# 植物因子によるマウス卵母細胞の成熟とブタ精子の運動性の抑制


| | |
|---|---|
| 誌名 | Japanese journal of veterinary science |
| ISSN | 00215295 |
| 巻/号 | 515 |
| 掲載ページ | p. 1045-1047 |
| 発行年月 | 1989年10月 |

# Inhibition of Mouse Oocyte Maturation and Porcine Sperm Motility by a Plant Compound

Eimei SATO
*Department of Animal Science, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan*
(Received 31 March 1989/Accepted 19 June 1989)

*Jpn. J. Vet. Sci.* 51(5): 1045–1047, 1989

KEY WORDS: oocyte maturation, sperm motility.

In most mammals, oogonia enter prophase of the first meiotic division and are arrested at the dictyate stage [8]. At the end phase of its growth period, each oocyte contains a single large nucleus designated as the germinal vesicle (GV) [3]. Upon ovulation resumption of meiosis occurs manifested by germinal vesicle breakdown (GVBD) [3, 8]. In mammals GVBD occurs spontaneously when oocytes are isolated from mature follicles and culture *in vitro* [14, 15]. The occurrence of spontaneous GVBD can be blocked by the addition of derivatized cyclic adenosine 3':5'-monophosphate (cAMP) or compounds that elevate intracellular cAMP levels [4, 5, 17]. These findings suggest that the meiotic arrest of oocytes is regulated by intraoocyte cAMP.

In addition to influencing meiosis, cAMP may play a role in sperm motility. During epididymal transit, sperm acquire the property of motility which is dependent upon the availability of cAMP [1, 2, 11, 12]. Moreover, phosphodiesterase inhibitors added *in vitro* prolong sperm motility by preventing breakdown of cAMP [1, 2, 12].

Recently, a novel nucleotide related to cAMP was isolated from higher plants [20]. This compound inhibits beef heart 3':5'-cyclic nucleotide phosphodiesterase and cAMP-dependent protein kinase [20, 21]. The physico-chemical characteristics of this compound is not that of plant lectins which induce the agglutination of ejaculated spermatozoa [13, 20]. Evidences will be presented showing that this plant compound is a potent inhibitor of mouse oocyte maturation *in vitro* by acting synergistically with dibutyryl cAMP (dbcAMP). In addition it was found to depress motility of porcine sperm motility *in vitro*.

The plant compound was prepared from the pith cells of *Nicotiana tabacum* as described by Wood *et al.* [21]. In summary the pith cells were extracted with 70% ethanol. The ethanol was removed under reduced pressure. The aqueous fraction was dialyzed against distilled water. The dialysate was fractionated by chromatography on a Dowex 1-x8 column by elution with 500 m*l* each of 0.005 N NCl, 0.01 N NCl and 750 m*l* of 0.1 N HCl. The active principle was eluted with 0.1 N HCl. The 0.1 N HCl fraction was lyophilized, resuspended in distilled water, neutralized and lyophilized again. The lyophilized product was assayed for inhibition of oocyte maturation and sperm motility.

The procedure for determining GVBD in mouse oocytes was described in previous reports [16, 17]. Oocytes were recovered from ovaries of prepubertal mice (ICR strain), suspended in modified KRB culture medium and placed in an incubator continuously flushed with an atmosphere of 95% air and 5% $CO_2$ [17].

Ejaculated boar (Landrace) spermatozoa were from the sperm-rich fraction of ejaculates collected manually with a dummy sow and filtrated through gauze. The seminal plasma was removed after centrifugation at 900 × g for 10 min. The spermatozoa were then washed twice at 25°C with Tyrode's solution, pH 7.4, and suspended in Tyrode's medium to a final concentration of $6\times10^4$ sperm/m*l*. The sperm suspensions were incubated at 37°C, and motility was assessed by visual examination under a phase-contrast microscope (×200).

The fraction eluted with 0.1 N HCl inhibited GVBD of mouse oocytes at concentrations of 0.5 to 2.5 mg/m*l* (Fig. 1). After the 3rd hour of incubation, 75.2% of control oocytes had undergone GVBD. The plant compound alone prevented GVBD in 14.3, 27.3 and 38.6% of the oocytes at concentrations of 0.5, 1.0 and 2.5 mg/m*l*, respectively. The inhibition was potentiated by dbcAMP at 20 $\mu$M (Fig. 1) although dbcAMP at this concentration had a slight inhibitory effect (15.2%).

The plant compound also inhibited motility of porcine sperm. In the control about 90% of spermatozoa remained motile after washing with Tyrode's solution. The percent of actively motile spermatozoa declined slightly during the 10 min incubation period to about 80%. The plant compound added to the culture medium at a final

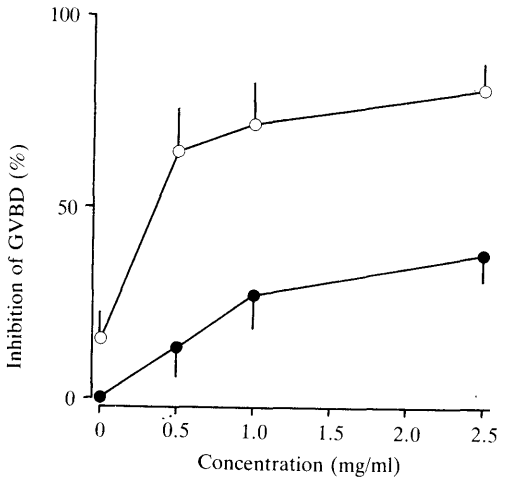


Fig. 1. Inhibition of the spontaneous dissolution of germinal vesicle of cumulus-free mouse oocytes in culture by the plant factor at varying concentrations. (○—○) with 20 $\mu$M dbcAMP; (●—●) without dbcAMP. Values are mean ± SE of triplicate determinations from two or more separate experiments. The percent inhibition of GVBD was calculated according to the following formula:

$$\% \text{ inhibition} = \frac{\% \text{ oocyte GVBD(cont.)} - \% \text{ oocyte GVBD(expt.)}}{\% \text{ oocyte GVBD(cont.)}} \times 100$$

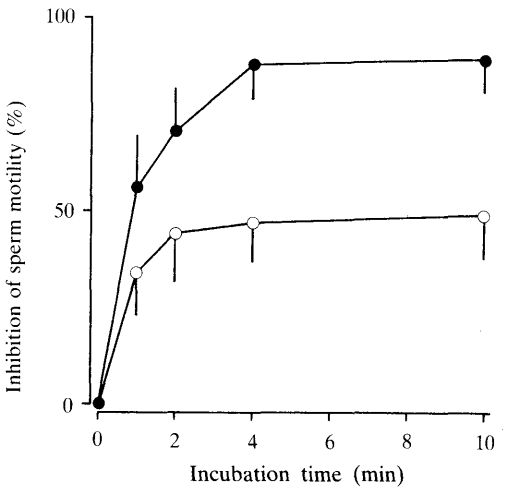


Fig. 2. Inhibition of porcine sperm motility by the plant factor. Sperm suspensions were incubated at 37°C. The concentrations of the plant factor were 1 mg/m*l* (○—○) and 2.5 mg/m*l* (●—●). Motility was assessed at the designated times for 10 min by light microscopy. Each point is the mean±SE determined with 4 or 5 ejaculated semen samples. The percent inhibition was calculated according to the following formula:

$$\% \text{ inhibition} = \frac{\% \text{ motile sperm(cont.)} - \% \text{ motile sperm(expt.)}}{\% \text{ motile sperm(cont.)}} \times 100$$

concentration of 1.0 and 2.5 mg/m*l* inhibited sperm motility by 48 and 89%, respectively, after 10 min of incubation (Fig. 2).

Oocytes isolated from mature follicles possess a large GV arrested at the dictyate stage of meiosis which will subsequently undergo dissolution when cultured *in vitro* [3]. The arrest of meiosis has been attributed to the intracellular content of cyclic nucleotides [5, 17]. This hypothesis is based on the finding that derivatized cAMP, e.g. dbcAMP or compounds that elevate intracellular cAMP, e.g., forskolin, cholera toxin, theophylline, 3-isobutyl-1-methylxanthine (IBMX), inhibit spontaneous maturation of mouse oocytes cultured *in vitro*. The present study clearly demonstrated that this plant compound inhibits mouse oocyte maturation. The physico-chemical characteristics of the cAMP-like compound identified in higher plants is similar to that of cyclic adenosine 3':5'-pyrophosphate (cAPP) [20]. We have shown that synthetic cAPP inhibits mouse oocyte maturation in combination with dbcAMP, and that a substance related to cAPP is present in bovine follicular fluid [18]. These findings indicate that compounds of this type are not restricted to the plant kingdom and occur in animal tissues and may act as a physiological regulator of cellular metabolism [18]. Porcine follicular fluid contains an inhibitor of mouse oocyte maturation which was identified as hypoxanthine [6, 7]. It acts synergistically with cAMP. The plant compound is not hypoxanthine [12].

Mammalian sperm acquire motility during their passage through the epididymis [11]. The organization of the motility apparatus is associated with the induction of protein kinase activity and accumulation of cAMP [12]. All of the components of the cyclic nucleotide system, *i.e.* adenylate cyclase, cAMP phosphodiesterase, cAMP-dependent protein kinase and phosphoprotein phosphatase, have been shown to be present and functional in the epididymal sperm of mammals [2, 9, 10, 19]. These enzymes may be essential for sperm motility. The addition of phosphodiesterase inhibitors to the medium failed to initiate motility in immobile sperm obtained from the caput epididymis [11].

Seminal plasma contains a factor influencing

sperm motility. This factor induces forward progression of sperm and increases intracellular cAMP [11]. This forward motility protein of seminal plasma in combination with phosphodiesterase inhibitors converts the ineffectual, flagellar activity exhibited by sperm from the caput epididymis to the forward progression pattern typical of caudal and ejaculated sperm [1]. The forward motility protein alone can not initiate motility, and requires a phosphodiesterase inhibitor to potentiate its action, indicating that cAMP is an obligatory metabolite for sperm maturation and motility. Thus, both components, cAMP and the forward motility protein, are required for the coordinated operation of the motility apparatus [1]. Since the plant factor inhibits phosphodiesterase activity, it should increase cAMP level and promote sperm motility. On the contrary, the plant factor blocked sperm motility. This sperm immobilizing effect may be related to its ability to block protein kinase activity [21]. The present findings supports the thesis that protein phosphorylation plays a role in sperm motility. The biochemical basis for the inhibition of sperm motility by the plant factor is under study.

ACKNOWLEDGEMENTS. The present study was supported in part by grant No. GA PS 8734 from the Rockefeller Foundation, U.S.A.

REFERENCES

1. Acott, T. S. and Hoskins, D. D. 1978. *J. Biol. Chem.* 253: 6744–6750.
2. Cascieri, M., Amann, P., and Hammerstedt, R. H. 1976. *J. Biol. Chem.* 251: 787–793.
3. Channing, C. P., Schaerf, F. W., Anderson, L. D., and Tsafriri, A. 1980. *Inter. Rev. Physiol.* 3: 117–201.
4. Cho, W. K., Stern, S., and Biggers, J. D. 1974. *J. Exp. Zool* 187: 383–386.
5. Dekel, N. and Beers, W. H. 1978. *Proc. Natl. Acad. Sci. U.S.A.* 75: 4369–4373.
6. Downs, S. M., Coleman, D. L., Ward-Bailey, P. F., and Eppig, J. J. 1985. *Proc. Natl. Acad. Sci. U.S.A.* 82: 454–458.
7. Downs, S. M. and Eppig, J. J. 1984. *Endocrinology* 114: 418–427.
8. Franchi, L. L., Mandle, A. M., and Zuckermann, I. S. 1962. pp. 1–88. *In*: The Ovary (Zuckerman, I. S. ed.), Academic Press, New York.
9. Gray, J. P., Drummond, G. I., Hardman, J. G., and Sutherland, E. W. 1976. *Arch. Biochem. Biophys.* 172: 20–30.
10. Hoskins, D. D., Casillas, E. R., and Stephens, D. T. 1972. *Biochem. Biophys. Res. Commun.* 48: 1331–1338.
11. Hoskins, D. D., Hall, M. L., and Munsterman, D. 1975. *Biol. Reprod.* 13: 168–176.
12. Hoskins, D. D., Stephens, D. T., and Hall, M. L. 1974. *J. Reprod. Fert.* 37: 131–133.
13. Nicolson, G. L., Usui, N., Yanagimachi, R., Yanagimachi, H., and Smith, J. R. 1977. *J. Cell Biol.* 74: 950–962.
14. Pincus, G. and Enzmann, E. V. 1935. *J. Exp. Med.* 62: 655–675.
15. Sato, E., Ishibashi, T., and Iritani, A. 1982. *Adv. Exp. Med. Biol.* 147: 161–173.
16. Sato, E. and Koide, S. S. 1984. *Differentiation* 26: 59–62.
17. Sato, E. and Koide, S. S. 1984.*J. Exp. Zool.* 230: 125–129.
18. Sato, E., Wood, H. N., Lynn, D. G., and Koide, S. S. 1985.*Cell Differ.* 17: 169–174.
19. Tang, F. Y. and Hoskins, D. D. 1975. *Biochem. Biophys. Res. Commun.* 62: 328–335.
20. Wood, H. N. and Lynn, D. G. 1981. *Differentiation* 18: 51–53.
21. Wood, H. N., Pomerantz, A. H., Binns, A. N., Allfrey, V. G., and Braun, A. C. 1978. *Proc. Natl. Acad. Sci. U.S.A.* 75: 4301–4305.

要　約

植物因子によるマウス卵母細胞の成熟とブタ精子の運動性の抑制(短報)：佐藤英明(京都大学農学部)——タバコ(*Nicotiana tabacum*)の髄からエタノールで抽出し，ダウエックス(*Dowex 1-x8*)カラムにかけ，0.1N 塩酸で溶出する分画を得たが，本分画にマウス卵母細胞の体外培養下での卵核胞崩壊やブタ精子の運動性を抑制する作用を認めた．また本分画の抑制作用はサイクリック AMP 誘導体の共存下で顕著に増強し，cAMP と共通の作用機序をもつと推察された．