KSDB 2015

Effects of caffeine on the vitrification of mouse mature oocyte and further embryonic development.

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CHA의과학대학교 일반대학원 의생명과학과

CHA 의과학대학교

동결보존

동결보존

세포를 초저온 상태(-196℃)에서 보관하여 생명활동을 일시적으로 중단시킨 후, 필요 시 해동하여 사용할 수 있도록 하는 방법.

기본 원리

세포의 고유한 형태를 유지하면서 내부의 수분을 고농도 동결보호제의 삼투압 원리를 이용하여 점진적으로 제거하고, 이로 인해 얼음 결정을 최소화 하여 세포를 보호함 (Kim et al., 2014).

동결보존

Science. 1972 Oct 27;178(4059):411-4.

Survival of mouse embryos frozen to -196 degrees and -269 degrees C.

Whittingham DG, Leibo SP, Mazur P.

Science Classic
AAAS

역사적 배경

1972 : 생쥐 배아를 동결 후 이식하여 산자 생산
1983-1984 : 인간 배아를 동결 후 임신, 출산
1985 : 생쥐 난자의 유리화동결
1986 : 인간 난자의 동결

동결보존의 중요성

- 과배란 유도 시술의 횟수를 감소시킴.
- 난자공여프로그램.
- 의학적 이유로 인한 늦은 배아 이식.
- 다태임신을 감소시켜주는 방법.
- 가임력 보존.

동결보존

난자의 동결보존 방법

완만동결

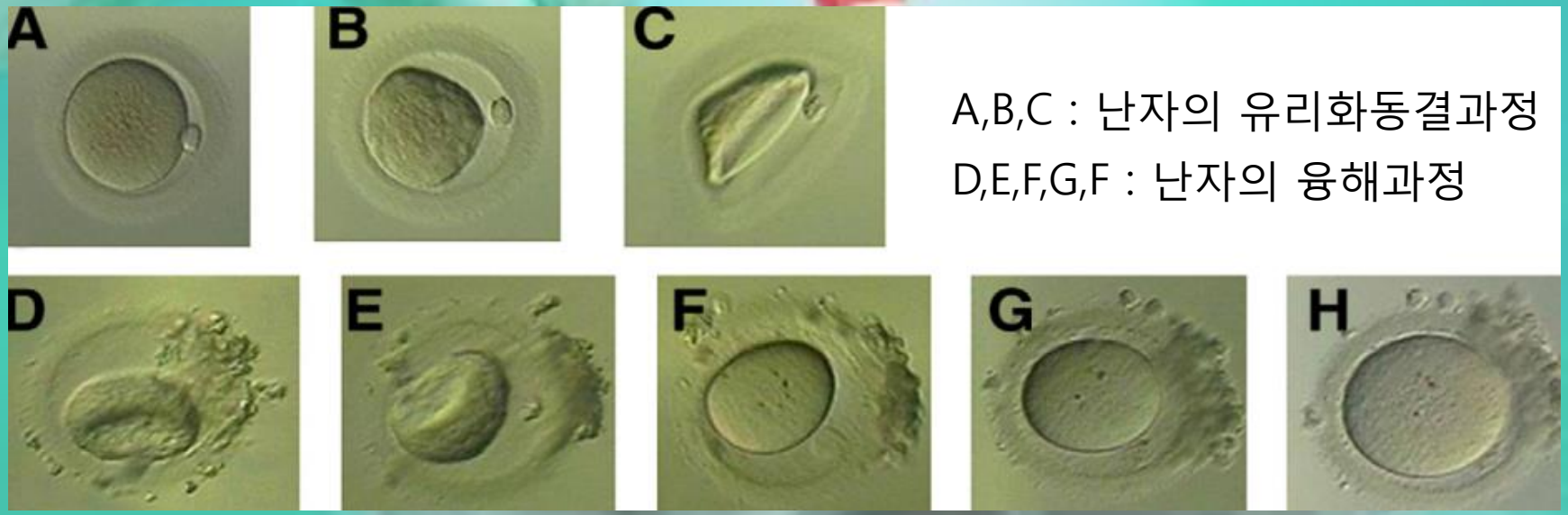
- 동결보호제에 의한 독성과 삼투압 영향이 낮은 편.
- 얼음 형성에 의한 세포질 손상, 많은 시간 소요.
- 고가의 비용, 낮은 생존율.

유리화동결

- 고농도의 동결 보호제 사용.
- 간편하며, 저렴한 비용, 빙결정형성의 최소화.

유리화동결과정과 문제점

고농도의 동결보호제의 사용, 삼투압 변화, 급속한 온도 변화로 인한 난자의 손상



A,B,C : 난자의 유리화동결과정
D,E,F,G,F : 난자의 융해과정

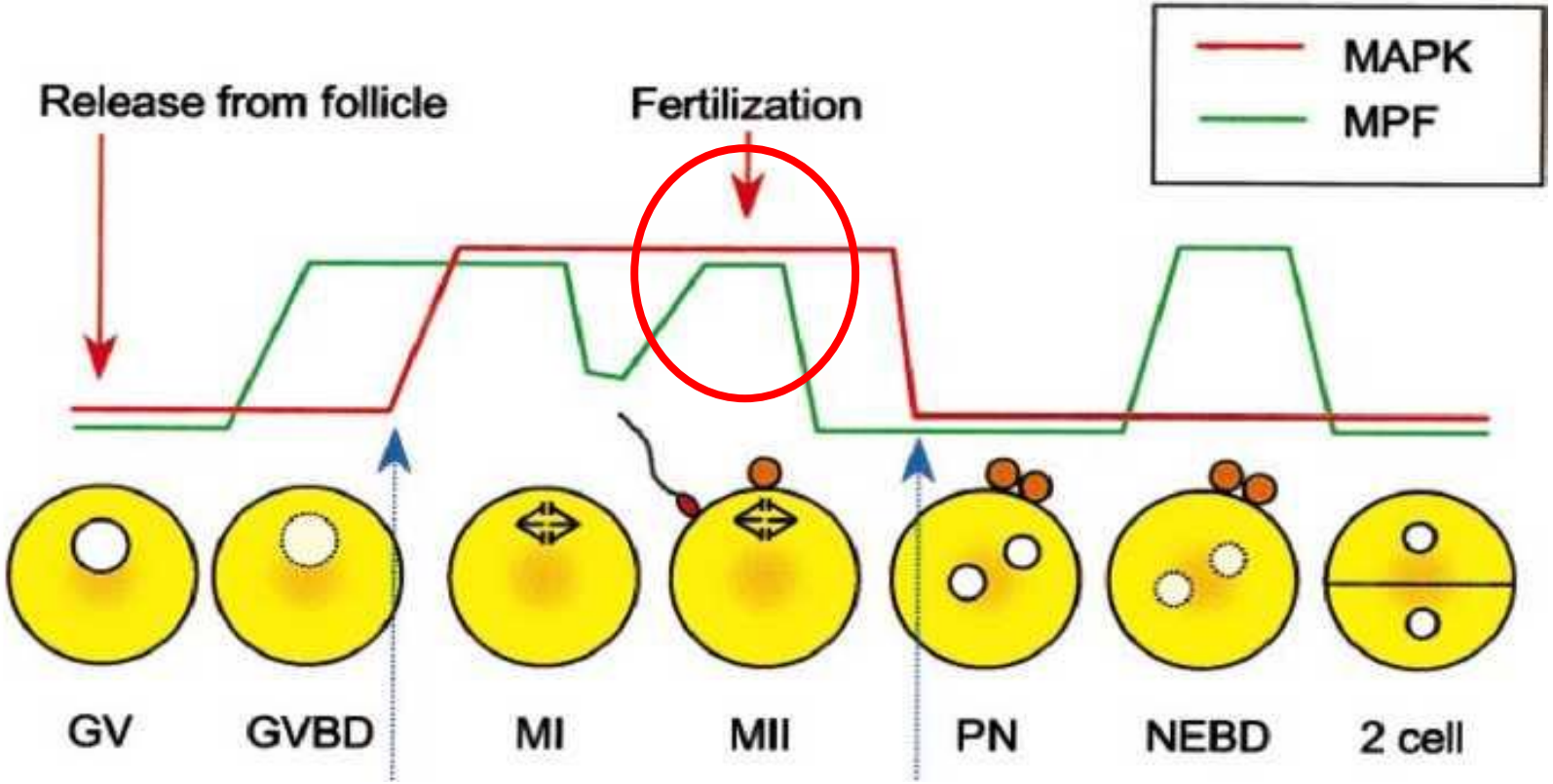
Minasi et al., Fertil Steril 2012

유리화동결에서 난자의 손상

CHA 의과학대학교

난자 성숙과 수정 후 배 발달

Maturation promoting factor (MPF), Mitogen-activated protein kinase (MAPK)



유리화동결 중 MPF와 MAPK의 손상

Cryo Letters. 2014 Nov-Dec;35(6):530-6.

Effect of caffeine treatment before vitrification on MPF and MAPK activity and spontaneous parthenogenetic activation of in vitro matured ovine oocytes.

Ariu F[1], Bogliolo L[2], Leoni G[3], Falchi L[2], Bebbere D[3], Nieddu SM[3], Zedda MT[3], Pau S[3], Ledda S[3].

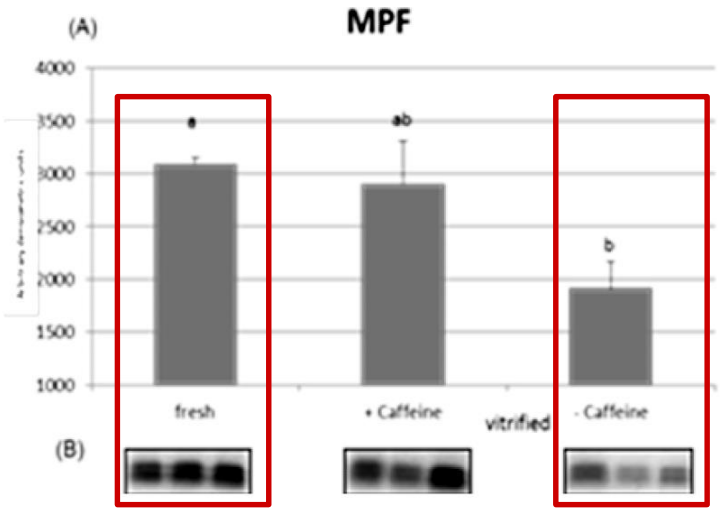


Figure 1. Effect of caffeine treatment on the activity of maturation promoting factor (MPF) of vitrified *in vitro* matured ovine oocytes. (A) Quantification by densitometry of MPF activity and (B) representative profiles of histone 1 (H1). a *vs* b $P \leq 0.05$.

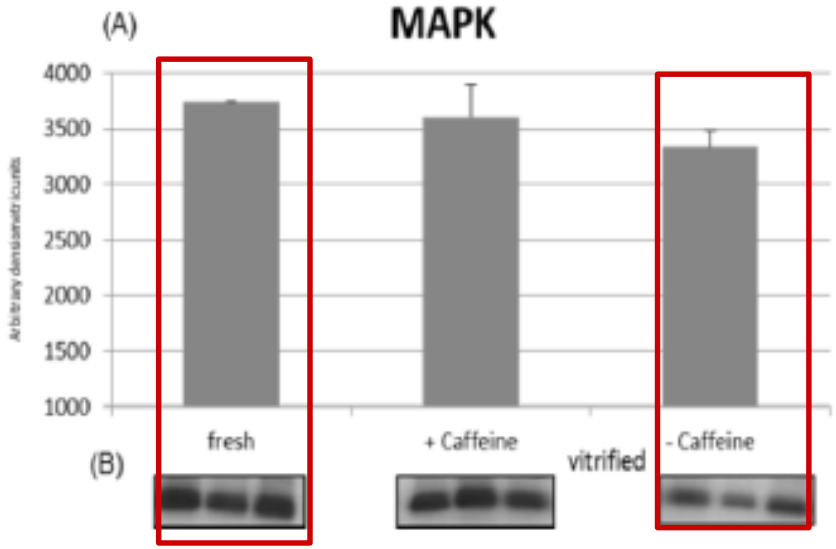
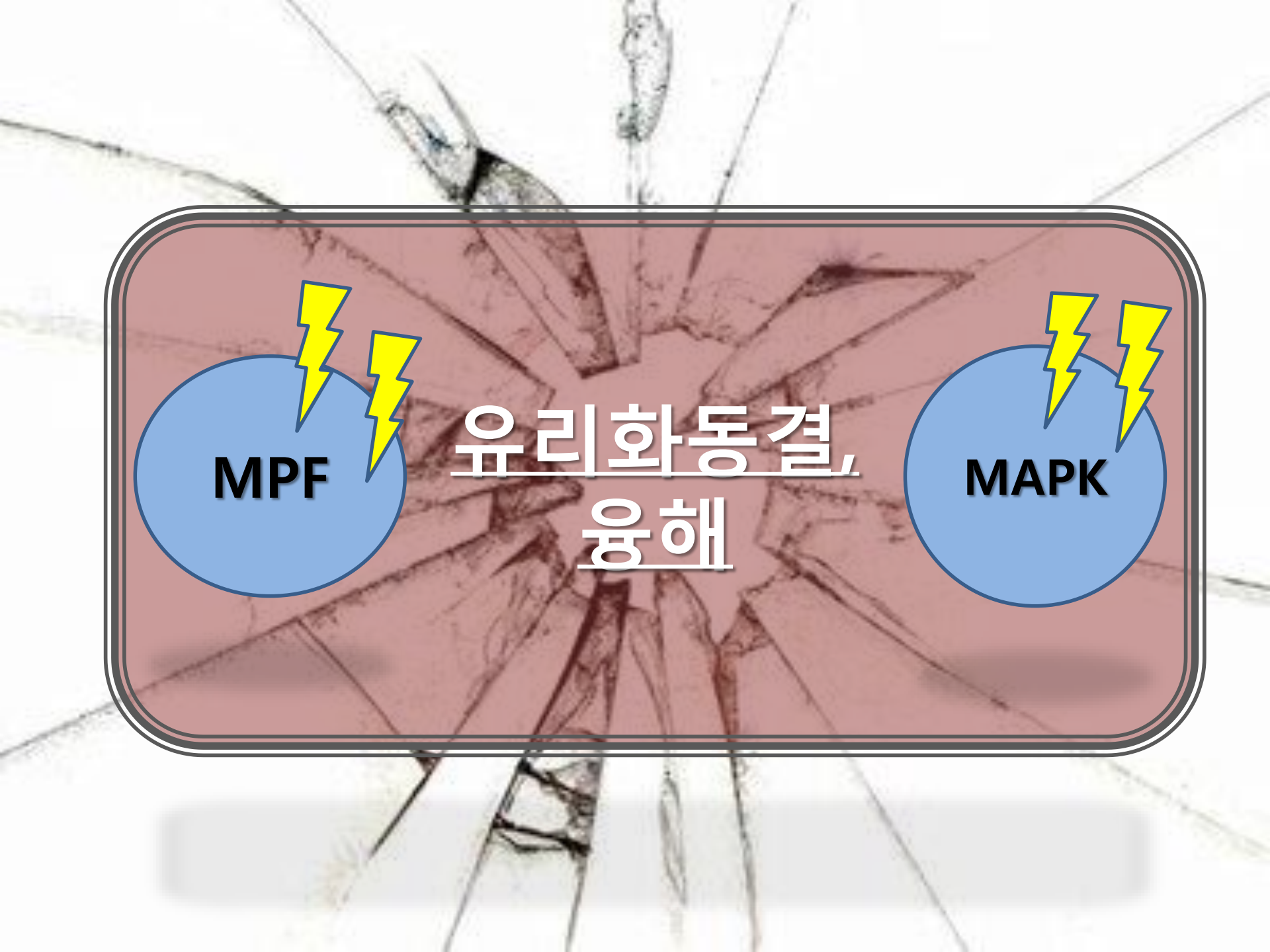


Figure 2. Effect of caffeine treatment on the activity of MAPK of vitrified in vitro matured ovine oocytes. (A) Quantification by densitometry of MAPK activity and (B) representative profiles of myelin basic protein (MBP).


MPF
유리화동결,
융해
MAPK

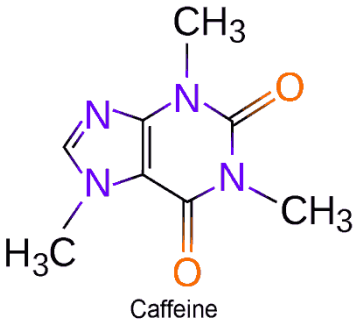
Human Reproduction Update, Vol.15, No.5 pp. 573–585, 2009
Advanced Access publication on May 8, 2009 doi:10.1093/humupd/dmp014

human reproduction update

Oocyte aging: cellular and molecular changes, developmental potential and reversal possibility

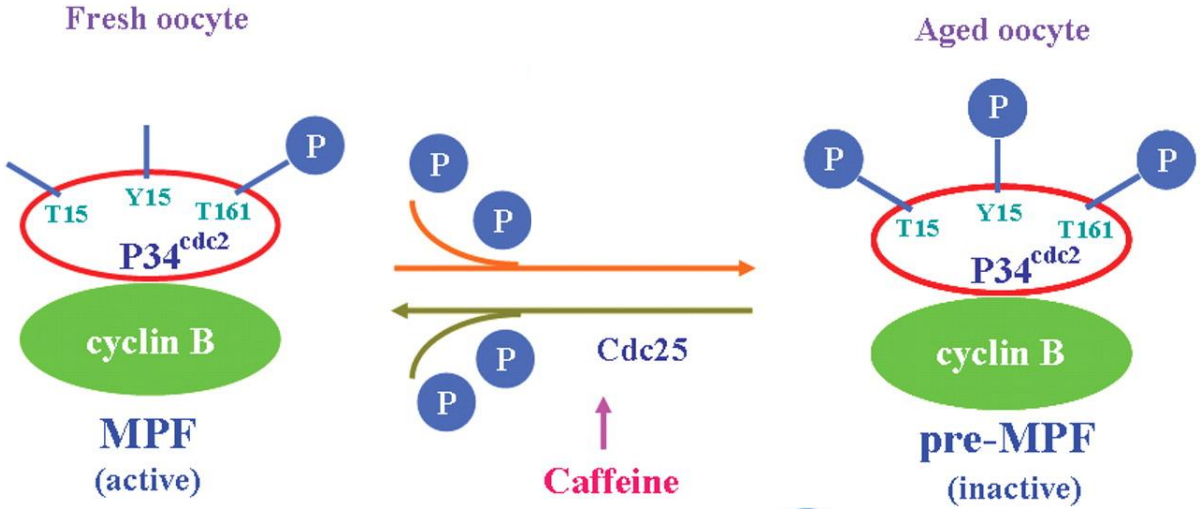
Yi-Liang Miao[1,2], Kazuhiro Kikuchi[3], Qing-Yuan Sun[1,4], and Heide Schatten[2,4]

Caffeine



Caffeine

- **Phosphatase Inhibitor,**
- **Pre- MPF → MPF activity.**



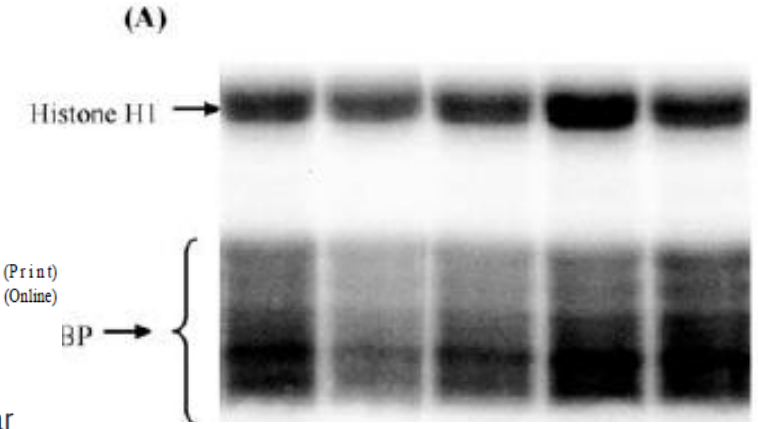
BIOLOGY OF REPRODUCTION **74,** 691–698 (2006)
Published online before print 21 December 2005.
DOI 10.1095/biolreprod.105.043885

Effects of Enucleation and Caffeine on Maturation-Promoting Factor (MPF) a Mitogen-Activated Protein Kinase (MAPK) Activities in Ovine Oocytes Used Recipient Cytoplasts for Nuclear Transfer[1]

Joon-Hee Lee and Keith H.S. Campbell[2]

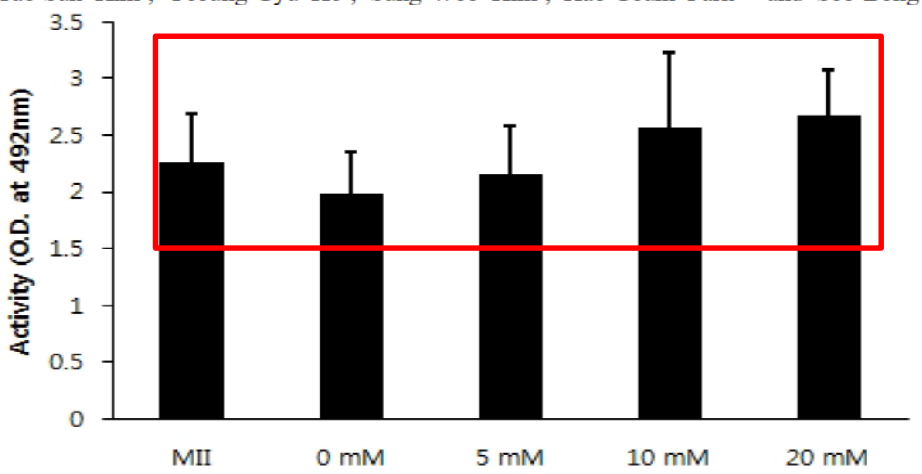
Reprod Dev Biol 36(4) : 261-267 (2012)
http://dx.doi.org/10.12749/RDB.2012.36.4.261

ISSN : 1738-2432 (Print)
ISSN : 2288-0151 (Online)



Effects of Caffeine on Maturation-Promoting Factor (MPF) Activity in Bovine Oocytes and on the Development of Somatic Cell Nuclear Transfer Embryos in White-Hanwoo

Joon-Hee Lee[1,2], Hee-Gyu Lee[1], Sang-Ki Baik[1], Sang-Jin Jin[1], Song-Yi Moon[1], Hye-Ju Eun[1], Tae-Suk Kim[1], Yeoung-Gyu Ko[3], Sung-Woo Kim[3], Hae-Geum Park[1,3] and Soo-Bong Park[3,†]



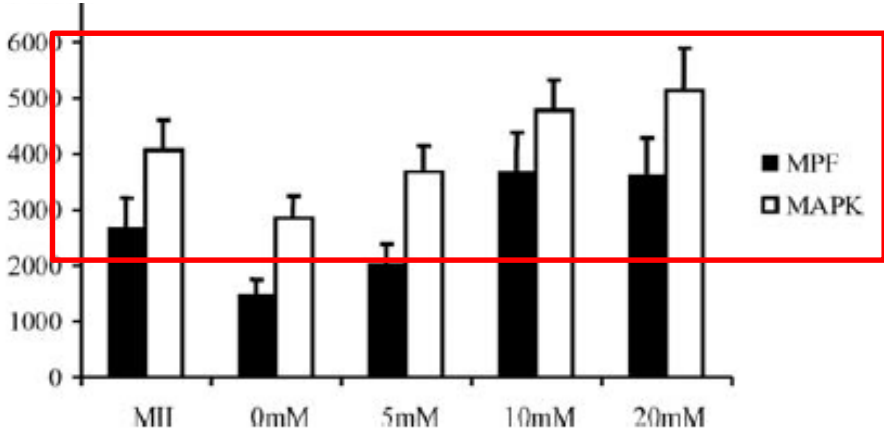


Fig. 2. Effects of caffeine on maturation-promoting factor (MPF) activity in bovine oocytes. Oocytes were denuded at 22~24 hpm, cultured in different concentrations (MII, 0 mM, 5 mM, 10 mM and 20 mM) of caffeine for 6 h and then collected in 5 μl sample buffer before snap freezing/thawing for cdc2 kinase activity. 10 oocytes were analyzed for each concentration of caffeine and th-ree replicates were performed. Bars represent mean±SEM.

Cryo Letters. 2014 Nov-Dec;35(6):530-6.

Effect of caffeine treatment before vitrification on MPF and MAPK activity and spontaneous parthenogenetic activation of in vitro matured ovine oocytes.

Ariu F[1], Bogliolo L[2], Leoni G[3], Falchi L[2], Bebbere D[3], Nieddu SM[3], Zedda MT[3], Pau S[3], Ledda S[3].

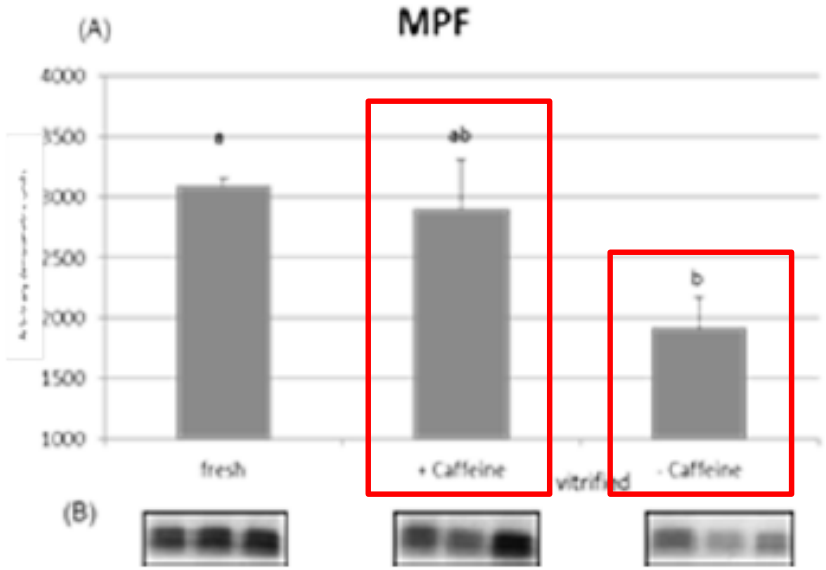


Figure 1. Effect of caffeine treatment on the activity of maturation promoting factor (MPF) of vitrified *in vitro* matured ovine oocytes. (A) Quantification by densitometry of MPF activity and (B) representative profiles of histone 1 (H1). a *vs* b $P \leq 0.05$.

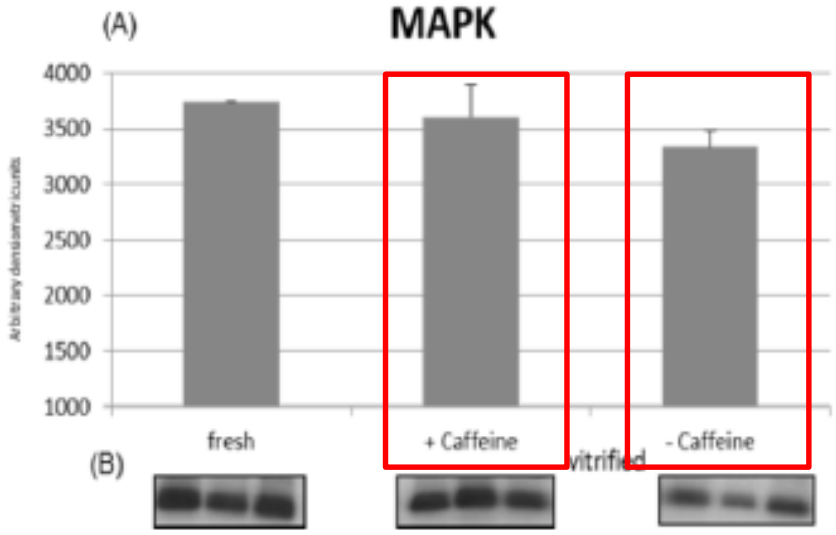
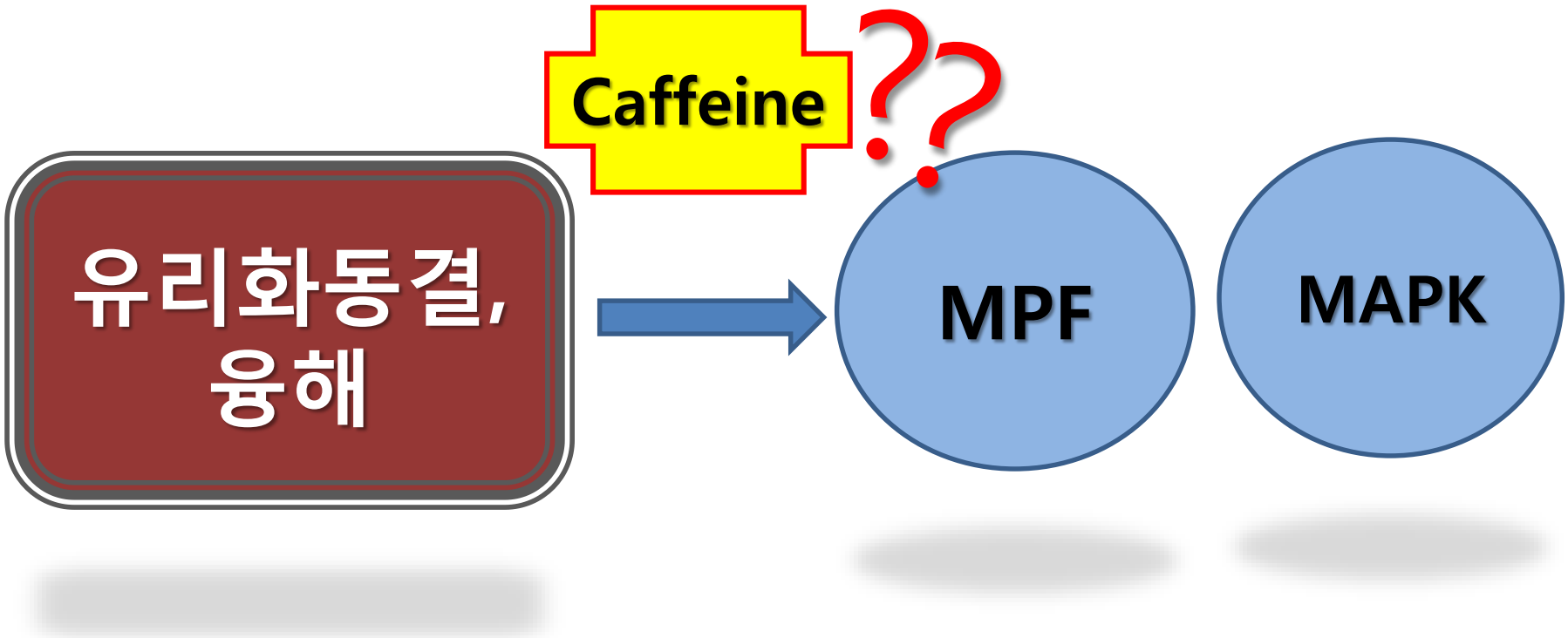


Figure 2. Effect of caffeine treatment on the activity of MAPK of vitrified in vitro matured ovine oocytes. (A) Quantification by densitometry of MAPK activity and (B) representative profiles of myelin basic protein (MBP).

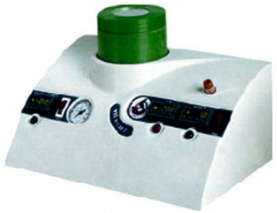
연구목적

생쥐 성숙난자의 유리화동결, 융해 시 Caffeine을 처리하였을 때 손상된 MPF와 MAPK 활성도의 회복 여부와 생존율 및 배반포 발달률에 영향을 미치는지 확인하고자 함.

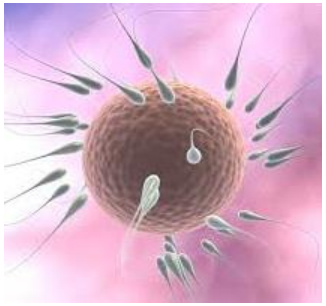


재료 및 방법

B6D2F1



Vit-master



- / + Caffeine

과배란유도 > 유리화동결 > 융해 > 2시간 전배양 > 체외수정/효소활성도측정

전배양
HTF + 10%SR

EXP 1. 체외수정 : HTF + 10% KSR
배반포 발달률: KSOM

EXP 2. MPF/MAPK 활성도
MPF : ELISA
MAPK : Western blot

유리화 동결

	전평형 용액	유리화동결 용액	융해 용액
HEPES + 20% KSR	7.5% EG 7.5% DMSO 2분 30초	15% EG 15% DMSO 0.5M sucrose 20초	0.5, 0.25, 0.125, 0M Sucrose 2분 30초

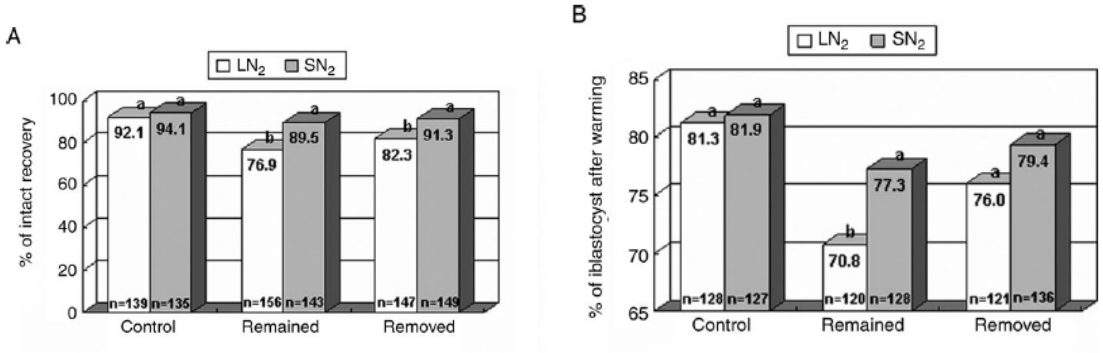
- / + Caffeine

Vit-master

Human Reproduction Vol.22, No.9 pp. 2509–2514, 2007
Advance Access publication on July 3, 2007
doi:10.1093/humrep/dem206

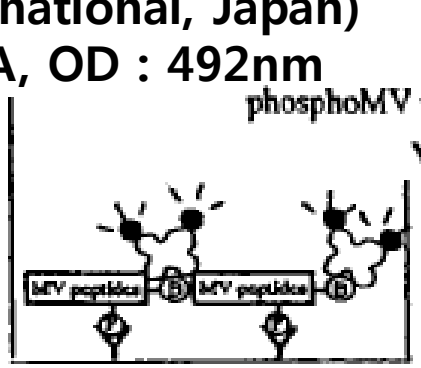
Effect of using slush nitrogen (SN_2) on development of microsurgically manipulated vitrified/warmed mouse embryos

Dong Ryul Lee[1,3], Yun Hee Yang[1], Jin Hee Eum[1], Jin Seong Seo[1], Jung Jae Ko[1], Hyung Min Chung[1,2] and Tae Ki Yoon[1]



MPF 활성도

- Non radioisotopic kit
- MESACUP cdc2/cdk1 kinase activity KIT (MBL International, Japan)
- ELISA, OD : 492nm



MAPK 활성도

- Western blot
- anti-pERK1/2, anti-ERK1/2 (abcam)
- Size : 42, 44 kDa

결과

Effect of caffeine on mouse oocyte vitrification and embryo development

Table. Effect of caffeine treatment during vitrification and warming of oocytes on survival, fertilization, cleavage, blastocyst rates after IVF.

Treatment		Conc. (mM)	Total No. of oocytes	Survival (%)[1]	Fertilization (%)	2C / 2PN (%)[2]	Blastocyst (%)
Fresh		0	65	65(100±0)	59(91.3±3.4)	56(95.1±1.7)	50(90.0±5.3)
Vitrified/warmed	Caffeine	0	64	61(95.3±1.6)	49(80.4±2.4)	44(89.7±4.0)	33(74.6±3.1)*
		1	65	61(93.8±0.1)	50(82.3±6.8)	47(94.4±1.9)	28(59.6±0.8)*
		10	68	65(95.3±1.6)	55(85.0±3.2)	52(94.7±1.8)	43(82.8±1.0)**
		20	63	51(81.0±2.4)*	36(70.8±4.5)*	35(96.9±3.1)	18(51.8±2.9)*

[1]Based on the number of warmed oocytes. [2]Based on the number of fertilization rate. [2]Based on the number of fertilization rate. Data are shown as means ± SEM . *4 replicates.

Effect of caffeine on mouse oocyte vitrification and embryo development

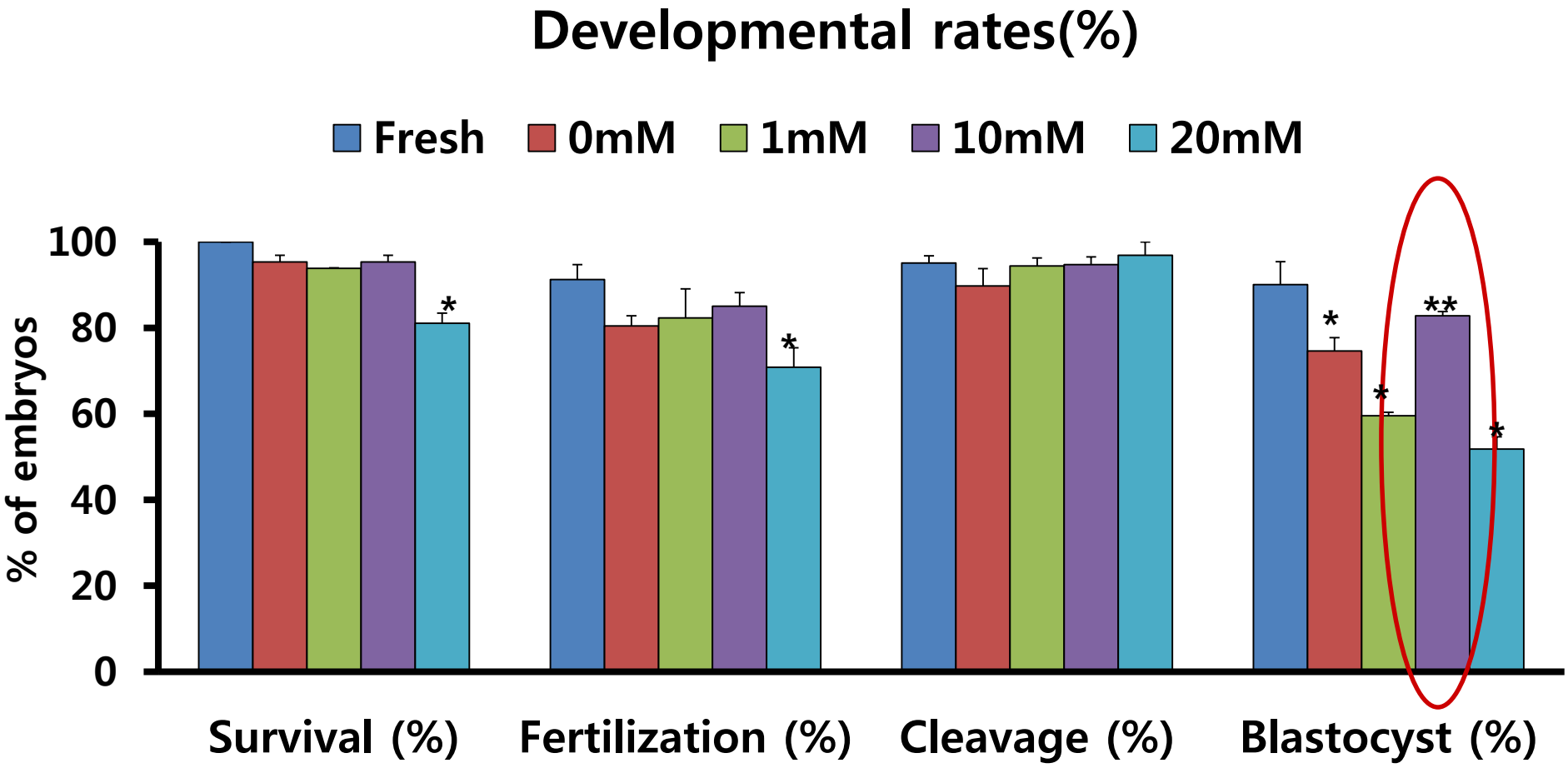


Figure 1. The effect of different caffeine (0, 1, 10, 20mM) concentration following vitrification and warming. * indicates differences between fresh and treatment ($P<0.05$), ** indicates differences between 0mM and 10mM caffeine treatment

Effect of caffeine on mouse oocyte vitrification and embryo development

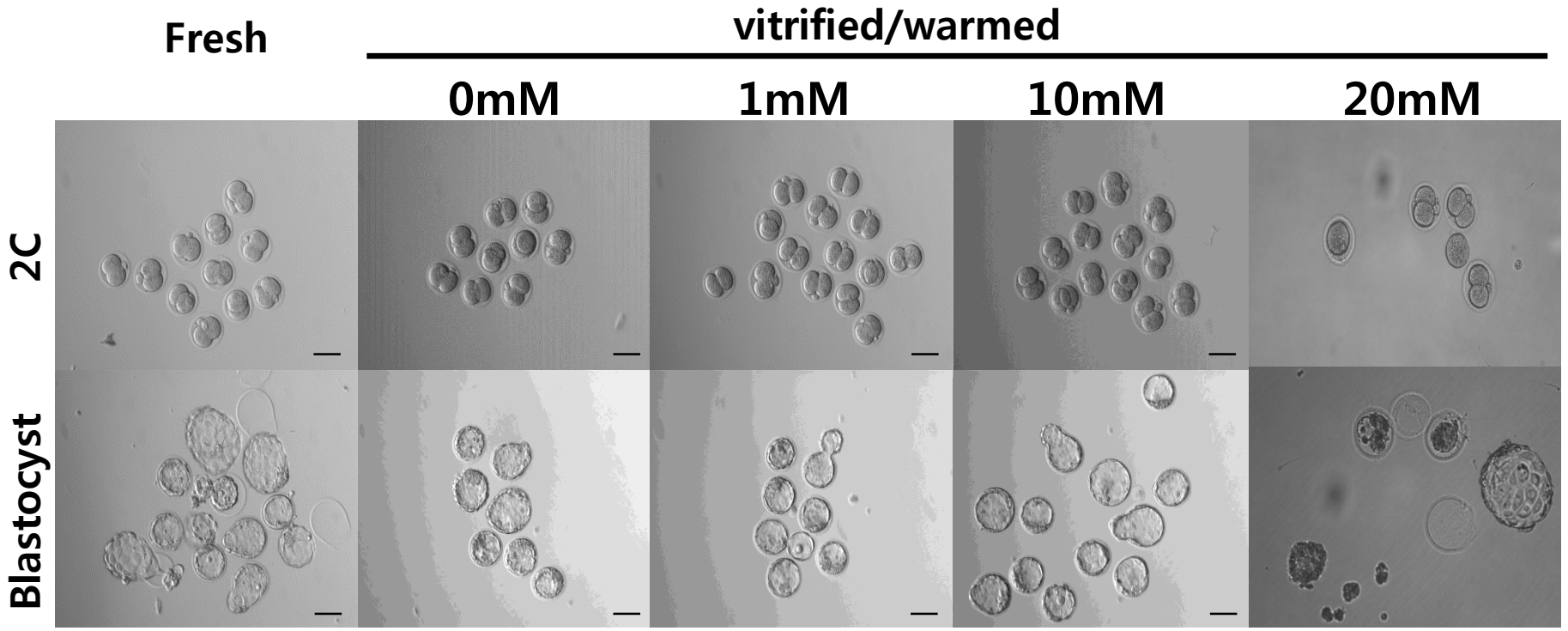


Figure 2. Effect of caffeine on mouse oocyte vitrification and embryonic development.

Effect of caffeine on mouse oocyte vitrification and embryo development

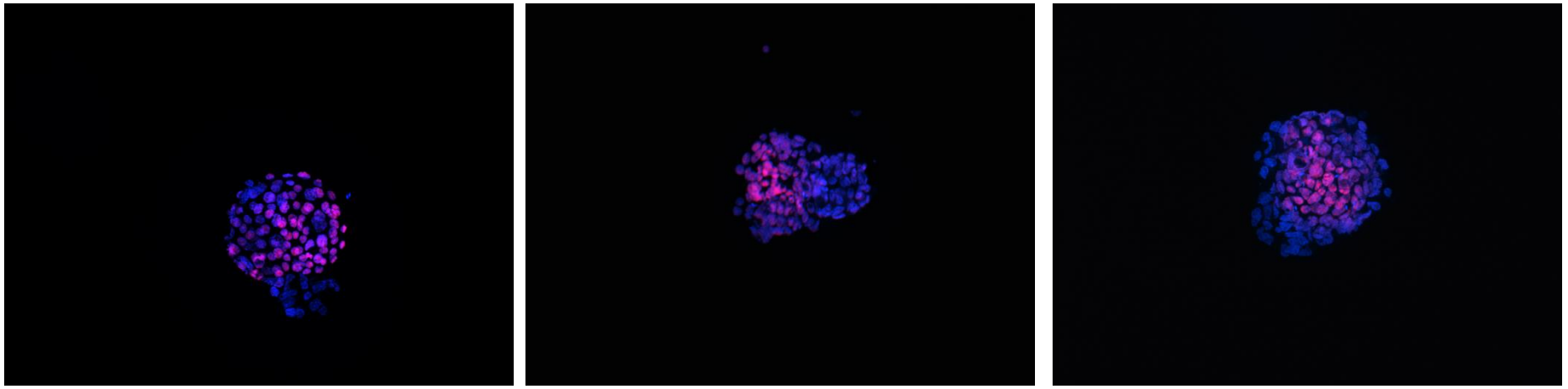
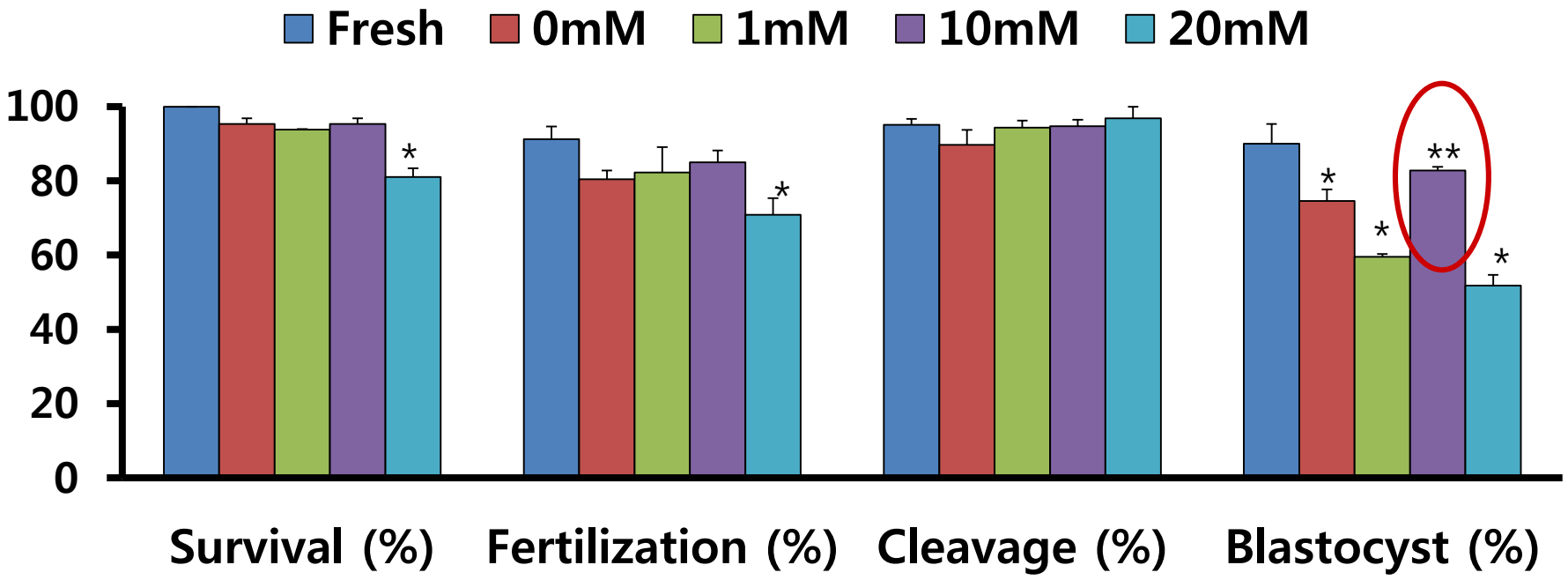
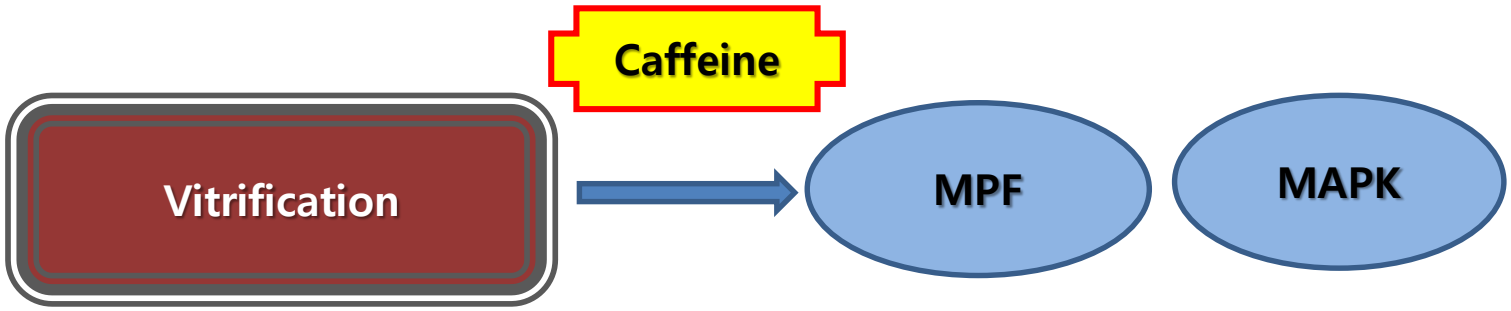


Figure 3. Differential staining of trophectoderm (TE) and inner cell mass (ICM) of blastocysts derived from Fresh , vitrified/warmed embryos.

Effect of caffeine on mouse oocyte vitrification and embryo development





Effect of caffeine on mouse oocyte vitrification and MPF activity

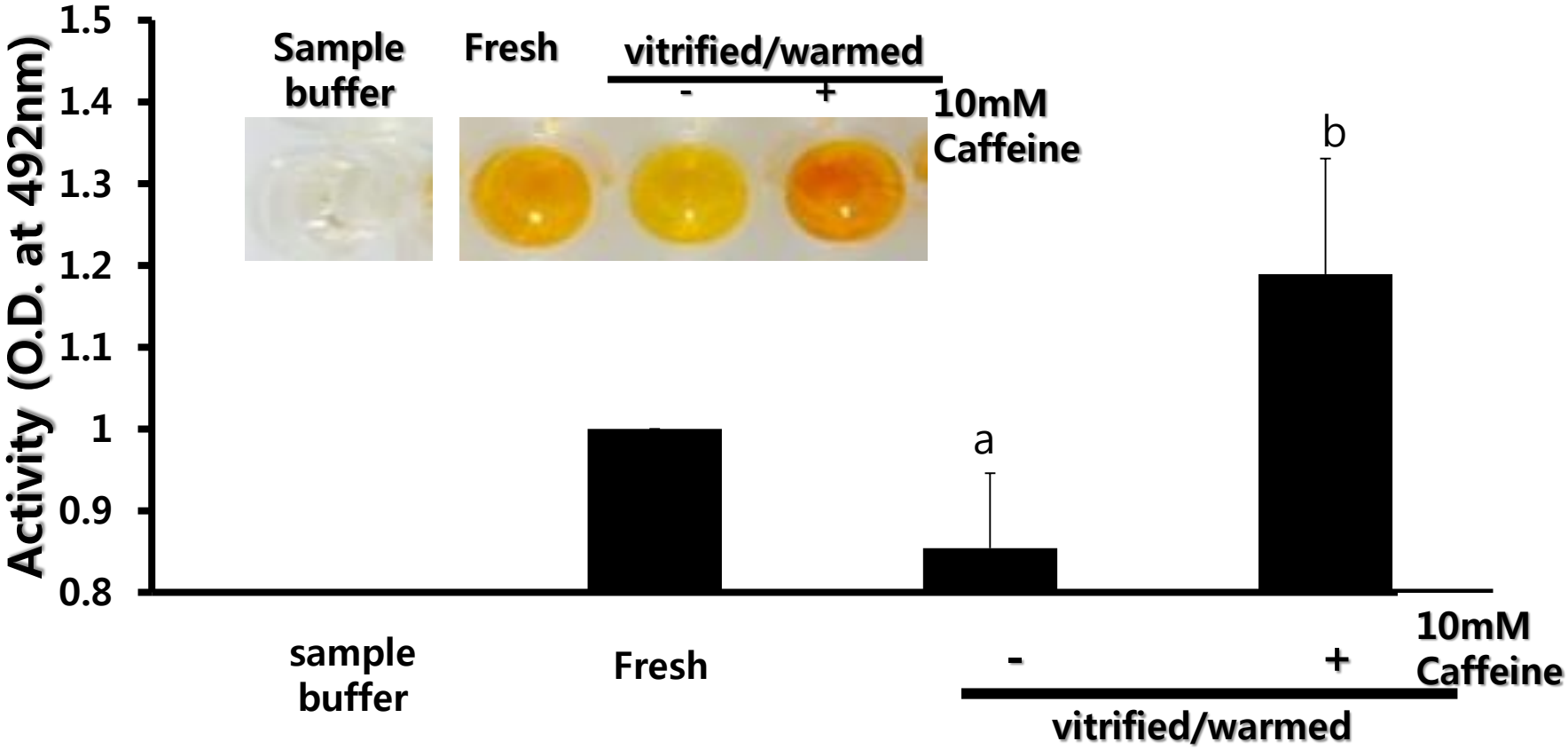


Figure 4. Effects of treatment of mouse oocytes with caffeine on MPF activity(O.D. at 492nm). Thirty oocytes were assayed at caffeine and three replicates were performed.

Effect of caffeine on mouse oocyte vitrification and MAPK activity

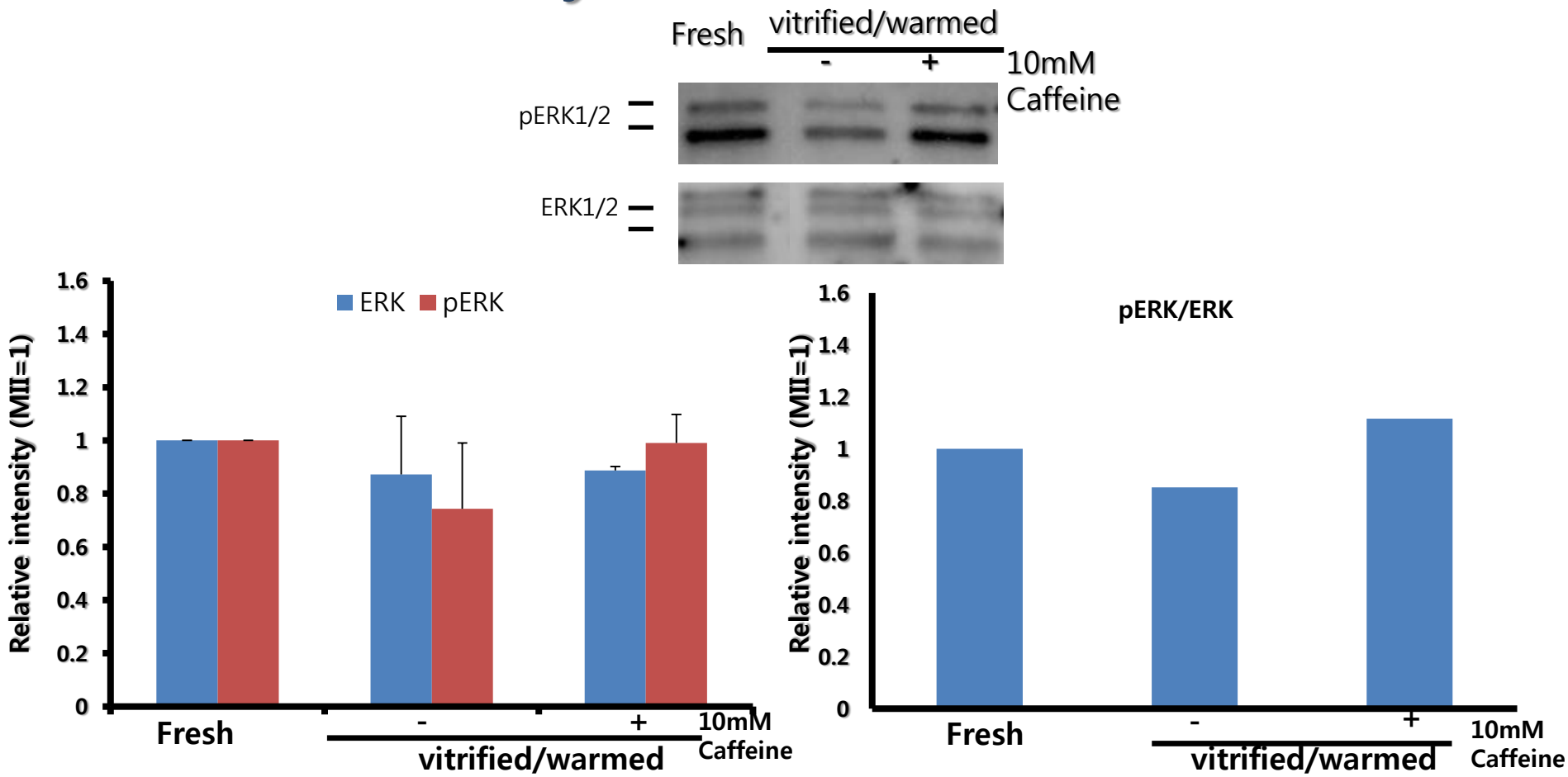


Figure 5. Effects of treatment of mouse oocytes with caffeine on MAPK activity. Twenty-five oocytes were assayed at caffeine and three replicates were performed.

결론

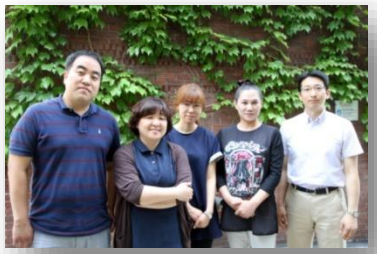
- 유리화동결, 융해시 MPF, MAPK 활성도가 감소되어지는 것을 확인함.

- Caffeine 처리시 유리화동결, 융해로 인해 감소되었던 MPF, MAPK 활성도가 회복되는 것을 확인함. 특히, MPF의 회복에 효과가 있음.

- 유리화 동결, 융해 동안 Caffeine을 처리하면 MPF, MAPK activity의 안정화에 영향을 주는것으로 보여지며, 이후 난자의 수정과 배아발달에 긍정적인 영향을 주는 것으로 보여짐.

Acknowledgement

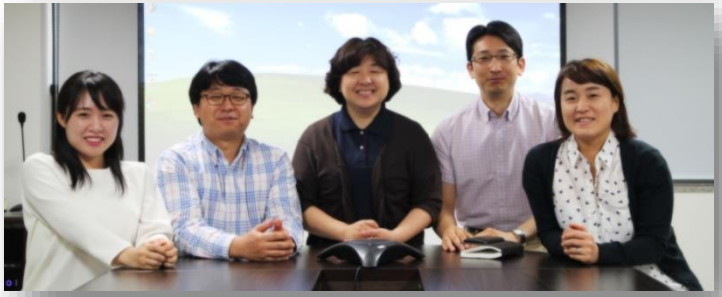
이 동 율



강남 기초의학연구소

신동혁
이진일
김민정

연구교수



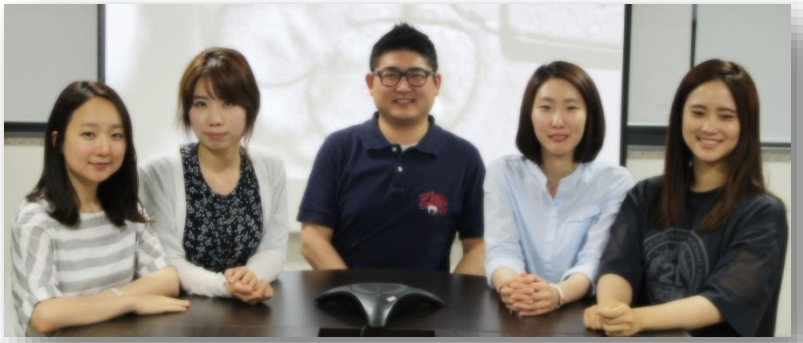
윤숙영
박세아
방재일
이아름

박사과정

설동원
신은영

석사과정

백지이
이민지
정다흰
김지나



This research was partly supported by a grant from the Korea Healthcare Technology R&D Projects, Ministry for Health, Welfare & Family affairs, Republic of Korea (A120080) and the BK21 PLUS Program (22A20130012640) through the NRF funded by the Ministry of Education, Korea.

감사합니다

예상질문

1) Human에서 이같은 실험을 해본적은?
2) GV상태에서 이같은 실험을 시도한적은?
3) Kit의 원리는?
4) 왜1mM 에서는 BL로의 발달률이 낮나?
5) 이외의 다른 추가실험을 한것은?

현재 진행중인 것은

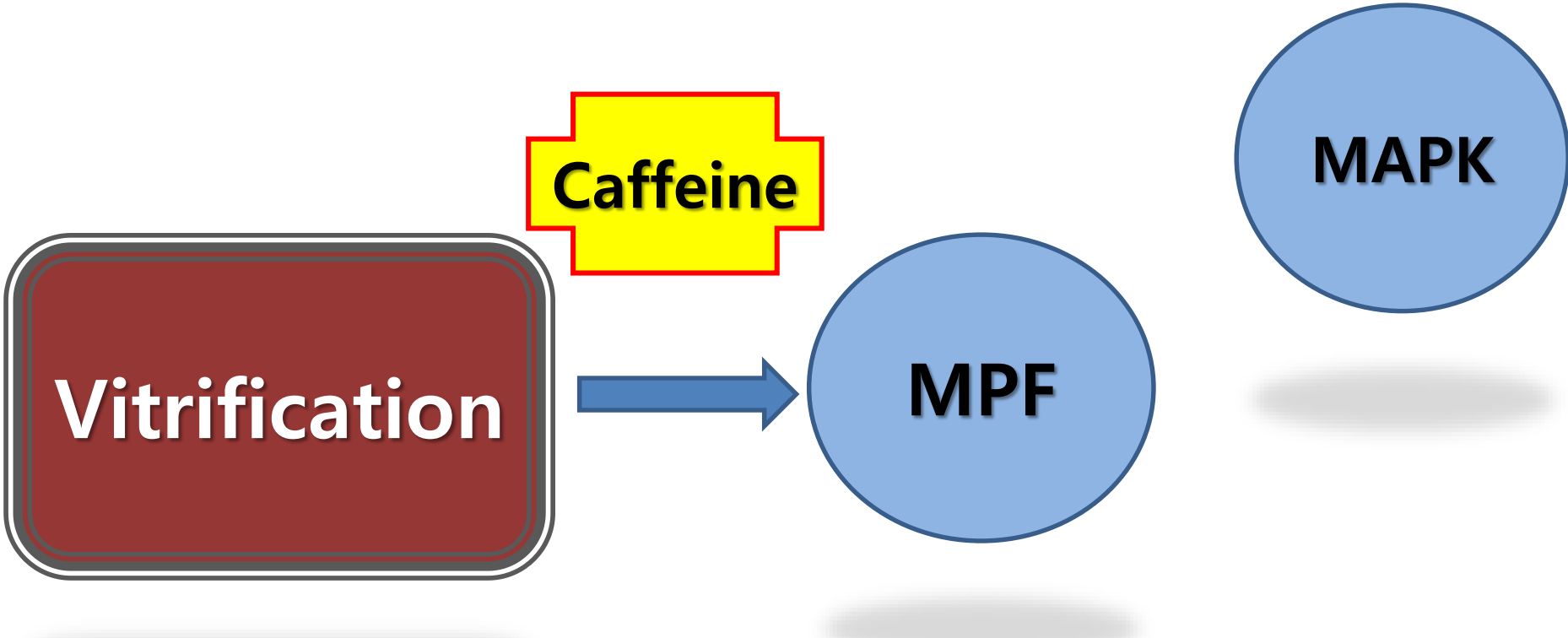
•NSET(Non surgical embryo transfer)을 이용한 각 그룹의 ET.

•해볼만한건...
•-----MII-----
•spindle check
•mitochondria potential
•ROS level
•IP3
•Ca2+
•cAMP
•Zinc
•--------------------

•------BL----차이가 없는데..
•apoptosis
•cell number(dapi로 모두다?)
•ET
•---------------

연구목적

Phosphatase inhibitor인 Caffeine을 처리하여 동결, 융해 시 손상되는 MPF, MAPK의 활성을 유지시킴과 동시에 더 나아가 향상된 수정률과 배반포 발달률을 여향을



Reprod Toxicol. 1992;6(4):309-18.

Caffeine effects on meiotic maturation in hamster oocytes in vitro.

Prather AL[1], Racowsky C.

Abstract

The effect of caffeine on meiotic maturation in cultured hamster oocytes was investigated. Meiotic status was scored from chromatin spreads of oocytes previously exposed to caffeine (0, 0.00017, 0.0017, 0.017, 0.17, 1.7, 2.4, 5.1, and 10.2 mM) for up to 20 h. While concentrations of caffeine less than 0.017 mM failed to affect significantly the onset of meiotic resumption, 0.0017 mM caffeine significantly decreased the proportion of oocytes progressing normally to telophase I-metaphase II, and concomitantly increased the proportion of both diploid MII and aneuploid oocytes. In addition, 0.17 to 10.2 mM caffeine induced a dose-dependent increase in the proportion of meiotically arrested oocytes, with less than 5% oocytes progressing normally through to the final stages of meiotic maturation at 10.2 mM caffeine. Taken together, these data show that caffeine at concentrations as low as 0.0017 to 0.017 mM interfere with progression of meiotic maturation, and that concentrations higher than 0.017 mM delay initiation of this process. Since caffeine peaks at 0.017 mM in the plasma of women following a cup of brewed coffee, we conclude that caffeine-induced perturbations of oocyte meiotic maturation may be responsible, at least in part, for the recently revealed correlation between caffeine intake and reduced fertility in women.

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Effects of Caffeine on Maturation-Promoting Factor (MPF) Activity in Bovine Oocytes and on the Development of Somatic Cell Nuclear Transfer Embryos in White-Hanwoo

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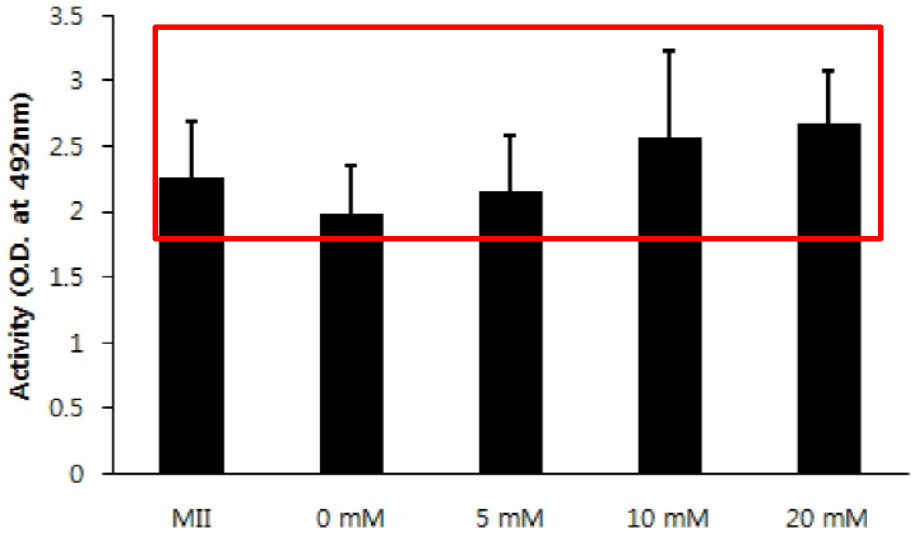

Fig. 2. Effects of caffeine on maturation-promoting factor (MPF) activity in bovine oocytes. Oocytes were denuded at 22~24 hpm, cultured in different concentrations (MII, 0 mM, 5 mM, 10 mM and 20 mM) of caffeine for 6 h and then collected in 5 μl sample buffer before snap freezing/thawing for cdc2 kinase activity. 10 oocytes were analyzed for each concentration of caffeine and three replicates were performed. Bars represent mean±SEM.