# Nuclear Maturation and Cortical Granule Distribution in Porcine Oocytes Treated with IBMX and dbcAMP

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**Summary**

We formerly reported in porcine oocytes that cortical granules (CGs) move to the cytoplasm immediately beneath the plasma membrane as nuclear maturation progresses, and that the movement of CGs and the resumption of meiotic division do not occur in those treated with olomoucine. Therefore, we suggested that CGs distribution in the cytoplasm is closely associated with nuclear maturation. However, whether olomoucine directly acts on the cytoplasm to inhibit such change in CGs distribution could not be determined. In the present investigation, the distribution of CGs was lectin-histochemically observed in porcine oocytes treated with IBMX or dbcAMP, which suppresses the nuclear maturation with the mechanism different from olomoucine, and also in porcine oocytes treated with olomoucine and dbcAMP, in order to examine the relationship between nuclear maturation and the distribution of CGs in the cytoplasm.

Nuclei of the oocytes cultured for 22 hrs with IBMX, dbcAMP, or olomoucine and dbcAMP were all in the germinal vesicle (GV) stage. The percentages of oocytes in the GV stage were significantly higher in the treated oocytes than in control oocytes. CGs in the oocytes treated with IBMX, dbcAMP, or olomoucine and dbcAMP were distributed in the cortical cytoplasm and also immediately beneath the plasma membrane. The distribution of CGs in such the treated oocytes did not differ from those of control oocytes. From these results, it was suggested that the changes in the distribution of CGs in the cytoplasm with oocyte maturation depend on the cAMP level in their cytoplasm rather than the progression of nuclear maturation.

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**Key words** : porcine oocyte, cAMP, distribution of cortical granule, lectin- histochemistry, nuclear maturation

It is reported that together with the nuclear maturation of oocytes, various changes occur in the cytoplasm. In porcine oocytes, changes in the distribution of cortical granules (CGs) with maturation have been studied under electron microscopy (Cran and Cheng, 1985; Yoshida *et al*., 1993) and histochemically with lectin (Yoshida *et al.*, 1993; Wang *et al*., 1997a; 1997b). CGs are distributed over the cortical cytoplasm in the germinal vesicle (GV)-stage oocyte collected from antral follicles, and the CGs move to the cytoplasm immediately beneath the plasma membrane as maturation progresses (Cran and Cheng, 1985; Yoshida *et al*., 1993; Wang *et al.*, 1997a; 1997b). In oocytes collected from antral follicles 20 hrs (Cran and Cheng, 1985) or 24 to 36 hrs (Yoshida *et al.*, 1993) after hCG injection and those cultured for 24 (Yoshida *et al.*, 1993) or 26 hrs (Wang *et al.*, 1997a; 1997b), most CGs are observed immediately beneath the plasma membrane.

We have also observed the nuclear maturation and the distribution of CGs in porcine oocytes, and inferred that the distribution of CGs altered almost in parallel with nuclear maturation, and CGs moved to the cytoplasm immediately beneath the plasma membrane in the metaphase Ⅰ (MⅠ) stage (Takano and Niimura, 2002). Furthermore, we have observed the distribution of CGs in porcine oocytes treated with 2-(2-hydroxyethylamino)-6-benzylamino-9-methylpurine (olomoucine), which was known to be an inhibitor of the activity of p34cdc2, a cyclin dependent kinase of MPF (Vesely *et al*., 1994; Abraham *et al.*, 1995), in order to determine the relationship between nuclear maturation and change in CG distribution in the cytoplasm. The resumption of nuclear maturation was completely inhibited in olomoucine-treated oocytes, and the movement of CGs to the cytoplasm immediately beneath the plasma membrane was also inhibited. Therefore, it was suggested that CGs distribution in the cytoplasm is closely associated with nuclear maturation (Takano and Niimura, 2002). However, whether olomoucine directly acts on the cytoplasm to inhibit such change in CGs could not be determined.

It is generally known that cAMP synthesized in cumulus cells by the stimulation of LH flows into the cytoplasm of oocytes through gap junctions between cumulus cells and oocytes, and plays some important roles in oocyte maturation (Petr *et al.*, 1991; Mattioli *et al.,* 1994; Funahashi *et al.*, 1997; Shimada and Terada, 2002; Shimada *et al*., 2002). We have reported that the amount of cAMP in porcine cumulus-oocytecomplexes (COCs) treated with olomoucine is significantly lower than that in non-treated COCs, and that the amount of cAMP in denuded oocytes (DOs) is similar between olomoucine-treated and non-treated ones (Takano and Niimura, 2004). Therefore, the reason for no change in CGs distribution in oocytes treated with olomoucine is thought to be the low cAMP level in their cytoplasm.

On the other hand, 3-isobutyl-1-methylxanthine (IBMX) is

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known to be an inhibitor of cAMP phosphodiesterase, which metabolizes cAMP to 5'-AMP, and has the effect to maintain cAMP at a high level in the cytoplasm (Shimada *et al*., 2002; Fan *et al*., 2002). It has been confirmed that maintaining the cAMP at a high level by IBMX treatment results in the suppression of resumption of maturation division in mammalian oocytes (Bilodeau *et al*., 1993; Tsafriri *et al*., 1996; Magnusson and Hillensjö, 1997; Sun *et al*., 1999; Hegele-Hartung *et al*., 2001). In addition, when cAMP level in the cytoplasm was maintained at a high level in oocytes treated with dibutyryl cAMP (dbcAMP), an analogue of cAMP, the rates of oocytes whose GVs had broken down significantly decreased (Petr *et al.,* 1991; Mattioli *et al.,* 1994; Funahashi *et al.,* 1997). From these facts, it is considered that there is a close relationship between nuclear maturation and cAMP level in the cytoplasm (Petr *et al*., 1991; Mattioli *et al*., 1994; Funahashi *et al*., 1997; Shimada and Terada, 2002).

In the present investigation, the distribution of CGs was lectin- histochemically observed in porcine oocytes treated with IBMX or dbcAMP, which suppresses the nuclear maturation with the mechanism different from olomoucine, and also in porcine oocytes treated with olomoucine and dbcAMP, in order to clarify the relationship between nuclear maturation and the distribution of CGs in the cytoplasm.

## MATERIALS AND METHODS

### Collection of COCs

Ovaries were obtained from prepubertal gilts at a local slaughterhouse and transported to the laboratory in 0.9 % NaCl solution maintained at 39 ℃. The ovaries were washed in 0.9 % NaCl solution containing 200 i.u./ml potassium penicillin G. COCs were aspirated from medium-sized follicles (3-6 mm in diameter) with a 21-gauge needle fixed to a 10-ml disposable syringe. Collected COCs were washed in PBS (pH 7.4) (Dulbecco and Vogt, 1954) and then in a culture medium composed of TCM-199 (Gibco BRL., NY, USA) supplemented with 10 % (v/v) porcine follicular fluid, 10 % (v/v) fetal calf serum (FCS; Gibco BRL.), 10 i.u./ml eCG (Serotropin; Teikoku Hormone Manufacturing Co. Ltd., Tokyo, Japan), 10 i.u./ml hCG (Gonatropin; Teikoku Hormone Manufacturing Co. Ltd.) and 0.001 % (w/v) estradiol-17$\beta$ (Wako Pure Chemical Industries, Osaka, Japan) (Yoshida *et al*., 1990).

### Observation of CG distribution and nuclear maturation in oocytes treated with IBMX or dbcAMP

Collected COCs were cultured for 22 hrs at 39 ℃ in the culture medium containing 500$\mu$M IBMX (Sigma Chemical Co., MO, USA) or 2.0 mM dbcAMP (Sigma Chemical Co.). IBMX was previously dissolved in dimethyl sulfoxide (DMSO) and then diluted with the culture medium up to 500$\mu$M. The concentration of DMSO in the culture medium was adjusted to 0.1 % (v/v), and COCs cultured for 22 hrs in the medium containing DMSO at 0.1 % or in the medium with no dbcAMP were used as controls.

After culture, cumulus cells were dispersed from the oocytes by pipetting in PBS containing 0.1 % hyaluronidase (Sigma Chemical Co.). In order to observe the distribution of CGs, the DOs were stained with PNA conjugated with FITC (E-Y Lab., CA, USA) according to the method of Ducibella et al. (1988). The DOs were immersed in PBS containing 0.2 % pronase (Sigma Chemical Co.) to dissolve the zonae pellucidae. The naked oocytes were washed in PBS and fixed in PBS containing 3.7 % paraformaldehyde for 30 min at room temperature. They were then washed 3 times in a blocking solution composed of 3 mg BSA (Sigma Chemical Co.), 7.51 mg glycine (Wako Pure Chemical Industries) and 1 ml PBS, immersed in PBS containing 0.1 % Triton X-100 (Nacalai Tesque, Kyoto, Japan) for 5 min at 20 ℃, and again placed in the blocking solution. These oocytes were finally immersed in a staining solution composed of 100$\mu$g PNA, 0.1 % Triton X-100, 3 mg BSA and 1 ml PBS for 30 min at 20 ℃. The stained oocytes were thoroughly washed in PBS containing 0.01 % Triton X-100 and 0.3 % BSA, and were placed in the center of four vaseline spots on a slide. A coverslip was then carefully placed on the vaseline spots and pressed gently to anchor the oocyte between the coverslip and the slide. Observation was carried out under an epifluorescence microscope (OPTIPHOT-2, Nikon, Tokyo, Japan). Degenerated oocytes were eliminated from the observation. In order to ensure the inhibition of resumption of meiotic division by the treatment of IBMX or dbcAMP, oocytes in which CGs had been observed were fixed in 25 % (v/v) acetic acid in ethanol for 48 hrs at room temperature, stained with 1.0 % aceto-orcein and then examined under a light microscope.

To determine the viability of oocytes treated with IBMX or dbcAMP, progression of nuclear maturation was also observed in those further cultured for 22 to 44 hrs in the medium without IBMX and dbcAMP.

### Observation of CG distribution and nuclear maturation in oocytes treated with olomoucine and dbcAMP

In order to investigate the distribution of CGs in oocytes in which resumption of meiotic division was inhibited and the high cAMP level in the cytoplasm was maintained, COCs collected from antral follicles were cultured for 22 hrs at 39 ℃ in the culture medium containing 400$\mu$M olomoucine (Sigma Chemical Co.) and 2.0 mM dbcAMP (Sigma Chemical Co.). Olomoucine was previously dissolved in DMSO and then diluted with the culture medium up to 400$\mu$M. The concentration of DMSO in the culture medium was adjusted to 0.37 % (v/v), and oocytes cultured for 22 hrs in the medium containing DMSO at 0.37 % were used as vehicle controls.

After culture, lectin-histochemical demonstration of CGs was performed by the same method as mentioned above. In order to ensure the inhibition of resumption of meiotic division by the treatment of olomoucine and dbcAMP, oocytes in which CGs had been observed were fixed in 25 % (v/v) acetic acid in ethanol for 48 hrs at room temperature, stained with 1.0 % aceto-orcein and then examined under a light microscope.

To determine the viability of the oocytes treated with olomoucine and dbcAMP, progression of nuclear maturation was observed in those further cultured for 22 to 44 h in the medium without olomoucine and dbcAMP.

**Statistical analysis**

The rates concerning nuclear maturation of cultured oocytes and the number of oocytes showing each CG distribution were statistically analyzed by Chi-square test.

## RESULTS

**CG distribution and nuclear maturation in oocytes treated with IBMX or dbcAMP**

When porcine oocytes cultured with IBMX or dbcAMP, and those cultured without IBMX and dbcAMP were stained with FITC-conjugated PNA, fluorescent granules showing the presence of CGs appeared in their cytoplasm. Distribution patterns of CGs differed among oocytes and could be classified into 3 types, as in previous our report (Takano and Niimura, 2002). In type Ⅰ, CGs were distributed over the cortical cytoplasm. In type Ⅱ, CGs were distributed in the cortical cytoplasm and also immediately beneath the plasma membrane. CGs in oocytes of type Ⅲ were all densely distributed just beneath the plasma membrane.

The changes in CG distribution and nuclei of porcine oocytes treated with IBMX or dbcAMP are shown in **Table 1**. Of oocytes cultured for 22 hrs in the medium containing IBMX or dbcAMP, 7 (5/72) and 7 % (5/75) showed the type Ⅰ distribution of CGs, and the remaining 93 % were all classified as types Ⅱ and Ⅲ. The percentages of control oocytes showing the types Ⅱ and Ⅲ distribution of CGs were 93 (78/84) and 95 % (70/74), showing no differences from those of IBMX-treated and dbcAMP-treated oocytes.

Of oocytes cultured for 22 hrs in the IBMX-free medium and in the dbcAMP-free medium, 3 (3/84) and 7 % (5/74) were in the GV stage, respectively, and the remaining 97 and 93 % were in the diakinesis to M Ⅱ stages, mostly in the M Ⅰ stage (75 and 70 %). On the other hand, all nuclei of the oocytes treated with IBMX or dbcAMP were in the GV stage. The percentages of oocytes in the GV stage were significantly higher in both the treated oocytes than in control oocytes ($P<0.05$). Therefore, it is confirmed that treatments of IBMX and dbcAMP are able to inhibit the resumption of nuclear maturation in porcine oocytes.

When the oocytes treated with IBMX or dbcAMP were further cultured for 22 hrs in the medium without IBMX and dbcAMP, most nuclei were in the M Ⅱ stage (90 %, 36/40 and 78 %, 28/36), suggesting that the ability of maturation in both IBMX-treated and dbcAMP-treated oocytes was sustained.

**CG distribution and nuclear maturation in oocytes treated with olomoucine and dbcAMP**

The changes in CG distribution and nuclei of porcine oocytes treated with olomoucine and dbcAMP are shown in **Table 2**. Of oocytes cultured for 22 hrs in the medium containing olomoucine and dbcAMP, only 1 % (1/77) showed the type Ⅰ distribution of CGs, and the remaining 99 % were all classified as types Ⅱ and Ⅲ. The percentage of control oocytes showing the types Ⅱ and Ⅲ distribution of CGs was 94% (74/79), showing no difference from that of the oocytes treated with olomoucine and dbcAMP.

Of oocytes cultured for 22 hrs in the medium without olomoucine and dbcAMP, 4 % (3/79) were in the GV stage, and the remaining 96 % were in the diakinesis to M Ⅱ stages, mostly in the M Ⅰ stage (76 %). On the other hand, all nuclei of the oocytes treated with olomoucine and dbcAMP were in the GV stage. The percentage of oocytes in the GV stage was significantly higher in the treated oocytes than in control oocytes ($P<0.05$). Therefore, it is confirmed that treatments of olomoucine and dbcAMP are able to inhibit the resumption of nuclear maturation in porcine oocytes.

When the oocytes treated with olomoucine and dbcAMP were further cultured for 44 hrs in the medium without olomoucine and dbcAMP, most nuclei were in the M Ⅱ stage (82 %, 28/34), suggesting that the ability of maturation in the treated oocytes was sustained.

## DISCUSSION

As previously mentioned, we confirmed in porcine

**Table 1**. The distribution of CGs and nuclei of porcine oocytes cultured with IBMX or dbcAMP

| Treatments | No. of oocytes examined | No. and (%) of oocytes with different types* of CG distribution | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ⅰ | | | | Ⅱ | | | | | Ⅲ | | | | |
| | | GV | Dia | M Ⅰ | Total | GV | Dia | M Ⅰ | M Ⅱ | Total | GV | Dia | M Ⅰ | M Ⅱ | Total |
| None | 84 | 1(1)# | 4(5) | 1(1) | 6(7)a | 2( 2) | 9(11) | 12(14) | 1(1) | 24(29)a | 0( 0) | 1(1) | 50(60) | 3(4) | 54(64)a |
| IBMX | 72 | 5(7) | 0(0) | 0(0) | 5(7)a | 30(42) | 0( 0) | 0( 0) | 0(0) | 30(42)a | 37(51) | 0(0) | 0( 0) | 0(0) | 37(51)a |
| None | 74 | 3(4)# | 1(1) | 0(0) | 4(5)a | 2( 3) | 6( 8) | 18(24) | 2(3) | 28(38)a | 0( 0) | 3(4) | 34(46) | 5(7) | 42(57)a |
| dbcAMP | 75 | 5(7) | 0(0) | 0(0) | 5(7)a | 33(44) | 0( 0) | 0( 0) | 0(0) | 33(44)a | 37(49) | 0(0) | 0( 0) | 0(0) | 37(49)a |

The oocytes were observed after 22 hrs of culture.

* Type Ⅰ : CGs were distributed over the cortical cytoplasm.
Type Ⅱ : CGs were distributed over the cortical cytoplasm and also immediately beneath the plasma membrane.
Type Ⅲ : CGs were distributed immediately beneath the plasma membrane.

GV : Germinal vesicle, Dia: diakinesis, M Ⅰ : metaphase Ⅰ, M Ⅱ : metaphase Ⅱ.

# The number of oocytes with percentages in parentheses.

Values with different superscripts in the same column in each experimental lot are significantly different ($P<0.05$).

**Table 2.** The distribution of CGs and nuclei of porcine oocytes cultured with olomoucine and dbcAMP

| Treatments | No. of oocytes examined | No. and (%) of oocytes with different types* of CG distribution | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ⅰ | | | | Ⅱ | | | | | Ⅲ | | | | |
| | | GV | Dia | M Ⅰ | Total | GV | Dia | M Ⅰ | M Ⅱ | Total | GV | Dia | M Ⅰ | M Ⅱ | Total |
| None | 84 | 1(1)# | 4(5) | 1(1) | 6(7)a | 2( 2) | 9(11) | 12(14) | 1(1) | 24(29)a | 0( 0) | 1(1) | 50(60) | 3(4) | 54(64)a |
| Olomoucine and dbcAMP | 77 | 1(1)# | 0(0) | 0(0) | 1(1)a | 34(44) | 0( 0) | 0( 0) | 0(0) | 34(44)a | 42(55) | 0(0) | 0( 0) | 0(0) | 42(55)a |

The oocytes were observed after 22 hrs of culture.

* Type Ⅰ : CGs were distributed over the cortical cytoplasm.
Type Ⅱ : CGs were distributed over the cortical cytoplasm and also immediately beneath the plasma membrane.
Type Ⅲ : CGs were distributed immediately beneath the plasma membrane.

GV: Germinal vesicle, Dia: diakinesis, M Ⅰ : metaphase Ⅰ, M Ⅱ : metaphase Ⅱ.

# The number of oocytes with percentages in parentheses.

Values with different superscripts in the same column are significantly different ($P < 0.05$).

oocytes that the start and the completion of CG movement into the cytoplasm immediately beneath the plasma membrane are closely related to the time at which GV breaks down and nuclei reach the M Ⅰ stage, and that the movement of CGs does not occur in those treated with olomoucine (Takano and Niimura, 2002). From these results, we suggested that the change in the distribution of CGs in the cytoplasm is associated with progression of nuclear maturation. However, whether olomoucine directly acts on the cytoplasm to inhibit the movement of CGs could not be determined. Also, the distribution of CGs in oocytes in which resumption of meiotic division is inhibited by maintaining the high cAMP level in the cytoplasm has not yet been clarified.

In the present investigation, we attempted to observe the distribution of CGs, using oocytes treated with IBMX or dbcAMP, which suppresses the nuclear maturation with the mechanism different from olomoucine, to determine the relationship between nuclear maturation and CGs distribution in the cytoplasm. The resumption of nuclear maturation was completely inhibited in the treated oocytes, whereas the movement of CGs to the cytoplasm immediately beneath the plasma membrane was not inhibited. It was also clarified that the resumption of nuclear maturation was completely inhibited in porcine oocytes treated with olomoucine and dbcAMP, while the movement of CGs was not inhibited. From these results, it was suggested that the change in CGs distribution in the cytoplasm with oocyte maturation depends on the cAMP level in their cytoplasm rather than the progression of nuclear maturation. It was also suggested that the high cAMP level in the cytoplasm is related to not only inhibition of nuclear maturation but also progression of cytoplasmic maturation.

It is generally known that cAMP synthesized in cumulus cells by the stimulation of LH flows into the cytoplasm of oocytes through gap junctions between cumulus cells and oocytes, and plays some important roles in oocyte maturation (Petr *et al.,* 1991; Mattioli *et al.*, 1994; Funahashi *et al.,* 1997; Shimada and Terada, 2002; Shimada *et al.,* 2002). It has been reported that the amount of cAMP in porcine COCs treated with olomoucine is significantly lower than that in non-treated COCs, and that the amount of cAMP in DOs is similar between olomoucine-treated and non-treated ones (Takano and Niimura, 2004). From these facts, we have suggested that cAMP contained in COCs is mostly derived from cumulus cells, and that synthesis of cAMP in cumulus cells and transfer of cAMP from cumulus cells to oocytes are inhibited by the treatment of olomoucine (Takano and Niimura, 2004). Therefore, we consider that the reason for no change in the distribution of CGs in olomoucine-treated oocytes is low cAMP level in their cytoplasm, and that the cAMP level is the important factor for the distribution of CGs in the cytoplasm of oocytes.

Since microfilaments are involved in the dynamics of CGs in porcine oocytes (Sun *et al*., 2001) and it was determined in the present study that GVBD was inhibited in the treated porcine oocytes with high cAMP level in the cytoplasm, while the movement of CGs to the cytoplasm immediately beneath the plasma membrane was not inhibited in those oocytes, cAMP is thought to be related not only suppression of GVBD in oocytes but also the dynamics of microfilaments in their cytoplasm. On the other hand, it has been reported that protein kinase A plays a role in bundling of actin filaments in the cytoplasm and its activity depends on cAMP (Glenn and Jacobson, 2002). Therefore, high cAMP level in the cytoplasm of treated oocytes is considered to be associated with the dynamics of microfilaments. However, the results of the present study do not provide the information needed to identify the relationship between cAMP and the dynamics of microfilaments in their cytoplasm. This issue should be further studied.

## REFERENCES

Abraham, R. T., M. Acquarone, A. Andersen, A. Asensi, R. Belle, F. Berger, C. Bergounioux, G. Burnn, C. Buquet-

Fagot, D. Fagot, N. Glab, H. Goudeau, M. Goudeau, P. Guerrier, P. Houghton, H. Hendriks, B. Kloareg, M. Lippai, D. Marie, B. Maro, L. Meijer, J. Mester, O. Mulner-Lorillon, S. A. Poulet, E. Schierenberg, B. Schutte, D. Vaulot and M. H. Verlhac. 1995. Cellular effects of olomoucine, an inhibitor of cyclin-dependent kinases. *Biol. Cell*., **83** : 105-120.

Bilodeau, S., M. A. Fortier and M. A. Sirard. 1993. Effect of adenylate cyclase stimulation on meiotic resumption and cyclic AMP content of zona-free and cumulus-enclosed bovine oocytes *in vitro*. *J. Reprod. Fert*., **97** : 5-11.

Cran, D. G. and W. T. K. Cheng. 1985. Changes in cortical granules during porcine oocyte maturation. *Gamete Res*., **11** : 311-319.

Ducibella, T., E. Anderson, D. F. Albertini, J. Aalberg and S. Rangarajan. 1988. Quantitative studies of changes in cortical granule number and distribution in the mouse oocyte during meiotic maturation. *Dev. Biol*., **130** : 184-197.

Dulbecco, R. and M. Vogt. 1954. Plaque formation and isolation of pure lines with poliomyelitis viruses. *J. Exp. Med*., **99** : 167-174.

Fan, H. Y., M. Y. Li, C. Tong, D. Y. Chen, G. L. Xia, X. F. Song, H. Schatten and Q. Y. Sun. 2002. Inhibitory effects of cAMP and protein kinase C on meiotic maturation and MAP kinase phosphorylation in porcine oocytes. *Mol. Repeod. Dev*., **63** : 480-487.

Funahashi, H., T. C. Cantley and B. N. Day. 1997. Synchronization of meiosis in porcine oocytes by exposure to dibutyryl cyclic adenosine monophosphate improves developmental competence following *in vitro* fertilization. *Biol. Reprod*., **57** : 49-53.

Glenn, H. L. and B. S. Jacobson. 2002. Arachidonic acid signaling to the cytoskeleton: the role of cyclooxygenase and cyclic AMP-dependent protein kinase in actin bundling. *Cell. Motil. Cytoskel*., **53** : 239-250.

Hegele-Hartung, C., M. Grützner, M. Lessl, C. Grøndahl, J. L. Ottesen and M. Brännström. 2001. Activation of meiotic maturation in rat oocytes after treatment with follicular fluid meiosis-activating sterol *in vitro and ex vivo*. *Biol. Reprod*., **64** : 418-424.

Magnusson, C. and T. Hillensjö. 1977. Inhibition of maturation and metabolism in rat oocytes by cyclic AMP. *J. Exp. Zool*., **201** : 139-147.

Mattioli, M., G. Galeati, B. Barboni and E. Seren. 1994. Concentration of cyclic AMP during the maturation of pig oocytes *in vivo* and *in vitro*. *J. Reprod. Fert*., **100** : 401-409.

Petr, J., L. Zetová and J. Fulka. 1991. Influence of dbcAMP on the inhibitory effect of cumulus cell factor(s). *Reprod. Nutr. Dev*., **31** : 135-140.

Shimada, M., N. Samizo, Y. Yamashita, K. Matsuo and T. Terada. 2002. Both $Ca^{2+}$-protein kinase C pathway and cAMP-protein kinase A pathway are involved in progesterone production in FSH- and LH-stimulated cumulus cells during *in vitro* maturation of porcine oocytes. *J. Mamm. Ova Res*., **19** : 81-88.

Shimada, M. and T. Terada. 2002. Role of cAMP in regulation of both MAP kinase and $p34^{cdc2}$ kinase activity during meiotic progression, especially beyond the M I stage. *Mol. Reprod. Dev*., **62** : 124-131.

Sun, Q. Y., L. Lai, K. W. Park, B. Kühholzer, R. S. Prather and H. Schatten. 2001. Dynamic events are differently mediated by microfilaments, microtubules, and mitogen-activated protein kinase during porcine oocyte maturation and fertilization *in vitro*. *Biol. Reprod*., **64** : 879-889.

Sun, Q. Y., Q. Lu, H. Breitbart and D. Y. Chen. 1999. cAMP inhibits mitogen-activated protein (MAP) kinase activation and resumption of meiosis, but exerts no effects after spontaneous germinal vesicle breakdown (GVBD) in mouse oocytes. *Reprod. Fert. Dev*., **11** : 81-86.

Takano, H. and S. Niimura. 2002. The relationship between nuclear maturation and cortical granule distribution in cultured porcine oocytes. *J. Mamm. Ova Res*., **19** : 21-25.

Takano, H. and S. Niimura. 2004. The amont of cAMP in porcine oocytes cultured with olomoucine. *Bull. Facul. Agric. Niigata Univ*., **57** : 15-20.

Tsafriri, A., S. Y. Chun, R. Zhang, A. J. Hsueh and M. Conti. 1996. Oocyte maturation involves compartmentalization and opposing changes of cAMP levels in follicular somatic and germ cells: studies using selective phosphodiesterase inhibitors. *Dev. Biol*., **178** : 393-402.

Vesely, J., L. Havlicek, M. Strnad, J. J. Blow, A. Donella-Deana, L. Pinna, D. S. Letham, J. Kato, L. Detivaud, S. Leclerc and L. Meijer. 1994. Inhibition of cyclin-dependent kinases by purine analogues. *Eur. J. Biochem*., **224** : 771-786.

Wang, W. H., M. Hosoe and Y. Shioya. 1997a. Induction of cortical granule exocytosis of pig oocytes by spermatozoa during meiotic maturation. *J. Reprod. Fert*., **109** : 247-255.

Wang, W. H., Q. Y. Sun, M. Hosoe, Y. Shioya and B. N. Day. 1997b. Quantified analysis of cortical granule distribution and exocytosis of porcine oocytes during meiotic maturation and activation. *Biol. Reprod*., **56** : 1376-1382.

Yoshida, M., D. G. Cran and V. G. Persel. 1993. Confocal and fluorescence microscopic study using lectins of the distribution of cortical granules during the maturation and fertilization of pig oocytes. *Mol. Reprod. Dev*., **36** : 462-468.

Yoshida, M., Y. Ishizaki and H. Kawagishi. 1990. Blastocyst formation by pig embryos resulting from *in-vitro* fertilization of oocytes matured *in vitro*. *J. Reprod. Fert*., **88** : 1-8.

# IBMXおよびdbcAMPで処置したブタ卵母細胞における核の成熟と表層粒の分布

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（平成19年12月3日受付）


## 要　約

以前我々は、ブタ卵母細胞において、表層粒は核の成熟に伴って細胞膜直下の細胞質に移動するとともに、核の成熟をオロモウシンで処置して阻止した卵母細胞ではこのような表層粒の分布変化はみられなかったことから、表層粒の分布変化と核の成熟とは密接に関係していることを推察して報告した。しかし、オロモウシンが表層粒の分布変化に直接関係しているのか否かは明らかにできなかった。そこで、オロモウシンとは異なった機構、すなわち細胞質のcAMPレベルを高く維持することによって核の成熟を抑制する作用のあるIBMXとdbcAMPを用いて、ブタの卵母細胞を処置し、表層粒の分布変化をレクチン組織化学的に観察した。

IBMX、dbcAMPあるいはオロモウシンとdbcAMPで22時間処置した卵母細胞において、核はすべてが卵核胞期にあり、これらの処置により成熟分裂が抑制されていることが確認された。一方、IBMX、dbcAMPあるいはオロモウシンとdbcAMPで22時間処置した卵母細胞において、表層粒は、細胞質の表層および細胞膜直下の細胞質に分布しており、対照の卵母細胞における分布と相違なかった。以上の結果から、細胞質における表層粒の分布は、核の成熟の進行よりも、細胞質のcAMPの量に依存して変化することが推察された。


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