

nicotine

The effects of nicotine on the mouse oocyte maturation in vitro

K.C. Sung, I.H. Bae

Department of Biology, College of Natural Sciences,
Sungshin Women's University, Seoul, Korea.

Objective: The present study was done to clarify the effects of nicotine and nicotine tartrate on the mouse oocyte maturation in vitro.

Method: GV(germinal vesicle) oocytes were isolated from Graafian follicle of ovaries with sharp needles under a stereomicroscope from female mouse of ICR strain(4weeks old).

Collected oocytes were cultured for 17 hours at 37 °C, 5% CO₂ in air and 100% humidified condition in incubator. New MHBS was the basic medium used in which nicotine, nicotine tartrate, and mecamlamine(antagonist of nicotinic acetylcholine receptor) were added depending on the experimental group. GV oocytes were cultured in one of these media.

Result: Nicotine(300 µM-5mM) had no effects on GVBD(germinal vesicle breakdown) compared to the control, but increasing concentration of nicotine led to an decrease in the first polar body formation. However, nicotine(10-500 µM) induced GVBD in a dose- dependent manner of GV oocytes in a medium containing dbcAMP.

Nicotine tartrate(50 µM-5mM) had no effects on GVBD compared to the control but, increasing concentration of nicotine tartrate led to an decrease in the first polar body formation.

Mecamlamine 10 µM added to the medium containing nicotine(300 µM-5mM) showed higher percentage of the first polar body formation compared to the nicotine(300 µM-5mM) treatment group.

Mecamlamine 10 µM added to the medium containing nicotine tartrate(50 µM-5mM) showed higher percentage of the first polar body formation compared to the nicotine tartrate(50 µM-5mM) treatment group.

Conclusion: The present study suggest that nicotine and nicotine tartrate have the harmful effects on the meiotic maturation of the mouse oocytes in vitro. However,

mecamylamine block harmful effects of nicotine and nicotine tartrate.

Key Words: Nicotine, Oocyte maturation, Nicotine tartrate

, (CO), nicotine, radioactive polonium, benzopyrene, dimethylbenzanthracene, dimethylnitromine, methylnaphthalene 4,000

가 , 2~4 , 5 , 6,7 , 8,9 , 10,11 , 12 (birth weight) 가 가 가 가 가 가 가 가 가 가 nicotine tar가 1970 가 nicotine 가 Hudson Timiras¹³ ,¹³ Card Mitchell¹⁴ (implantation) .¹⁴ Hammer Mitchell¹⁵ (embryo growth) , (implantation) (parturition) .¹⁵ Navarro ¹⁶ nicotine (nicotinic receptor)가 가 .¹⁶ , Maritz^{17,18} nicotine (placenta) . Pekarsky ¹⁹ nicotine 가 Balling Beier²⁰ (preimplantation embryo) nicotine DNA , DiCarlantonio Talbot²¹

nicotine nicotine tartrate가
 , nicotinic acetylcholine
 nicotine mecamylamine nicotine, nicotine tartrate
 , nicotine Ca^{2+} 가
 dbcAMP nicotine
 .
 1.
 14 , 10
 Swiss albino ICR
 4
 ,
 (Wild M5A, Swiss) (26G needle)
 mouth
 controlled micropipette (germinal vesicle; GV)
 22
 2.
 microdroplet (60 × 15mm, Falcon) 30 μ
 equilibrated mineral oil(light oil, Sigma)
 (Forma Scientific, Model 3130) 2 , 20-25
 Nicotine nicotine ethyl alcohol mineral oil
 4 well 가 (4-well multidish, Nunclon, Denmark)
 well 300 μ l 2 ,
 20-25 open culture , 4 well 가 3-4ml
 37 , 5% CO_2
 95% 가 100% 가
 3.

Modified Hanks' Balanced Salt Solution²²(MHBS)
 280mOsm New MHBS
 . NaCl; 98.7mM, KCl; 5.3655mM, MgSO₄;
 0.8118mM, Na₂HPO₄; 0.3358mM, KH₂PO₄; 0.1533mM, glucose; 5.551mM, phenol red;
 10mg/l, penicillin-G; 100units/ml, streptomycin; 52mg/l Milli-Q(Millipore
 SA-67120, France) 10 stock solution , Na-Lactate(Sigma),
 Na-pyruvate(Sigma) CaCl₂(Sigma) 100 stock solution
 . NaHCO₃(Sigma) 0.4% bovine
 serum albumin(BSA, Sigma) , stock solution
 1 . pH 7.3-7.4
 280-290mOsm²³ .
 dbcAMP dPBS(Dulbecco's phosphate-buffered saline) 20 stock solution
 (-20) , 가 0.2mM
 .
 nicotine(C₁₀H₁₄N₂, M.W. 162.2, Sigma) ethyl alcohol(Carlo Erba,
 Italy) 300 stock solution , nicotine tartrate(C₁₀H₁₄N₂
 2C₄H₆O₆, M.W. 462.4, Sigma) Milli-Q 100 stock solution
 . Nicotinic acetylcholine (antagonist)
 mecamlamine(C₁₁H₂₁N HCl, M.W. 203.8, Sigma) Milli-Q 300
 stock solution .

4.
 (immature oocyte) 4 (germinal
 vesicle breakdown; GVBD) 17 1 (first polar body)
 (Interference contrast microscope, Labovet, Leitz. Germany)
 1 .
 (germinal vesicle; GV) ,
 (GVBD) . 1 (first polar body)
 , (degenerated oocyte)
 (condensed oocyte) (fragmentation) .
 0.4% PVP(polyvinylpyrrolidone, Sigma)가
 dPBS washing 0.4% PVP 1% glutaraldehyde가 bisbenzamide solution
 (Hoechst 33342, 100µg/ml, Sigma) , dPBS washing
 mounting IX 70 (Olympus, Japan) UV filter
 .

5.

1
 1
 spss/pc⁺(version 3.0) student's t-test

1. nicotine
 17
 (New MHBS) nicotine 4
 4 nicotine
 13 1
 , nicotine

(1) Nicotine

nicotine 300 μ M, 500 μ M, 1mM, 5mM 4
 Table 1 Nicotine 300 μ M, 500 μ M, 1mM, 5mM

(2) Nicotine

1
 4 nicotine 300 μ M
 M, 500 μ M, 1mM, 5mM 13 Table 2
 300 μ M 1 80.6% 79.6%
 1mM 5mM 1 nicotine 가 가 57.1, 16.1%
 (P<0.001), 500 μ M 91.3% 1
 500 μ M nicotine 가 가
 가 (shrinkage) (Fig. 3), Hoechst
 (pyknotic chromosome) (Fig. 4). , 5mM 39%
 (P<0.001).

2. dbcAMP

nicotine
 dbcAMP
 GV(germinal vesicle) nicotine
 dbcAMP
 dbcAMP nicotine

Table 3 nicotine 10, 100, 500 μ M 4
 nicotine 가 가 0% 10.5, 23.5, 26.7%
 (P<0.001).

17 10, 100, 500 μ M 1 nicotine 가 가
 0% 9.5, 21.1, 25% (P<0.001), 1mM

1 가 , 5% .

3. nicotine tartrate
Nicotine nicotine tartrate가
nicotine tartrate
4 17 1
50 μ M, 500 μ M, 5mM nicotine tartrate 17
Table 4 .
4 , 50 μ M, 500 μ M, 5mM
가 .
17 , nicotine tartrate 가 50 μ M, 500 μ M, 5mM
가 1 80.7% 60.4, 56.2, 50%
($P<0.001$).

4. Mecamylamine
nicotine
Nicotinic acetylcholine (antagonist) mecamylamine 10 μ
M nicotine 1
Mecamylamine 10 μ M
, mecamylamine 10 μ M 4
가 mecamylamine 10 μ M
nicotine 300 μ M, 500 μ M, 1mM, 5mM 13
Table 5 mecamylamine 10 μ M nicotine 300 μ M
1 ($P<0.05$), nicotine 500 μ M
1 . Nicotine 1mM 5mM
69.6, 11.1% 1 ($P<0.05$).
Fig. 1 mecamylamine 10 μ M nicotine 300 μ M
nicotine 300 μ M (Table 2) 1 79.6%
96.1% 가 ($P<0.001$), mecamylamine 10 μ M
nicotine 1mM nicotine 1mM (Table 2) 1
57.1% 69.6% 가 , mecamylamine 10 μ M
nicotine 5mM nicotine 5mM (Table
2) 39% 9.3% ($P<0.001$).

5. Mecamylamine
nicotine tartrate
Mecamylamine 10 μ M nicotine tartrate 4
17 1
Mecamylamine 10 μ M
, mecamylamine 10 μ M nicotine tartrate

Table 6	mecamylamine 10 μ M	nicotine tartrate 50 μ
M- 5mM	4 , nicotine tartrate 5mM	
	(P<0.05), 50, 500 μ M	
	17	1

Fig. 2 , mecamylamine 10 μ M nicotine tartrate 50 μ M

17 1 nicotine tartrate 50 μ M

(Table 4) 60.4% 72.3% 가 , mecamylamine 10 μ M

nicotine tartrate 500 μ M 1 nicotine

tartrate 500 μ M (Table 4) 56.2% 79.4% 가

(P<0.001). mecamylamine 10 μ M nicotine tartrate 5mM

1 nicotine tartrate 5mM (Table 4) 50%

78.4% 가 (P<0.001).

nicotine nicotine tartrate가

0.1 - 1 μ M nicotine nicotine (0.1 - 1 μ M)

Nicotine 300 μ M - 5mM 4

Nicotine 300 μ M - 5mM 17 가

, nicotine 1 4

nicotine 300 μ M - 5mM 13

, nicotine 500 μ M 17 1

80.6% 91.3% 가 , nicotine 300 μ M, 1mM, 5mM

가 가 79.6, 57.1, 16.1% 1

, 500 μ M nicotine 가 가

가 가 , Hoechst (pyknotic

chromosome)가 , 5mM 39%

nicotine toxic .

Nicotine nicotinic acetylcholine ,
 acetylcholine muscarinic acetylcholine nicotinic acetylcholine
 가 . Muscarinic acetylcholine muscarine ,
 nicotinic acetylcholine ion-channel-linked (skeletal
 muscle cell) (nerve cell) nicotine . Nicotine
 nicotinic acetylcholine Ca^{2+} 가 ,
 24 ~ 27 가 , Ca^{2+} , 28 ~ 30
 . 31 ~ 33 cAMP agonist
 N⁶,O²-dibutyryl adenosine 3',5'-cyclic monophosphate(dbcAMP)
 34 Ca^{2+} 가 가 dbcAMP 가
 가 . 31 dbcAMP
 nicotine , nicotine dbcAMP
 가 dbcAMP nicotine
 nicotine 가 10, 100, 500 μ M 가 10.5, 23.5,
 26.7% 가 .
 nicotine nicotinic acetylcholine 가
 nicotine Ca^{2+} 가 .
 nicotine tartrate nicotine
 . Nicotine tartrate 50 μ M, 500 μ M, 5mM 4
 , 17 1
 80.7% 60.4, 56.2, 50.0% 가 가
 . , nicotine 5mM 39%
 가 , nicotine nicotine tartrate toxic .
 Nicotinic acetylcholine mecamlamine nicotine nicotinic
 acetylcholine 가 가 35 ~ 37
 mecamlamine nicotine .
 Mecamlamine 10 μ M 4
 . Nicotine
 mecamlamine 10 μ M 4
 mecamlamine 10 μ M
 nicotine 300 μ M-5mM 1 .
 Nicotine 300 μ M 1 mecamlamine 10 μ M
 82.4% 96.1% , nicotine 1mM 5mM
 1 69.6, 11.1% .
 Nicotine , mecamlamine 10 μ M
 nicotine 300 μ M. 1mM 1 nicotine
 79.6, 57.1% 96.1, 69.6% 가 , 5mM 39.0%
 9.3% . Mecamlamine nicotine
 nicotinic acetylcholine 가 가

, mecamlamine nicotine tartrate
 . Nicotine tartrate가
 mecamlamine 10 μM nicotine tartrate 50 μ
 M-5mM 4 17 1

Nicotine tartrate mecamlamine 10 μM
 nicotine tartrate 50 μM, 500 μM, 5mM 1
 nicotine tartrate 60.4, 56.2, 50.0% 72.3, 79.4, 78.4%
 가 . , mecamlamine 가 nicotine tartrate

nicotine ,
 dbcAMP nicotine
 nicotinic acetylcholine mecamlamine nicotine
 nicotine tartrate nicotinic
 acetylcholine 가 가 .
 가 .

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Conc. of nicotine (μ M)	Total No. of oocytes	No. of oocytes (%)	
		4 hours	
		GV	GVBD
0	98	5.1 \pm 2.3	94.9 \pm 2.3
300	74	13.5 \pm 2.4	86.5 \pm 2.4
500	109	14.7 \pm 6.0	85.3 \pm 6.0
1000	102	3.9 \pm 5.8	96.1 \pm 5.8
5000	100	11.0 \pm 4.0	89.0 \pm 1.8

Table 1. Effects of nicotine on GVBD of the mouse GV oocytes.

Data are presented as mean \pm SD and four replications were done.

Abbreviation: GV, germinal vesicle; GVBD, germinal vesicle breakdown

Conc. of nicotine (μ M)	Total No. of oocytes (GVBD)	No. of oocytes(%)		
		17 hours		
		GVBD	PB	Deg
0	129	19.4 \pm 9.1	80.6 \pm 9.1	0
300	103	20.4 \pm 6.7	79.6 \pm 6.7	0
500	126	8.7 \pm 7.0	91.3 \pm 7.0*	0
1000	112	42.9 \pm 9.0	57.1 \pm 9.0**	0
5000	118	44.9 \pm 3.3	16.1 \pm 4.3	39.0 \pm 5.8

Table 2. Effects of nicotine on the meiotic maturation of the mouse GVBD oocytes^a.

Data are presented as mean \pm SD and five replications were done.

^aGVBD oocytes were transferred to nicotine contained media after culture for 4hrs in New MHBS medium.

Conc. of nicotine (μ M)	Total No. of oocytes	No. of oocytes(%)				
		4 hours		17 hours		
		GV	GVBD	GV	GVBD	PB
0	145	100 \pm 0	0	100 \pm 0	0	0
10	105	89.5 \pm 15.0	10.5 \pm 15.0	87.6 \pm 16.1	2.9 \pm 5.6	9.5 \pm 11.1**
100	166	76.5 \pm 9.4	23.5 \pm 9.4	69.9 \pm 10.3	9.0 \pm 0.9	21.1 \pm 10.8**
500	116	73.3 \pm 10.8	26.7 \pm 10.8	66.4 \pm 9.6	8.6 \pm 8.9	25.0 \pm 13.1**
1000	101	100 \pm 0	0	95.0 \pm 4.1	5.0 \pm 4.1*	0

Abbreviation: GVBD, germinal vesicle breakdown; PB, polar body; Deg, degeneration

*, ** Significantly different from the control(0 μ M), *P<0.05, **P<0.001.

Table 3. Effects of nicotine on the meiotic maturation of the mouse oocytes in the presence of dbcAMP(0.2mM).

Data are presented as mean \pm SD and five replications were done.

0: New MHBS media + dbcAMP 0.2mM

Abbreviation: GV, germinal vesicle; GVBD, germinal vesicle breakdown; PB, polar body

*, ** Significantly different from the control(0 μ M), *P<0.05, **P<0.001.

Conc. of nicotine tartrate (μ M)	Total No. of oocytes	No. of oocytes (%)				
		4 hours		17 hours		
		GV	GVBD	GV	GVBD	PB
0	124	10.5 \pm 2.4	89.5 \pm 2.4	4.8 \pm 1.4	14.5 \pm 1.9	80.7 \pm 2.9
50	101	10.9 \pm 5.2	89.1 \pm 5.2	3.0 \pm 1.8	36.6 \pm 2.7	60.4 \pm 1.0*
500	105	5.7 \pm 1.4	94.3 \pm 1.4	2.9 \pm 1.9	40.9 \pm 1.9	56.2 \pm 1.9*
5000	102	10.7 \pm 3.3	89.3 \pm 3.3	9.8 \pm 2.4	40.2 \pm 11.4	50.0 \pm 9.2*

Table 4. Effects of nicotine tartrate on the meiotic maturation of the mouse oocytes.

Data are presented as mean \pm SD and four replications were done.

Abbreviation: GV, germinal vesicle; GVBD, germinal vesicle breakdown; PB, polar body

* Significantly different from the control(0 μ M), *P<0.001.

Conc. of nicotine (μ M)	Total No. of oocytes (GVBD)	No. of oocytes(%)		
		17 hours		
		GVBD	PB	Deg
0	108	17.6 \pm 2.6	82.4 \pm 2.6	0
300	102	3.9 \pm 0.7	96.1 \pm 0.7*	0
500	101	14.8 \pm 1.6	85.2 \pm 1.6	0
1000	102	30.4 \pm 2.4	69.6 \pm 2.4*	0
5000	108	79.6 \pm 1.5	11.1 \pm 1.4	9.3 \pm 0.1**

Table 5. Effects of mecamlamine on effects of nicotine on the meiotic maturation of the mouse GVBD oocytes^a.

Data are presented as mean \pm SD and four replications were done.

^aGVBD oocytes were transferred to nicotine contained media after culture for 4hrs

in mecamlamine 10 μ M contained medium.

0: New MHBS media + mecamlamine 10 μ M

Abbreviation: GVBD, germinal vesicle breakdown; PB, polar body; Deg,

Conc. of nicotine tartrate (μ M)	Total No. of oocytes	No. of oocytes (%)				
		4 hours		17 hours		
		GV	GVBD	GV	GVBD	PB
0	102	4.9 \pm 3.5	95.1 \pm 3.5	3.9 \pm 4.2	19.6 \pm 0.9	76.5 \pm 4.8
50	101	11.9 \pm 2.4	88.1 \pm 2.4	5.9 \pm 1.1	21.8 \pm 3.0	72.3 \pm 1.9
500	102	8.8 \pm 1.1	91.2 \pm 1.1	0	20.6 \pm 1.9	79.4 \pm 1.9
5000	102	17.6 \pm 4.5	82.4 \pm 4.5*	8.8 \pm 1.3	12.8 \pm 2.5	78.4 \pm 1.2

degeneration *, ** Significantly different from the control(0 μ M), *P<0.05, **P<0.001.

Table 6. Effects of mecamlamine on effects of nicotine tartrate on the meiotic maturation of the mouse oocytes.

Data are presented as mean \pm SD and four replications were done.

0: New MHBS media + mecamlamine 10 μ M

Abbreviation: GV, germinal vesicle; GVBD, germinal vesicle breakdown; PB, polar body

* Significantly different from the control(0 μ M), *P<0.01.

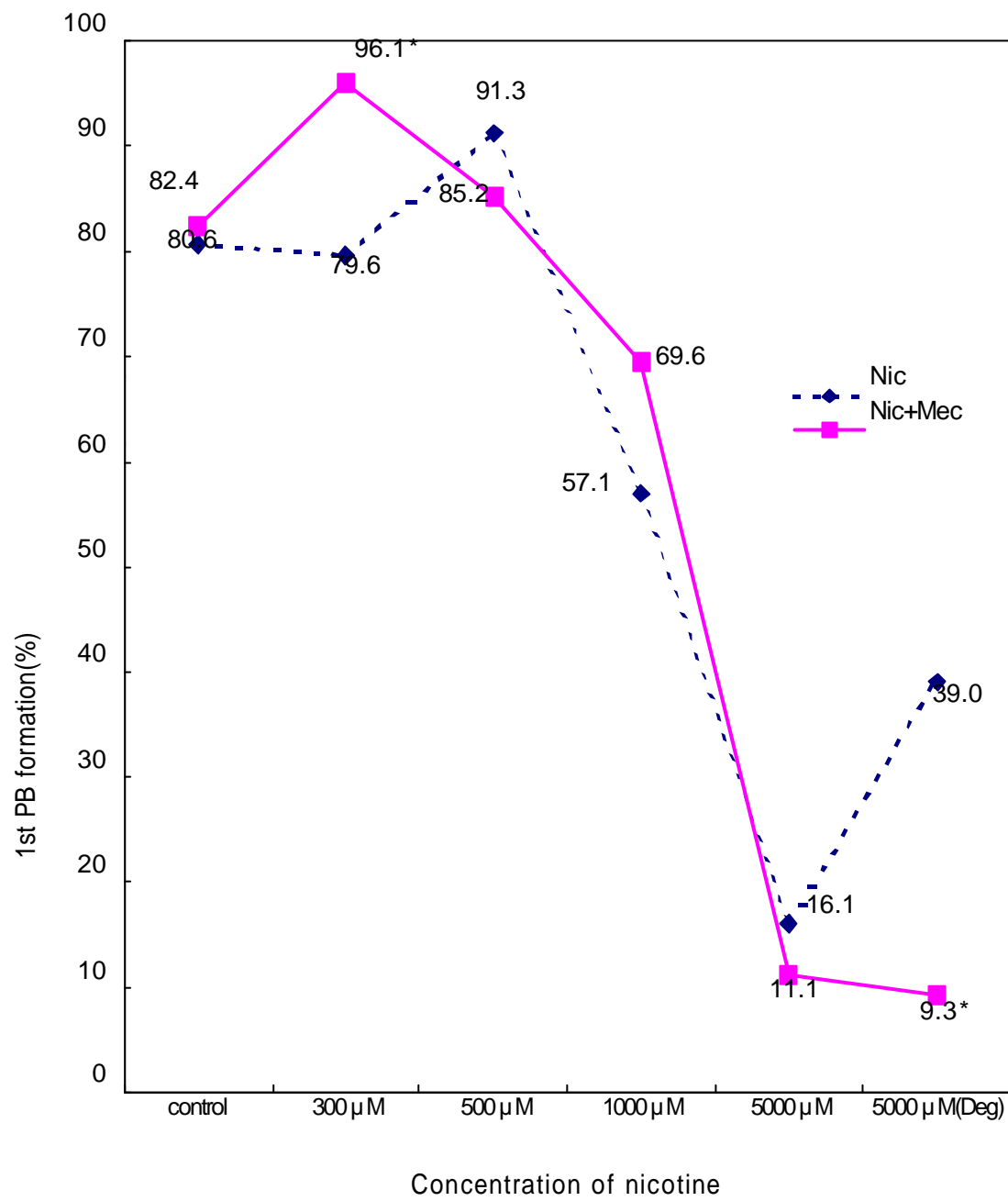


Fig. 1. Effects of nicotine(Nic) on the mieotic maturation of mouse GVBD oocytes in the absence or presence of mecamlamine(Mec, 10 µM).

* Significantly different from the control, *P<0.001.

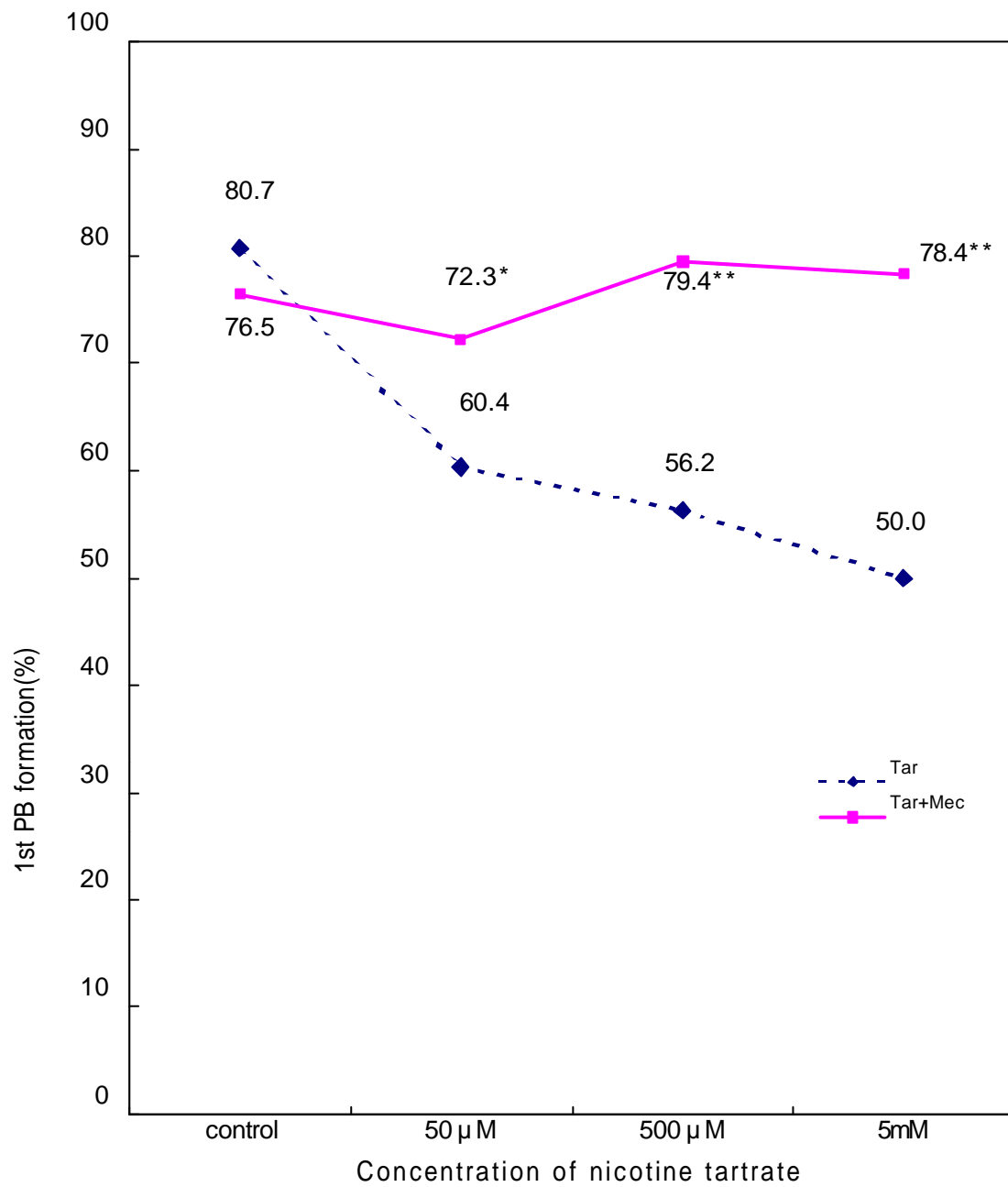


Fig. 2. Effects of nicotine tartrate(Tar) on the meiotic maturation of mouse oocytes in the absence or presence of mecamylamine(Mec, 10 μ M).

*, ** Significantly different from the control, * $P < 0.05$, ** $P < 0.001$.

Fig. 3. Light micrographs of mouse oocytes cultured for 17hrs in the presence of nicotine($\times 400$).

A. Control(New MHBS), B. Nicotine 500 μ M, C. Nicotine 5mM

(PB, polar body; Deg, degeneration; , indicates oocyte with shrunken cytoplasm)

Fig. 4. Fluorescence micrographs of mouse oocytes stained with Hoechst 33342 cultured for 17hrs in the presence of nicotine($\times 300$)
A. Control(New MHBS), B. Nicotine 500 μ M, C. Nicotine 5mM

(MII, metaphase II; , indicates oocyte with pyknotic chromosome)