行政院國家科學委員會專題研究計畫 成果報告

著床前胚胎發育過程中蛋白質巰基亞硝基化及硝基酪氨酸所扮演的角色

研究成果報告(精簡版)

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Introduction

Nitric oxide (NO) is a highly diffusible molecule and plays an important role in mammalian reproductive function, including folliculogenesis, fertilization, and implantation (Chwalisz and Garfield, 2000). The endogenous NO is produced by conversion of oxygen and L-arginine to NO and L-citrulline, which was catalyzed by three isoforms of nitric oxide synthase (NOS), namely, the neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) (Griffith and Stuehr, 1995). All three isoforms are expressed in preimplantation mouse embryos and function as a regulator of embryo development (Tranguch *et al.*, 2003). When the activity of NOS was inhibited, the embryo development would cease making progress. These results suggested that NO is essential for pre-implantation embryo development.

The physiological effect of NO on early embryo development is not clear, although NO has been recognized as a major regulator for cell respiration and mitochondrial metabolism in several cell lines (Moncada and Erusalimsky, 2002). The respiratory electron transport chain in mitochondria is the target regulated by NO (Moncada and Erusalimsky, 2002). The mitochondria of early stage of embryos are thought to be 'immature' and inactive in the early embryos; however, it has been demonstrated that a steady level of ATP is maintained by active mitochondrial oxidative phosphorylation in the mouse oocytes (Dumollard *et al.*, 2004). Whether the mitochondrial activity is regulated by NO in the early embryos has not been investigated.

Our previous report indicated that the follicular levels of NO was associated with poor embryo quality (Lee *et al.*, 2004). Actually, NO is also a notorious free radical and an important bioregulator of apoptosis (Chung *et al.*, 2001). Embryo fragmentation in early cleavage embryos was regarded as a consequence of apoptosis in humans and mice (Chen *et al.*, 2001;Jurisicova *et al.*, 1996). Furthermore, previous reports suggested that NO might through mitochondria- dependent pathway to induce apoptosis or fragmentation during in vitro development for the preimplantation embryos (Chen *et al.*, 2001;Lee *et al.*, 2004). The effect of NO on the mitochondrial activity and related apoptosis in preimplantation embryos needs further elucidation.

The culture environment optimization for pre-implantation embryo development is an essential part for the success of assisted reproductive technology cycles (Loutradis *et al.*, 2000). The optimal culture condition for in vitro embryo growth is still under intensive investigation. The free radical, including reactive oxygen species and reactive nitrogen species, is detrimental to cell survival and responsible for the fragmentation of embryo (apoptosis) (Lee *et al.*, 2004;Chung *et al.*, 2001). Therefore, the supplementation of antioxidants into culture medium may be able to against the deleterious effect of excess amount of NO without impairment of the development potential of embryos. This study is designed to investigate the relationship between NO/NOS and mitochondrial metabolism in preimplantation mouse embryo and also tried to identify the possible antidote against the apoptotic effect of NO during in vitro culture.

Materials and methods

One-cell embryo collection and in vitro culture

Six- to eight-week-old B6CBF1 female mice were superovulated with intraperitoneal injection of 5 IU pregnant mare's serum gonadotropin (PMSG; Sigma, St Louis, MO, USA), followed 48 hr later by 5 IU human chorionic gonadotrophin (HCG; Serono, Bari, Italy). The mice were placed into the same cage

overnight with males of the same strain. Vaginal plug was checked on the next morning as the evidence of mating. Mated mice were sacrificed by cervical dislocation 24 hr later subsequent to administration of HCG. One-cell embryos (zygotes) were released from excised oviducts into modified human tubal fluid (mHTF) medium. Cumulus cells were removed by exposure to 80 IU/mL hyaluronidase (Sigma).

One-cell embryos were cultured in 1 ml of mHTF medium supplemented with sodium nitroprusside (SNP; Sigma) or N^G-nitro-L-arginine (L-NA; Sigma). The embryos were cultured under oil in groups of 10-15 embryos for up to 96 hours. The media were freshly prepared and utilized for 96 hours at 37^{o}C in a humidified atmosphere containing 5% CO_2. Embryo development was monitored at an interval of 24 hours. The number of embryos developing to the expanded blastocyst stage was assessed after 96 hours of culture. The 2-cell block is defined as the development of embryos arrests at the 2-cell stage without apparent degeneration for 48 hours.

Analysis of mitochondrial membrane potential

Oxidative phosphorylation and ATP production is driven by an electrochemical gradient which is created by pumping protons from inside the mitochondrial membrane to outside. This process establishes mitochondrial membrane potential (MMP) and helps to estimate the metabolic function through measure of differences in MMP. The potential sensitive fluorescence dye 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazoyl carbocyanine iodide (JC-1, DePsipher^{TM} Kit TA700; R&D Systems Inc., USA) was used to measure the activity of mitochondria (Reers *et al.*, 1991;Reers *et al.*, 1995). The dye is a lipophilic cationic dye that enters the inner mitochondrial matrix in its monomeric form when the mitochondrial membrane is polarized. When the mitochondrion has a high mitochondrial membrane potential, the dye across the membrane and forms J-aggregates, which fluorescence red under UV light. If the membrane potential is low, the dye remains in the monomeric form and appear green.

The JC-1 dye was prepared from stock solutions according the manufacture's recommended concentration (1 µl DePsipher^{TM}, 900 µl ultrapure water, 100 µl reaction buffer and 10 µl stabilizer) directly prior to use. The solution was pre-warmed in a 37^{0}C water bath and then was put into the culture medium with embryos for 25 min for staining in a humidified incubator at 37^{0}C, 5% CO_2. Subsequent to staining, the embryos were washed individually with PBS two times and placed in microdrops of mHTF medium under light mineral oil (Sigma) in culture dish at 37^{o}C. A Leica TCSNT laser scanning confocal microscope (LSCM, Leica) was used for all experiment.

Assay of apoptosis

Apoptosis assay for blastomeres was performed with terminal deoxynucleotidyl transferase (TdT) mediated dUTP nick end labeling (TUNEL) assay. The TUNEL was utilized to label DNA strand breaks, which is an apoptotic event subsequent to cleavage of genomic DNA. A commercial kit, In Situ Cell Death Detection Kit, Fluorescein (Roche Applied Science, Penzberg, Germany) was applied to detect DNA stand breaks. The procedure was modified from our previous report (Huang *et al.*, 2005).

S-Nitrosylation proteins in embryos

We modified the method for detecting S-nitrosoproteins in situ as described previously (Yang and Loscalzo, 2005; Jaffrey *et al.*, 2001). This method depends on first blocking thiols with a rapidly acting thiol-reactive agent S-methyl methanethiosulfonate (MMTS, Sigma), followed by reducing the S-nitrosothiols with ascorbate (Sigma), after which the thiols generated by ascorbate reduction are labeled with a fluorescent derivative of methanethiosulfonate (MTSEA-Texas red, Toronto research Chem). Specifically, cells were first

fixed in methanol (Romil) at -20℃ for 10 min. Thiol groups were then blocked with 200mM MMTS (chosen for its rapid reaction kinetics) in 80% methanol containing 100 mM Hepes (pH 7.7, Gibco BRL), 1 mM EDTA (Gibco BRL), and 0.2 mM neocuproine (Sigma) named HEN_methanol, at 50℃ for 30 min. The cells were then washed four times with HEN_methanol, after which they were incubated with 0.2 mM ascorbate and 0.2 mM MTSEA-Texas red in HEN_methanol at room temperature for 1 h. Excess dye was removed by washing the cells repeatedly with methanol. Cell mitochondria were stained with 20 μM MitoTracker Green FM (Invitrogen, molecular probes) for 20 min then washed four times with PBS (Gibco). Stained cells fixed to slides were then treated with Prolonged Antifade Mounting Medium. Fluorescent images were taken with a Leica TCSNT confocal microscope. Eight fields magnified _400 were analyzed per embryo. Fluorescence intensity was quantified by subtracting background fluorescence, then integrating the image with the photoshop software.

The addition of antioxidants and analysis of blastocyst development

To counteract the apoptotic action of NO on the development of mouse embryos, the culture media were further supplemented with the following antioxidants. First, Mn(III) tetrakis(4-benzoic acid) porphyrin choloride (TBAP; Cayman Chemical, Ann Arbor, MI, USA) is a superoxide dismutase (SOD) mimetic agent and is able to dismuation of the superoxide radical (O_2^-) (Faulkner *et al.*, 1994). Second, the peroxynitrite scavenger ebselen (EBS; Sigma) at 10 μ M was utilized in order to eliminate the detrimental reactive nitrogen species, peroxynitrite (Lee *et al.*, 2002). Third, glutathione methyl ester (GSH; Sigma) at 1 mM was applied to the culture medium to reduce the oxidative stress and to prevent the S-nitrosylation action of NO on potential enzymes (Beltran *et al.*, 2000).

The number of embryos developing to the expanded blastocyst stage (blastocyst formation rate) was assessed after 96 hours of culture. In order to evaluate the afterward development potential of the blastocysts derived from above culture conditions, the total cell numbers and apoptotic cell numbers were calculated after DAPI and TUNEL stain for DNA and apoptotic DNA fragmentation, respectively. In addition, the amount of ATP for individual blastocyst was also measured.

Statistical analysis

The rate of blastocyst formation was tested for significance using the χ^2-test or Fisher's exact test determined by the condition. JC-1 staining intensity data were subjected to Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks followed by Dunn's test to evaluate the significance of the results within pairs of groups. All the analyses were performed using the Statistical Package for the Social Sciences (version 14.0; SPSS Inc., Chicago, IL, USA). A confidence level of $p < 0.05$ was considered to constitute the limit of statistical significance.

Results

The development of pre-implantation mouse zygotes after exposure to variable concentration of NO donor (SNP) and NOS inhibitor (L-NA) during in vitro culture was demonstrated in Table I. When the concentration of SNP was greater or equal to 1 μ M, no blastocyst formed under such culture condition. The supplementation of SNP into culture medium suppressed the rate of blastocyst formation and elevated the rate of embryo degeneration, while the application of L-NA also inhibited the in vitro development of mouse embryos. Most of the embryos (83.3%) arrested at two cell stage of development at the level of 25 μ M L-NA (Table I).

In order to elucidate the effect of NO/NOS on the pre-implantation embryo development and

metabolism of mitochondria, inner membrane potential of mitochondria and occurrence of apoptosis in 2-cell stage embryos were investigated. The membrane potential was presented as the intensity of red fluorescence under UV light (Fig. 1A). The supplementation of SNP would induce reduction of inner membrane potential of mitochondria in a dose-dependent manner at the 2 cell stage (Fig. 1C). However, the addition of L-NA at the concentration of arresting embryo development did not significantly affect the inner membrane potential of mitochondria at the 2 cell stage of embryos (Fig. 1D).

The TUNEL test was utilized to demonstrate the occurrence of apoptosis here. Almost no evidence of apoptosis was demonstrated by TUNEL stain at 2-cell stage embryos. Nonetheless, the supplementation of SNP into culture medium induced apoptosis of preimplantation mouse embryos with degeneration (Fig 2A), while L-NA did not cause degeneration of mouse embryos until 48 hours later.

The nitric oxide-mediate protein modification were detected by antibody against protein S-nitrosylation at 2 cell stage. The addition of SNP induced elevated level of S-nitorsyaltion, while the levels of S-nitrosylation was lower in L-NA treated embryos. The addition of SNP and LNA resulted in naturalization of S-nitrosylation, which was similar to controls.

The addition of antioxidants (TBAP, GSH and EBS in this study) into culture environment after exposure to NO donor was evaluated for the capability of such antioxidants to reverse the detrimental effect of SNP on mouse zygote development (Fig. 3). The blastocyst formation rate in the control groups were around 90% in each experiment. The supplementation of 0.1 μ M SNP reduced the blastocyst formation rate to a level around 20%. The supplementation of TBAP resulted in a little reduction of blastocyst formation rate (Fig 3A). However, the addition of GSH was not harmful for the development of mouse embryos (Fig 3B). Nonetheless, the TBAP and GSH were able to reverse the adverse effect of SNP on embryo development (Fig 3A and 3B). Unfortunately, EBS was not able to against the deleterious effect of SNP on preimplantation embryos in this culture system. Actually, the supplementation of EBS itself into culture medium exhibited obviously detrimental effect on blastocyst formation (Fig 3C).

The cell number within each formed blastocyst was at a similar level demonstrated by DAPI stain (Table 2). The occurrence of apoptosis in individual blastomeres was revealed by TUNEL assay. Interestingly, those blastocysts treated with NO donor exhibited a higher percentage of apoptotic cells than those cultured with supplementation of TBAP, GSH, SNP+TBAP, and SNP+GSH (Table 3).

Discussion

The results of this study demonstrated that SNP (a donor of NO) was detrimental for preimplantation embryo development and would evoke embryo fragmentation and apoptosis. In contrast, L-NA (an inhibitor of NOS) arrested mouse embryo development at 2-cell stage but did not directly induce embryo degeneration. These data confirmed that NO was an important regulator for early development of embryos (Chen *et al.*, 2001). The supplementation of NO to culture medium would induce arrest of development (Joo *et al.*, 1999) and apoptosis of embryos (Chen *et al.*, 2001;Tranguch *et al.*, 2003). However, inhibition of NO production by NOS inhibitor would also impair the development of embryos (Tranguch *et al.*, 2003). In order to reverse the effect of NO on embryo growth, direct inhibition of NO production in embryos by NOS inhibitors was not an effective way.

In this study, the NO donor SNP exerted a direct effect on mitochondrial membrane potential in a dose-dependant manner (Fig. 1). The disruption of mitochondrial membrane potential was viewed as an early sign of apoptosis (Barroso *et al.*, 2006). The occurrence of apoptosis was further confirmed by TUNEL assay

in those degenerated cleavage stage embryos or blastocysts (Fig. 2 and Fig. 3). The results were similar to our previous finding in humans that exposure to a high level of follicular fluid NO was associated with poor quality of embryos (high levels of fragmentation) (Lee *et al.*, 2004). Furthermore, our previous study indicated that the detrimental effect of NO from follicular fluid was not through Fas-related apoptosis pathway during embryo development (Lee *et al.*, 2004). This study further supported that the detrimental effect of NO on embryos acted through mitochondria- dependent apoptosis pathway.

On the other hand, NOS inhibitor L-NA did not exert direct effect on mitochondrial membrane potential, but L-NA reduced the level of protein S-nitrosylation . During the culture process, those embryos exposure to L-NA most arrested at the 2 cell stage. The 2-cell block phenomenon occurred after the supplementation of NOS inhibitors in this study. The observed results were consistent with that reported at 2003 by Tranguch (Tranguch *et al.,* 2003). The adverse effect of L-NA might be not through mitochondrial- dependant apoptotic pathway. Our results further confirmed that endogenous NO might be necessary for early embryo development. Direct inhibiting NOS activity and then abolishing NO production was also detrimental in terms of embryo development.

In most mammalian species, mitochondria are exclusively maternally derived and oocyte mitochondria display some unique features, such as spherical, a very dense matrix, a low number of cristae and possible low metabolic activity (Jansen and de Boer, 1998). Although this type of ‘immature’ mitochondria in mammalian fertilized eggs are different from the ‘mature’ mitochondria in somatic cells, it has been reported that the so called ‘immature’ mitochondria are involved in the early phase of oxidative stress-induced apoptosis (Liu *et al.,* 2000) . Our data suggested that NO was able to cause decline of mitochondrial membrane potential in early embryos and then induce apoptosis in degenerated embryos and even in the developed blastocysts.

As the mouse embryos cleaved from one-cell embryos to blastocysts, the mitochondrial activity (evident by mitochondrial membrane potential) changed gradually (Acton *et al.,* 2004). The elevation of the ratio high- to low-polarized mitochondria was observed in 2 cell embryos and concurrent with mouse genome activation (Acton *et al.,* 2004). Furthermore, the NO was the major regulator of mitochondrial respiration (Moncada and Erusalimsky, 2002) and endogenous NO has been reported to trigger mitochondria biogenesis in several type of somatic cells (Nisoli *et al.,* 2003). By this physiological way, endogenous NO may be the essential regulator of mitochondrial activity or biogenesis during this period of embryo development. Unfortunately, our experiments only revealed that the supplementation of SNP exhibited apoptotic effect on the embryo development instead of increasing mitochondrial metabolism. In contrast, totally inhibiting NO production by L-NA brought deleterious effect to the embryos and the results supported that endogenous NO in early embryos are necessary for development.

The apoptotic effect of NO has been proposed to be a consequence of excessive production of peroxynitrate or hydroxyl peroxide in mitochondrial respiratory complex (Moncada and Erusalimsky, 2002). The antioxidant agents that would eliminate such reactive oxygen species might be the candidates to alleviate the deleterious effect of NO and improve the embryo development in vitro. The oviducts are the major in vivo environment for sperm-oocyte interaction and zygote development; both developmental stages are vulnerable to oxidative stress or excessive free radial. Several kinds of antioxidants, such as glutathione, were discovered within tubal fluid and enzymes, like superoixde dismutase, glutathione reductase were expressed in tubal epithelium (Guerin *et al.,* 2001). The results of this study indicated that TBAP (superoxide scavenger) and reduced form of glutathione were able to reverse the toxic effect of NO. Base on these reports, antioxidants

might be a beneficial supplement to culture medium for in vitro development of embryos in IVF/ICSI cycles.

Nitric oxide has been reported to be an important modulator for mitochondrial oxidative phophorylation. Specifically, NO could reversible inhibit cytochrome C oxidase (complex IV) and then complex I (Beltran *et al.*, 2000) in the respiratory chain. Although NO has been able to regulate the activity of mitochondrial metabolism, high level of NO would induce irreversible modification in complex I and then disruption of the mitochondrial membrane potential. Subsequently, the apoptosis pathway was activated and resulted in fragmentation and degeneration of embryos.

The apoptotic effect of NO could not be erased by TBAP in a previous report for fibroblast in a hypoxia state (Lee *et al.*, 2002). The different effect might be resulted from different kind of cells used or culture environment. On the other hand, the preoxynitrate scavenger ebselen was unable to opposite the apoptotic effect of NO on embryos, which was similar with previous report (Lee *et al.*, 2002).

The embryo exposed to NO and antioxidants showed the capability of development competence to blastocyst and mitochondrial metabolism for ATP production. In addition, the total cell number and the percentage of apoptotic cell of the SNP-GSH treated and SNP-TBAP blastocysts did not differ from those of the control group. These results suggested that the blastocysts were normal not only in morphological criteria, but also in terms of mitochondrial activity within the blastocyst. Subsequent to supplementation of antioxidants, the embryos might escape from the deleterious effect of NO.

In conclusion, the apoptotic effect of NO on embryo development is closely related to the mitochondrial membrane potential. Total abolishing NO production by NOS inhibitor is also deleterious to embryo development, although the mitochondrial membrane potential is not directly affected. The supplement of TBAP (superoxide scavenger) and reduced form glutathione to NO-exposed embryos was able to keep going on development, adequate proliferation of blastomeres, and ATP production in blastocysts. Further studies would be necessary in order to extend the results to in vitro culture of human embryos in IVF/ICSI cycles, especially for those patients characterized with endometriosis and hydrosalpinx.

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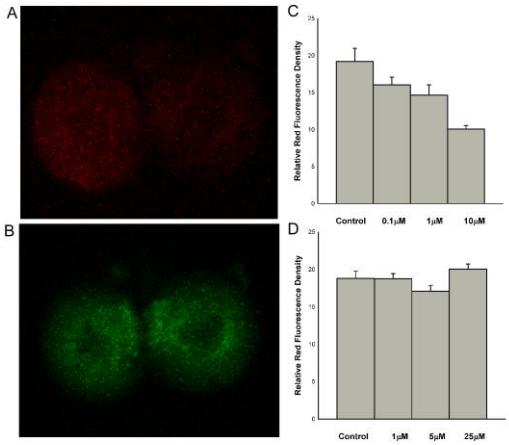

Figure 1. The effect of nitric oxide on mitochondrial membrane potential. A and B) MMP was presented as the intensity of red/green fluorescence under UV light. C) Supplementation of SNP would induce reduction of inner membrane potential of mitochondria in a dose-dependent manner at the 2 cell stage. D). Addition of L-NA at the concentration of arresting embryo development did not significantly affect the inner membrane potential of mitochondria at the 2 cell stage of embryos.

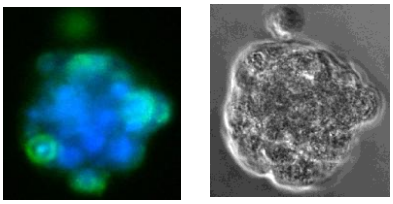

Figure 2. The TUNEL test was utilized to demonstrate the occurrence of apoptosis. The supplementation of SNP into culture medium induced apoptosis of preimplantation mouse embryos (morula stage) with degeneration

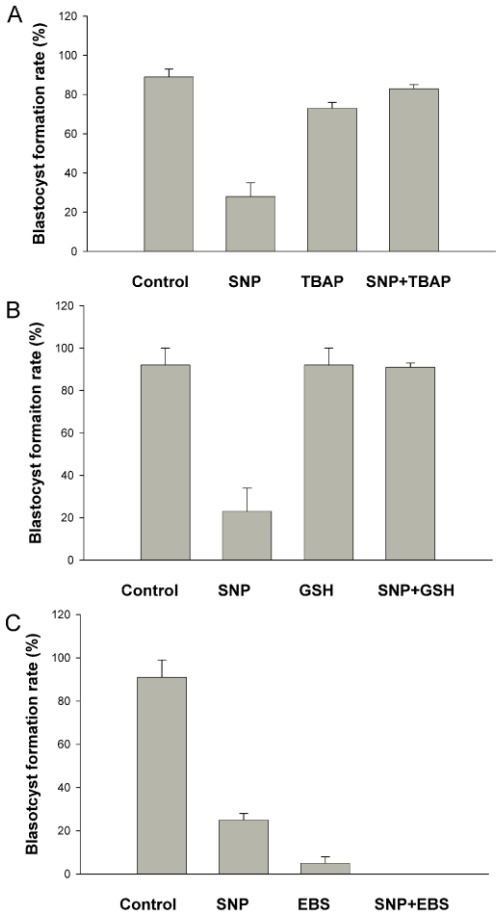

Figure 3. The addition of antioxidants (TBAP, GSH and EBS in this study) into culture environment after exposure to NO donor was evaluated for the capability of such antioxidants to reverse the detrimental effect of SNP on mouse zygote development. The blastocyst formation rate in the control groups were around 90% in each experiment. The supplementation of 0.1 μ M SNP reduced the blastocyst formation rate to a level around 20%. A) The supplementation of TBAP resulted in a little reduction of blastocyst formation rate. B) The addition of GSH was not harmful for the development of mouse embryos Both the TBAP and GSH were able to reverse the adverse effect of SNP on embryo development. C) EBS was not able to against the deleterious effect of SNP on preimplantation embryos in this culture system.

Table I. Outcome of pre-implantation development of mouse embryos after exposure to nitric oxide donor (sodium nitroprusside, SNP) and nitric oxide synthase inhibitor (L-NA).

	Rate of 2-cell block (%)	Degeneration rate (%)	Blastocyst formation rate (%)
Control	(0)	(1)	24/28 (85.3)
0.1μM SNP	7/48 (14.6)	(12)	9/51 (17.6)
1μM SNP	(19.5)	(20)	0/42 (0)
10μM SNP	(18.7)	22/48 (45.8)	0/48 (0)
Control	0/(0)	(0)	25/30 (83.3)
1μM L-NA	(3.3)	(4.2)	8/24 (33.3)
5μM L-NA	(41.7)	(24)	2/24 (8.3)
25μM L-NA	(83.3)	(43.3)	0/26 (0)

Table II. The number of cells, amount of ATP production, and percentage of TUNEL-positive blastomeres in the blastocysts after exposure to NO donor with or without addition of antioxidants.

Variable	Control	SNP	GST	TBAP	SNP+GST	SNP+TBAP
Number of blastocysts	30	28	30	24	27	27
Number of blastomeres	63.7±2.3	51.3±1.7[a]	61.6±3.2	57.5±4.1	58.0±1.9	60.0±1.5
Number of apoptotic cells	2.9±0.4	4.3±0.7	2.1±0.4	2.1±0.4	3.1±0.8	2.2±0.3
Proportion of apoptotic cells (%)	4.6±0.7	8.5±1.3[a]	3.6±0.8	4.0±0.9	5.4±1.3	3.8±0.5
Amount of ATP production	±	±	±	±	±	±

Data are presented as Mean±SEM

[a] Statistically significant ($P < 0.05$) after Kruskal Wallis test

無衍生研發成果推廣資料

98 年度專題研究計畫研究成果彙整表

計畫主持人：李宗賢	計畫編號：98-2314-B-040-012-
計畫名稱：著床前胚胎發育過程中蛋白質巰基亞硝基化及硝基酪氨酸所扮演的角色	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	0	1	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	1	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	無

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：□已發表 □未發表之文稿 ■撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限）

此研究首度證實小鼠著床前鼠胚已經有蛋白質巯基亞硝基化的現象發生，而一氧化氮提供試劑或一氧化氮合成酶抑制劑則會干擾這個現象，進而影響鼠胚的發育。而 Glutathione 在此的角色則是可以降低一氧化氮對粒腺體或內質網蛋白質巯基亞硝基化的作用，進而使胚胎在高濃度一氧化氮的壓力下能正常發育。