Effect of BPA and Nicotine on *In Vitro* Maturation of Porcine Oocytes

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BPA 및 Nicotine 첨가가 돼지 난자의 체외 성숙에 미치는 영향

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SUMMARY

본 연구는 BPA 및 nicotine 첨가 농도와 배양 시간이 돼지 난자의 체외성숙에 미치는 영향을 조사하였다. 0.02~10.0 nM BPA와 0.5~10.0 mM nicotine이 첨가된 TCM-199 배양액에서 40~52시간 난자를 배양했을 때 체외성숙율을 조사하였다. BPA 농도가 높을수록 체외성숙율이 유의적으로 낮게 나타났다. 0.05~10.0 nM BPA를 첨가한 TCM-199 배양액에서 난자를 44시간 배양했을 때 체외성숙율은 각각 40.0±4.1%, 24.0±4.7%, 10.0±5.3%, 6.0±3.2%, 0.0±0.0%로서 첨가 농도가 증가할수록 낮은 체외성숙율을 나타냈다. 난자를 0.5~10.0 mM nicotine를 첨가한 TCM-199 배양액에서 44시간 배양했을 때 체외성숙율은 각각 44.0±4.5%, 24.0±4.2%, 18.0±4.9%, 8.0±2.2%, 0.0±0.0%로서 대조군(52.0±4.5%)에 비해 낮은 체외성숙율을 나타냈다. 난자를 0.5 nM BPA와 2.5 mM nicotine을 첨가한 TCM-199에서 40~52시간 배양했을 때 체외성숙율은 8.3±2.1%~26.0±3.9% 및 11.2±2.2%~28.6±3.9%로서, 44시간 배양이 다른 배양시간보다 가장 높은 체외성숙율을 나타냈다.

(Key words : PBA, nicotine, IVM rate, porcine oocyte)

INTRODUCTION

Nuclear transfer has been considered very important because genetically modified pigs might be able to provide organs and tissues for xerotransplantation. However, in the pig, the viability of nuclear transfer embryos is poor, with an extremely low rate of maturation, fertilization and cloned piglet production.

Bisphenol A (BPA), a raw material of polycarbonate and epoxy resins (i.e. dental sealants and lacquer coating of food cans), is a widely used chemical proposed to have estrogenic activity (Krishnan *et al.*, 1993). Smoking causes spontaneous abortions, perinatal mortality, stillbirths, and fetal malformations, and induces decreased sperm density, reduced testosterone secretion and an increase in morphologically abnormal spermatozoa (Arabi and Moshtaghi, 2005; Tuormaa, 1995), and show that maternal smoking increases the zona pellucida thickness of oocytes and embryos (Shiloh *et al.*, 2004) and results in reduced fertilization and pregnancy rates (Klonoff-Cohen *et al.*, 2001; Augood *et al.*, 1998; Joesbury *et al.*, 1998). Baldwin and Racowsky (1998) reported a dose-dependent decrease in the proportion of mouse embryos reaching the blastocyst stage following *in vitro* treatment with 0.5~5.0 mM nicotine. Miceli *et al.* (2005) reported that nicotine induces a sort of luteal insufficiency by inhibiting progesterone release. Nicotine also induces embryonic malformations mediated by apoptosis from increasing intracellular Ca and oxidative stress (Zhao and Reece, 2005; Bishun *et al.*, 1972). Racowsky *et al.* (1989) reported that when hamster oocytes were cultured in medium containing 5 mM/l nicotine, the oocytes blocked in MI and of oocytes with disruption of homologue segregation at anaphase I were increased. In mice, nicotine reduces ovulation rate and increases the frequencies of premature centromere separation and premature anaphase (Maihes *et al.*, 2005, 2000).

Many of the references citied used a rodent model to determine the effects of nicotine on meiosis. The information on humans is based mainly upon cumulative clinical studies rather than *in vitro* oocyte assays because of ethical issues.

In the present study were carried out to investigate the effect of BPA and nicotine concentration and culture time on *in vitro* maturation of porcine oocytes.

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MATERIALS AND METHODS

1. Collection of Oocytes

Porcine ovaries were collected at a local slaughter house and transported to the laboratory in physiological saline containing 100 μl/ml penicillin G and streptomycin sulfate 100 ug/ml at 25~30℃. Oocytes were aspirated from medium size follicles with an 21 gauge fixed to a 10 ml disposable syringe. The cumulus-oocytes complexes (COSs) that had an evenly distributed cytoplasm and washed three times in oocyte maturation medium containing hormonal supplements.

2. *In Vitro* Maturation of Oocytes

Then each group of 50 COCs was cultured in 500 μl of maturation medium, which had previously been covered with mineral oil and equilibrated in a humidified atmosphere of 5% CO_2 and 95% air at 38℃. After culturing for 44 h, COCs were washed three times in the maturation medium without hormonal supplements. The basic medium used for oocyte maturation were TCM-199 (Whittaker, U.S.A.) supplementation with 2 IU/ml의 hCG(Sigma, U.S.A.) and 10% FCS (Sigma, U.S.A.). COCs were cultured in TCM-199 media with supplemented with 0.05~10.0 nM BPA (Sigma, U.S.A.), 0.5~10.0 mM of nicotine (Sigma, U.S.A.). BPA were prepared by dissolving the compounds in ethanol.

3. Assessment of Meiotic Stage

Oocytes were fixed in acetic acid : ethanol (1 : 3) solution for 24 h then stained using with 1% acetoorcein (Sigma, U.S.A.) or 10 μg/ml bisbenzimide (Hoechst 33342, Sigma, U.S.A.) and observed under an fluorescence microscope. The judgement of oocytes maturation *in vitro* was carried out depending on the criteria of maturation by cell and nuclear division.

4. Statistical Analysis

The results were expressed by treatment as mean ± SD. For comparison of means, Duncan's multiple verification was performed using SAS package of general Linears Model procedures (SAS Institute).

RESULTS AND DISCUSSION

1. Effect of BPA on IVM Rate

This experiment was carried out to investigate the effect of BPA concentration on IVM rate of porcine oocytes were shown in Table 1. The IVM rate of oocytes cultured in TCM-199 supplementation with 0.05~10.0 nM BPA for 44 h were 40.0±4.1%, 24.0±4.7%, 10.0±5.3%, 6.0±3.2%, 0.0±0.0%, respectively. The IVM rate of oocytes cultured in TCM-199 supplementation with BPA was significantly lower cultured non supplementation of BPA (52.0±4.5%). BPA affects porcine oocyte maturation rate in a dose-dependent manner. The experimental animal was different but, the above result was similar than Baek *et al.* (2007) reported that the GVBD rate of longchin goby (Chasmichthys dolichognathus) oocytes cultured in salt solution containing with HEPES showed that low concentrations of BPA triggered GVBD depending on the stage of oocyte development.

2. Effect of Nicotine on IVM Rate

This experiment was carried out to investigate the effect of nicotine concentration on IVM rate of porcine oocytes were shown in Table 2. The IVM rate of oocytes cultured in TCM-199 medium supplementation with 0.5~10.0 mM nicotine for 44 h were 44.0±4.5%, 24.0±4.2%, 18.0±4.9%, 8.0±2.2%, 0.0±0.0%, respectively. Nicotine affects porcine oocyte *in vitro* maturation rate in a dose-dependent. This result were significantly lower than the control group (52.0±4.5%). The experimental animal was different but, the above result was similar or lower than Liu *et al.* (2006) reported that the IVM rate of bovine oocyte cultured in TCM-199 medium supplementation with 5.0 or 10.0 mM nicotine for 44 hrs were 18.0±6.2%, 0.0±0.0%, respectively. Also, This study showed that nicotine affected bovine oocyte maturation rates in a dose-dependent manner. When nicotine concentrations were 2.0 mM or higher, it significantly reduced maturation rates.

Table 1. The IVM rate of oocytes cultured in TCM-199 supplementation with BPA for 44 h

BPA (nM)	No. of oocytes examined	No. of at the stage of			Rate of IVM (%)
		GV	GVBD	MII	
Control	50	6	18	26	52.0±4.5[a]
0.05	50	18	12	20	40.0±4.1
0.5	50	20	10	12	24.0±4.7[b]
1.0	50	16	7	5	10.0±5.3[b]
5.0	50	12	7	3	6.0±3.2[b]
10.0	50	4	0	0	0.0±0.0[b]

[a,b] Values within column with different superscript ($p<0.05$).

Table 2. The IVM rate of oocytes cultured in TCM-199 supplementation with nicotine for 44 h

Nicotine (mM)	No. of oocytes examined	No. of at the stage of			Rate of IVM (%)
		GV	GVBD	MII	
Control	50	6	18	26	52.0±4.5[a]
0.5	50	11	17	22	44.0±4.5
1.0	50	18	9	12	24.0±4.2[b]
2.5	50	15	17	9	18.0±4.9[b]
5.0	50	12	9	4	8.0±2.2[b]
10.0	50	7	0	0	0.0±0.0[b]

[a,b] Values within column with different superscript ($p<0.05$).

3. Effect of Concentration and Culture time on IVM Rate

This experiment was carried out to investigate the effects of BPA, nicotine concentration and culture time on IVM rate of porcine oocytes were shown in Table 3. The IVM rate of oocytes cultured in TCM-199 medium supplementation with 0.5 nM BPA for 40~52 hrs were 8.3±2.1%~26.0±3.9%, respectively. The IVM rate of oocytes cultured in TCM-199 medium supplementation with 2.5 mM nicotine for 44 or 72 h were 11.2±2.2%~28.6±3.9%, respectively. The IVM rate of oocytes cultured for 44 h in TCM-199 medium supplementation with 0.5 nM BPA or 2.5 mM nicotine were higher than those of other culture time. This result was similar to Liu *et al.* (2006) reported that the MII rate of bovine oocyte cultured in TCM-199 supplementation with 5.0 or 10.0 mM nicotine for 22 h were 24.4~0.0%, respectively. Oocytes at GVBD and metaphase I stages were less affected by nicotine at 5.0 and 10.0 mM concentrations than GV-stage oocytes. In mice, nicotine reduces ovulation rate and perturbs the maturation rate *in vivo* (Maihes *et al.*, 2000).

CONCLUSION

The study were carried out to investigate the effects of BPA and nicotine concentration and culture time on *in vitro* maturation of porcine oocytes. Oocytes were cultured in maturation TCM-199 media with supplementation with 0.02~10.0 nM BPA or 0.5~10.0 mM of nicotine for 40~52 h.

1. The IVM rate of oocytes cultured in TCM-199 supplementation with 0.05~10.0 nM BPA for 44 h were 40.0±4.1%, 24.0±4.7%, 10.0±5.3%, 6.0±3.2%, 0.0±0.0%, respectively. The IVM rate of oocytes cultured in TCM-199 supplementation with BPA was significantly lower than that of oocytes cultured without BPA (52.0±4.5%).
2. The IVM rate of oocytes cultured in TCM-199 supplementation with 0.5~10.0 mM nicotine for 44 hrs were 44.0±4.5%, 24.0±4.2%, 18.0±4.9%, 8.0±2.2%, 0.0±0.0%, respectively. Nicotine affects porcine oocyte IVM rate in a dose-dependent manner.
3. The IVM rate of oocytes cultured in TCM-199 medium supplementation with 0.5 nM BPA or 2.5 mM nicotine for 40~52 hrs were 8.3±2.1%~26.0±3.9% and 11.2±2.2%

Table 3. Effect of BPA, nicotine concentration and 40~52 h incubation time on IVM rate of oocytes

Supplementation		Incubation time	No. of oocytes examined	No. of at the stage of			Rate of IVM (%)
				GV	GVBD	MII	
Control		44	50	6	16	28	52.0±4.7
BPA	0.5	40	45	12	15	8	17.8±2.7
		44	50			13	26.0±3.9
		48	45	9	5	6	13.3±2.8
		52	48			4	8.3±2.1
NT	2.5	40	46	12	9	10	21.7±3.2
		44	49			14	28.6±3.9
		48	47	11	3	11	22.9±4.1
		52	45			5	11.2±2.2

* NT : nicotine.

~28.6±3.9%, respectively. The IVM rate of oocytes cultured for 44 hrs in TCM-199 medium supplementation with 0.5 nM BPA or 2.5 mM nicotine were higher than those of other culture time.

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