits, 100 iu of hCG was administered intravenously to determine a series of intraoocyte cAMP contents after the induction of maturation *in vivo*. The relationship between the intraoocyte cAMP concentrations and the percentage of oocytes achieving germinal vesicle breakdown after exposure to hCG *in vivo* was compared with that in ovaries perfused with forskolin at 10^{-4} mol l^{-1} .

Cyclic AMP determinations

The cAMP contents of the follicles and oocytes in ovaries perfused with forskolin or in hCG-treated ovaries were determined using the Yamasa cyclic nucleotide assay kit (Yamasa Shoyu Co., Ltd, Chiba, Japan) as previously described (Kunitada *et al.*, 1978; Oda *et al.*, 1990). The follicles were excised and frozen immediately in liquid nitrogen. The sample was stored in liquid nitrogen until cAMP was measured by radioimmunoassay. The follicular tissue was homogenized with 1 ml of cold 6% trichloroacetic acid (TCA) using a glass homogenizer. After centrifugation, the TCA extract was washed four times with ether that was saturated with water. The supernatants were removed and dried down under a stream of air in a water-bath at 75°C. The cyclic nucleotide isolated from tissues (0.1 ml) was succinylated by adding 0.1 ml of dioxane which contained succinic anhydride and triethylamine. Samples were then mixed with 0.1 ml of ^{125}I -labelled succinyl cAMP tyrosine methyl ester and 0.1 ml of diluted anti-cAMP antiserum. Two different aliquots of each dissolved ovarian extract were measured in duplicate. The sensitivity of this assay was 10 fmol per tube.

At the end of the perfusions of various duration after exposure to hCG *in vivo*, the cumulus-enclosed oocytes were collected in a minimum volume of medium 199 containing 0.1 mmol 3-isobutyl-1-methylxanthine l^{-1} (IBMX, Sigma Chemical Co., St Louis, MO). Cumulus cells were removed from oocytes with a solution of 0.5 mg hyaluronidase ml^{-1} and gentle agitation with a narrow-bore pipette. Ten to twenty oocytes were transferred into polypropylene tubes in 20 μl of 1 mol HCl l^{-1} . Repeated freezing and thawing (three times) of the oocytes in liquid nitrogen was used to break the membranes and release the cAMP content into the HCl solution. A similar volume of HCl solution without the oocytes was treated to obtain control values for intraoocyte cAMP determinations. Each sample was succinylated by adding 20 μl of dioxane that contained succinic anhydride and triethylamine. After 10 min at room temperature the mixture was diluted with 60 μl of imidazole buffer. These solutions served as the samples of intraoocyte cAMP determination for radioimmunoassay. The sensitivity of this assay was 1 fmol per tube.

Statistical analysis

Data for the maturity of cumulus-enclosed oocytes and the concentrations of progesterone and cAMP are presented as the mean $\pm$ SEM. Statistical significance was determined using analysis of variance (ANOVA). The χ squared test with Yates' correction was used to assess per cent degeneration and per cent germinal vesicle breakdown of follicular oocytes.

Results

Oocyte maturation in cultured cumulus-enclosed oocytes

The nuclear structure of cumulus-enclosed oocytes of rabbits began to disappear after 3–4 h of incubation, indicating resumption of meiosis. By 6 h of incubation, most of the oocytes cultured in

control medium (93.8%) showed evidence of meiotic resumption with germinal vesicle breakdown. In contrast, the exposure of cumulus-enclosed oocytes to forskolin prevented the resumption of meiosis (Fig. 1). Furthermore, forskolin inhibited spontaneous oocyte maturation in a dose-dependent manner. At 4 h of incubation, meiotic arrest was observed in 68.8% and 48.1% of cumulus-enclosed oocytes in the presence of forskolin at 10^{-4} mol l^{-1} and 10^{-5} mol l^{-1} , respectively. However, this inhibition was attenuated as the duration of incubation increased. By 6 h of incubation, 83.8% of the oocytes had resumed meiosis despite the presence of 10^{-4} mol l^{-1} forskolin. No difference was observed in the percentage of follicular oocytes displaying germinal vesicle breakdown when forskolin-treated and control groups were compared after incubation for 6 h at each dose.

Fig. 1. Effect of forskolin on the spontaneous maturation of cumulus-enclosed oocytes. Isolated cumulus-enclosed oocytes were cultured with or without forskolin. Oocytes were examined after 3, 4 and 6 h of incubation. Data are expressed as the mean $\pm$ SEM of at least six different experiments. Values with the same letters are significantly different: ^{a,b} $P < 0.005$; ^c $P < 0.01$; ^d $P < 0.02$; ^e $P < 0.05$.

Oocyte maturation in the perfused rabbit ovary

Ovulation did not occur in rabbit ovaries during 12 h of perfusion with forskolin at concentrations of 10^{-4} , 10^{-5} or 10^{-6} mol l^{-1} in the absence of gonadotrophin stimulation. The percentage of follicular oocytes achieving germinal vesicle breakdown was significantly ($P < 0.001$) increased in response to forskolin at a concentration of 10^{-4} mol l^{-1} compared with controls (83.8% germinal vesicle breakdown with forskolin; 5.6% germinal vesicle breakdown in controls). Furthermore, forskolin at a concentration of 10^{-5} or 10^{-6} mol l^{-1} also significantly increased the meiotic maturation of follicular oocytes. Forskolin enhanced the meiotic maturation of follicle-enclosed oocytes in a dose-dependent manner (Fig. 2). Although the percentage of follicular oocytes that showed evidence of degeneration was increased in forskolin-treated ovaries

at all concentrations, compared with each group of control ovaries, these differences were not significant (forskolin 10^{-4} mol l^{-1} 18.9%, 10^{-5} mol l^{-1} 25.0%, 10^{-6} mol l^{-1} 17.6%, control 15.2%).

Fig. 2. Percentage of follicular oocytes achieving germinal vesicle breakdown after 12 h of perfusion with medium alone or with forskolin at a concentration of 10^{-4} mol l^{-1} , 10^{-5} mol l^{-1} or 10^{-6} mol l^{-1} . Data are the mean of six different ovaries from at least four experiments. Values with asterisks differ significantly from control ovaries perfused with medium alone: * $P < 0.005$; ** $P < 0.001$.

Cyclic AMP content of follicles

A significant increase in the cAMP content of the follicle was observed in the forskolin-stimulated perfused ovary. Treatment with forskolin enhanced cAMP accumulation in the follicle in a dose-dependent manner (Fig. 3). The maximal effective dose of forskolin was attained at a concentration of 10^{-4} mol l^{-1} . Forskolin at a concentration of 10^{-4} mol l^{-1} in the perfusate increased the cAMP concentration in follicular tissue within 30 min (Fig. 4). Cyclic AMP accumulation reached a maximum 1 h after exposure to forskolin. A decrease in the ability of the rabbit follicles to respond to forskolin *in vitro* was observed as early as 2 h after the onset of perfusion. At 4 h after exposure to forskolin, the perfused rabbit follicles could generate only half of the maximum concentration of cAMP. The concentration of follicular cAMP returned to the basal concentration observed in unstimulated ovaries by 12 h of perfusion. Thus, the production of cAMP in response to forskolin was attenuated as the duration of perfusion increased. Similarly, addition of hCG to the perfusate significantly increased the intrafollicular cAMP level. However, the concentration of intrafollicular cAMP differed significantly between forskolin-treated and hCG-treated ovaries at 0.5, 1, 2 and 4 h of perfusion.

Relationship between cAMP content and meiotic maturation of oocytes

Meiotic maturation of the follicle-enclosed oocytes was initiated 4 h after exposure to forskolin at 10^{-4} mol l^{-1} (Fig. 5). The stimulation of germinal vesicle breakdown in follicle-enclosed oocytes reached its plateau after 8 h of perfusion. The intracellular content of cAMP in oocytes derived from unstimulated ovarian follicles was 0.36 ± 0.08 fmol per oocyte. The intraoocyte cAMP content increased significantly within 30 min and reached its maximum (4.9 ± 1.3 fmol per oocyte) 2 h

Fig. 3. Effect of forskolin on intrafollicular cAMP content. Ovaries were perfused with or without forskolin. Cyclic AMP levels in mature follicles greater than 1.5 mm in diameter were determined after 1 h of perfusion. Values are expressed as the mean $\pm$ SEM. Six ovaries were studied for each dose of forskolin.

Fig. 4. Changes in cAMP content of follicles during 12 h of perfusion. Ovaries were perfused with forskolin at a concentration of 10^{-4} mol l⁻¹ (●—●) or with 50 iu of human chorionic gonadotrophin (hCG) (○—○). At least four ovaries were examined at each interval for each treatment. Values are expressed as the mean $\pm$ SEM.

after exposure to 10^{-4} mol forskolin l⁻¹. Thereafter, the cAMP content of oocytes decreased abruptly and returned to the pretreatment value 6 h after forskolin administration. This fall in the intraoocyte cAMP content of follicle-enclosed oocytes was followed by their meiotic maturation. After exposure to hCG *in vivo*, the meiotic maturation of oocytes in the follicles was detected initially within 2 h (Fig. 6). Although the time course of oocyte maturation in the follicles exposed to hCG *in vivo* was earlier than that in ovaries perfused with forskolin, the percentage of oocytes achieving germinal vesicle breakdown did not differ significantly between the two groups at each

time point after exposure to the stimulus. Furthermore, the alterations of intraoocyte cAMP concentrations after exposure to hCG *in vivo* paralleled those observed in the ovaries perfused with forskolin.

Fig. 5. Relationship between the alteration of intraoocyte cAMP content and meiotic maturation of follicle-enclosed oocytes in ovaries perfused with forskolin. Ovaries were perfused with 10^{-4} mol l $^{-1}$ forskolin. The maturity of oocytes and intraoocyte cAMP content were examined at various times after perfusion of ovaries. The results are presented as mean $\pm$ SEM of at least six replicates of three different experiments. Values in parentheses represent the numbers of oocytes analysed.

Fig. 6. Relationship between changes in intraoocyte cAMP content and meiotic maturation of follicle-enclosed oocytes in ovaries exposed to human chorionic gonadotrophin (hCG) *in vivo*. The maturity of oocytes and intraoocyte cAMP content were examined at various times after administration of hCG (100 iu) *in vivo*. The results are presented as means $\pm$ SEM of at least six replicates of three different experiments. Values in parentheses represent the numbers of oocytes analysed.

Discussion

Data derived from culture of cumulus-enclosed oocytes with forskolin are consistent with a previous report that dibutyryl cAMP inhibits the spontaneous maturation of rabbit oocytes (Hosoi *et al.*, 1989). However, most cumulus-enclosed oocytes cultured with forskolin resumed meiosis as the incubation time was increased. Treatment of oocytes with forskolin resulted in a transient and reversible block of the resumption of meiosis *in vitro*. Since a decrease in cAMP in oocytes is known to correlate with the resumption of meiosis (Schultz *et al.*, 1983b), an alternative explanation would be that the cAMP content of cumulus-enclosed oocytes may decrease with increasing time of incubation. These observations suggest that cAMP may play a significant role in the intrafollicular arrest of oocyte meiosis in rabbits as it does in other mammalian oocytes (Cho *et al.*, 1974; Dekel & Beers, 1978; Freter & Schultz, 1984; Racowsky, 1985). However, the inhibition by cAMP of oocyte maturation has been demonstrated only with data from experiments studying the spontaneous maturation of cumulus-enclosed and denuded oocytes (Cho *et al.*, 1974; Magnusson & Hillensjö, 1977; Dekel & Beers, 1978, 1980; Freter & Schultz, 1984; Aberdam *et al.*, 1987).

Dekel *et al.* (1988) recently showed that cAMP has a dual role in regulating oocyte maturation. Lower concentrations of the nucleotide act to maintain meiotic arrest whereas higher concentrations mediate gonadotrophin-induced resumption of meiosis. In the follicle, however, the possibility that a transient rise in cAMP is necessary for initiation of meiotic maturation of follicle-enclosed oocytes has previously been reported (Ekholm *et al.*, 1984; Hubbard, 1985). Forskolin, which activates adenylate cyclase, has been shown to mimic the effects of gonadotrophin on the follicle, stimulating both cAMP accumulation and oocyte maturation in rats and hamsters (Dekel & Sherizly, 1983; Hubbard, 1985). Although spontaneously maturing oocytes serve as useful models for studying oocyte maturation, there is evidence suggesting that follicle-enclosed oocytes behave in a physiologically different manner from cumulus-enclosed and denuded oocytes when stimulated by LH *in vitro* (Moor *et al.*, 1981; Ekholm *et al.*, 1984; Hubbard, 1985). Conclusions regarding the role of cAMP drawn from data in experiments that study the spontaneous maturation of cumulus-enclosed and denuded oocytes may require critical evaluation for the physiological regulation of maturation of mammalian oocytes.

The perfused rabbit ovary has been used to evaluate the effects of specific phenomena associated with oocyte maturation while the microenvironment of oocytes is controlled (Yoshimura *et al.*, 1985, 1986; Hosoi *et al.*, 1989). Exposure of the perfused rabbit ovary to forskolin accelerated the resumption of meiosis in follicle-enclosed oocytes in proportion to the accumulation of cAMP in the preovulatory follicles. These results are supported by the observation that forskolin can induce maturation of follicle-enclosed oocytes in rats (Dekel & Sherizly, 1983; Ekholm *et al.*, 1984). We demonstrated earlier that exposure to dibutyryl cAMP for 2 h in the two-step perfusion model significantly accelerated meiotic maturation in a dose-related manner. However, this stimulatory effect was not observed when follicle-enclosed oocytes were continuously exposed to dibutyryl cAMP during 12 h of perfusion or cultured with medium alone in the absence of dibutyryl cAMP (Hosoi *et al.*, 1989). The initiation of meiotic maturation could be observed only after removing dibutyryl cAMP from the perfusate, thus avoiding the inhibition by continuous exposure to dibutyryl cAMP. These data imply that high, sustained concentrations of cAMP are responsible for the maintenance of meiotic arrest but exposure to high level of cAMP is a prerequisite for initiation of meiotic maturation.

Forskolin increased cAMP formation of preovulatory rabbit follicles. The increase in both intrafollicular and intraoocyte cAMP concentrations within 30 min of exposure to forskolin was followed by a rapid decline at 4 h. The fall in the intraoocyte cAMP concentration resulted in the initiation of meiotic maturation of follicle-enclosed oocytes. The similar relationships between the intraoocyte cAMP concentrations and the percentage of oocytes achieving germinal vesicle breakdown were observed in ovaries perfused with forskolin *in vitro* and in ovaries exposed to hCG *in*

vivo. These data clearly demonstrated that forskolin can mimic the effects of hCG, stimulating both the intracellular cAMP content and the meiotic maturation of follicle-enclosed oocytes. All doses of forskolin that stimulated intrafollicular cAMP synthesis also accelerated the resumption of meiosis. The ability of forskolin to accelerate the resumption of meiosis in a dose-dependent manner suggests that increased cAMP concentrations may be a signal for the resumption of meiosis rather than being responsible for intrafollicular arrest. Gonadotrophin-induced maturation in follicle-enclosed oocytes may be a cAMP-mediated phenomenon.

The results of this study may be applied to resolve the paradoxical observation that cAMP inhibits the spontaneous maturation of cumulus-enclosed oocytes, whereas the gonadotrophin surge stimulates both the formation of cAMP in preovulatory follicles and the resumption of meiosis in follicle-enclosed oocytes. Continuously high cAMP concentrations inhibit oocyte maturation in both cumulus-enclosed and follicle-enclosed oocytes. The ability to accelerate the resumption of meiosis is not observed when oocytes, regardless of whether they are enclosed by cumulus or follicles, are continuously exposed to high cAMP concentration. In contrast, transient exposure of the preovulatory follicles to a high cAMP concentration accelerates the resumption of meiosis in follicle-enclosed oocytes. The initiation of meiotic maturation can be observed when the cAMP concentration in the intrafollicular environment is reduced abruptly after a transient increase. The inhibition of cumulus-enclosed oocytes by forskolin is reversible and most oocytes achieve germinal vesicle breakdown after incubation for 6 h. Alternatively, increases in both intrafollicular and intraoocyte cAMP concentrations may be an essential prerequisite for the resumption of meiosis during the process of ovulation.

In conclusion, we found that forskolin postponed the spontaneous maturation *in vitro* of cultured cumulus-enclosed oocytes in rabbits in a similar way to that observed in other mammals. In perfused rabbit ovaries, doses of forskolin that stimulate intrafollicular cAMP synthesis also accelerate the resumption of meiosis. Furthermore, forskolin can mimic the effects of gonadotrophin, stimulating both the intracellular cAMP content and meiotic maturation of follicle-enclosed oocytes. These data provide evidence that changes in cAMP concentrations that follow the gonadotrophin surge play a significant role in the resumption of meiosis in follicle-enclosed oocytes. A transiently increased intrafollicular cAMP concentration may be an essential prerequisite for meiotic maturation during ovulation.

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