EFFECT OF NITROGLYCERIN ON IN VITRO MATURATION OF SHEEP OOCYTES

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ABSTRACT

This Study was conducted to investigate the effects of different concentrations of nitroglycerine (NTG) (0, 0.05, 0.1, 0.15, 0.3 AND 0.5 µM) supplied with two types of culture media (Roswell Park Memorial Institute – 1640; RPMI- 1640 and simple medium for assisted reproductive technology; SMART on *in vitro* maturation (IVM) of sheep oocytes. This study was executed in the laboratories of the Institute of Embryo Research and Infertility Treatment / AL- Nahrain University during the period from December, 2009 to June, 2010. The ovine ovaries were used as a source of oocytes. oocytes were collected using aspiration technique. One thousand and three hundred and twenty four oocytes were collected from 844 ovaries obtained from local abattoir. Most of recovered collected oocytes were immature (1139 oocytes). A significant ($p< 0.05$) increase in IVM oocytes with using 0.05 and 0.1 µM of NTG supplied with RPMI – 1640 as compared with control and other treated groups . On the other hand, using of SMART in comparison with 0.05µ M NTG lead to an obvious ($p < 0.05$) increases in IVM oocytes as compared with the control and remaining treated groups. In conclusion, using 0.05 and 0.1µM of NTG within RPMI-1640 medium produces improvement in the percentages of IVM as well as Enrichment 0.05µM of NTG to SMART medium produced increases percentages of IVM, while supplemented 0.5 µM of NTG to RPMI-1640 and SMART media produced decreases percentages of IVMP

*part of M.Sc. thesis of second auther .

تأثير النايتروكليسرين في انضاج بويضات الاغنام مختبرياً

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المستخلص

أجريت هذه التجربة بهدف بيان تأثير أستخدام تراكيز مختلفه من النايتروكلسرين 0.5 و 0.3 و 0.15 و0.1 و0.05 و0 مايكرومول مضافه الى نوعين من الاوساط الزرعيه SMART وRPMI-1640 في أنضاج البويضات الاغـــنـــــام مختبريا" . نفذت التجربة في معهد أبحاث الاجنه وعلاج العقم التابع لجامعة النهرين للفتره من كانون الاول 2009 ولغاية حزيران 2010 . أستخدمت مبايض الاغنام كمصدر للبويضات اذ تم جمع 1324 بويضه من 844 مبيض. وتم جمع البويضات بستخدام تقنية الشفط وكانت معظم البويضات المسحوبة هي بويضات غير ناضجة اذ بلغت 1139. حوالي 10-5 بويضات لكل قطرة 1 ملليتر من الوسط الزرعي المخصص لهذة المجموعة (RPMI-1640وSMART) مع تركيز النايتروكلسرين مضافاً اليه 10IU من الهرمون المشيمي البشري (HCG) و 5IU من هرمون مصل الفرس الحامل (PMSG) 1µg/mL من هرمون الاستروجين وحضنت البويضات في الاطباق الزرعية لمدة 24 ساعة في حاضنة مجهزة ثنائي اوكسيد الكربون بتركيز 5% و درجة حرارة 38.5 مئوية مع رطوبة عالية (95%) كانت هنالك زياده معنويه ($p<0.05$) في أنضاج البيوض مختبريا" عند أستخدام 0.1 و0.05 مايكرومول من النايترو كلسرين مع الوسط الزرعي RPMI–1640 مقارنة مع مجموعة القياس والمعاملات الاخرى قيد الدراسه . ، أدى أستخدام الوسط الزرعي SMART المضاف اليه 0.05 مايكرومول من النايتروكلسرين الى حدوث زياه معنويه في أنضاج البويضات مختبريا" مقارنه مع معاملة القياس والمعاملات الاخرى . يمكن الاستنتاج أن التركيز المنخفض للنايتروكلسرين 0.05 و 0.1 مايكرومول مضافاً الى الوسط الزرعي RPMI–1640 له تأثير أيجابي على أنضاج البويضات مختبريا"وكذلك اضافة 0.05 مايكرومول نايتروكلسرين الى الوسط الزرعي SMART ادى الى زيادة أنضاج البويضات مختبريا"بينما اضافة النايتروكلسرين الى كلا الوسطين الزرعين RPMI–1640 وSMART كان لة تأثير سلبي في أنضاج بويضات الاغنام مختبريا.

*جزء من رسالة ماجستير الباحث الثاني

Introduction

Nitroglycerine (NTG) is colorless oil, soluble in alcohols but insoluble in water. The molecular formula of NTG is $C_3H_5 (ONO_2)_3$, it has a high nitrogen content (18.5%) and contains more than enough oxygen atoms to oxidize the carbon and hydrogen atoms when nitrogen is liberated, so it is one of the most powerful explosives, the NTG was affected by formation of NO by the mitochondrial aldehyde dehydrogenase (mtALDH) (1) and glutathione S-transferases (GST) (2), those enzymes are available in several mammalian cells such as sperm and oocyte (3). The process of nuclear maturation in the oocyte begins with germinal vesicle breakdown (GVBD). The GVBD is typically observed 4-8 hours after the pre ovulatory LH peak and is characterized by the recession or breakdown of the nuclear membrane (4). Following GVBD, the oocyte resumes meiosis and progresses through metaphase I (MI), anaphase I, and telophase I, resulting in the extrusion of the first polar body (5). The oocyte then arrests again at the metaphase II (MII) stage of meiosis. It is at the MII stage that the oocyte is ovulated and ready for fertilization (6). A growing amount of experimental data indicates that NO can induce its biological effects even via cGMP-dependent pathways (binding to heme-containing proteins other than sGC) (7). However, It is documented that cGMP has an important role in maintaining the meiotic arrest of oocytes (8). The exact mechanisms through which NO influences oocyte maturation have not been reported. Amidi *et al* (9) found that a complete prevention of GVBD was only obtained after exposure to high concentration of SNP for 1–5 hours. This effect is very similar to that of for skolin, a stimulator of adenylate cyclase (AC), which can stimulate cumulus cells to produce a positive signal (10). The objective of this study was to inspect the influence of different NTG concentrations supplied with two types of culture media (RPMI-1640 and SMART) on oocytes IVM.

Materials and Methods

The first experiment aimed to study the effect of six concentrations of NTG (control, 0.05, 0.1, 0.15, 0.3, and 0.5 μM) on IVM of oocytes in sheep, while the second experiment aimed to study the influence of two types of culture media (SMART, RPMI-1640) on the results of, IVM in sheep. This study was undertaken using oocyte collected from ovarian follicles of ewes which were slaughtered in AL-Shualla local abattoir. Both ovaries were collected from each animal, immediately after slaughtering and placed into glass tubes contained normal saline solution (0.9% NaCl) supplemented with antibiotics (100IU/mL penicillin and 100 μg/mL streptomycin), and placed it into thermos at 30-35°C . Ovaries were transported to the laboratory within at least

2h. From all visible follicles on the ovarian surface with 2-6 mm diameter, oocytes were collected using aspiration technique. Oocytes were washed three times in culture medium containing 5% human serum albumin (HAS) to remove substances in follicular fluid, than, about 5-10 oocytes per droplet (1mL) from culture medium allocated to this group (RPMI-1640,SMART)with concentrations of NTG, supplied with 10 IU/mL hCG, 5 IU/mL PMSG and 1µg/mL estradiol and cultured in four well Petri dish and covered by liquid paraffin was incubated for about 24 h in CO_2 incubator (5% CO_2) at 38.5°C with high humidity (95%).

Statistical analyses

The data were statistically analyzed using SPSS/PC version 10 software (SPSS, Chicago). IVM percentages were analyzed using complete randomized design (CRD). The Statistical model was

$Yij = \mu + Ti + eij.$

Where Yij= dependent variables (IVM %), µ= overall mean, Ti= effect of treatments (NTG, 0, 0.05, 0.1, 0.15, 0.3, 0.5µM and RPMI-1640 and SMART media, eij= error term. Differences among means were computed using the Duncan multiple ranges test (11).

Results and Discussion

In this study 1324 oocytes were collected from 844 ovaries obtained from local abattoir. Most of recovered collected oocytes were immature oocytes (1139 oocytes).The addition of NTG (0.05µM T2 and 0.1µM T3) to RPMI-1640 medium significant increased ($P< 0.05$) in percentage of IVM, while 0.5µM of NTG was significant declined ($P< 0.05$) IVM percentage as compared to control and other treated groups (Table 1).

Table 1. Percentages of *in vitro* maturation using RPMI-1640 medium enriched with different concentrations of NTG (Mean±S.E).

concentrations of NTG / Parameters	Control	0.05µM	0.1 µM	0.15 µM	0.3 µM	0.5 µM
NO. of immature oocytes	58	68	70	58	67	61
No. of matured oocytes	36	45	48	34	31	15
IVM (%)	61.818 b ±0.93	66.111 a ±0.90	68.402 a ±1.33	58.666 c ±0.22	46.250 d ±1.34	24.523 e ±0.94

* * Means with different superscripts within each row are significantly different ($P<0.05$).

Addition of 0.05µM NTG; to SMART medium significant increased (P< 0.05) IVM percentage, In contrast, significant decline (P< 0.05) in the percentage of IVM using of 0.5µM NTG was noted as compared to control and other treated groups (Table 2) Non significant differences were observed between RPMI-1640 and SMART media for their effects on IVM percentage with three NTG concentrations (0, 0.05 and 0.15 µM) (Figure 1). On the other hand, higher (P < 0.05) IVM percentage were obtained using RPMI-1640 medium compared with SMART medium when supplied 0.1,0.3 and 0.5 µM of NTG (Figure 1).

Table 2. Percentages of *in vitro* maturation using SMART medium enriched with different concentrations of NTG (Mean±S.E).

concentration of NTG / Parameters	Control	0.05µM	0.1 µM	0.15 µM	0.3 µM	0.5 µM
NO. of immature oocy	**177**	**120**	**105**	**115**	**125**	**115**
No. of matured oocytes	**105**	**79**	**67**	**65**	**51**	**15**
IVM (%)	**59.359 c ±1.48**	**65.914 a ±0.98**	**63.666 b ±1.40**	**56.584 c ±0.723**	**40.7142 d ±1.646**	**12.962 e ±1.23**

* Means with different superscripts within each row are significantly different (P<0.05).

Different concentrations of NTG were used to investigate the effects on IVM Amidi *et al* 9) showed that neither NOS inhibitors nor low concentration of SNP has a harmful effect on oocyte morphology, but high concentration of SNP significantly increase the number of a typical oocytes compared with the control. These results revealed that the concentration of intracellular NO could be critical factor in cell survival and function. At low concentrations, NO transmits extra cellular signals to its intracellular targets and regulates meiosis progression of oocytes just as in other eukaryotic cells; when at high concentrations it harms oocyte greatly by its derivates (12).

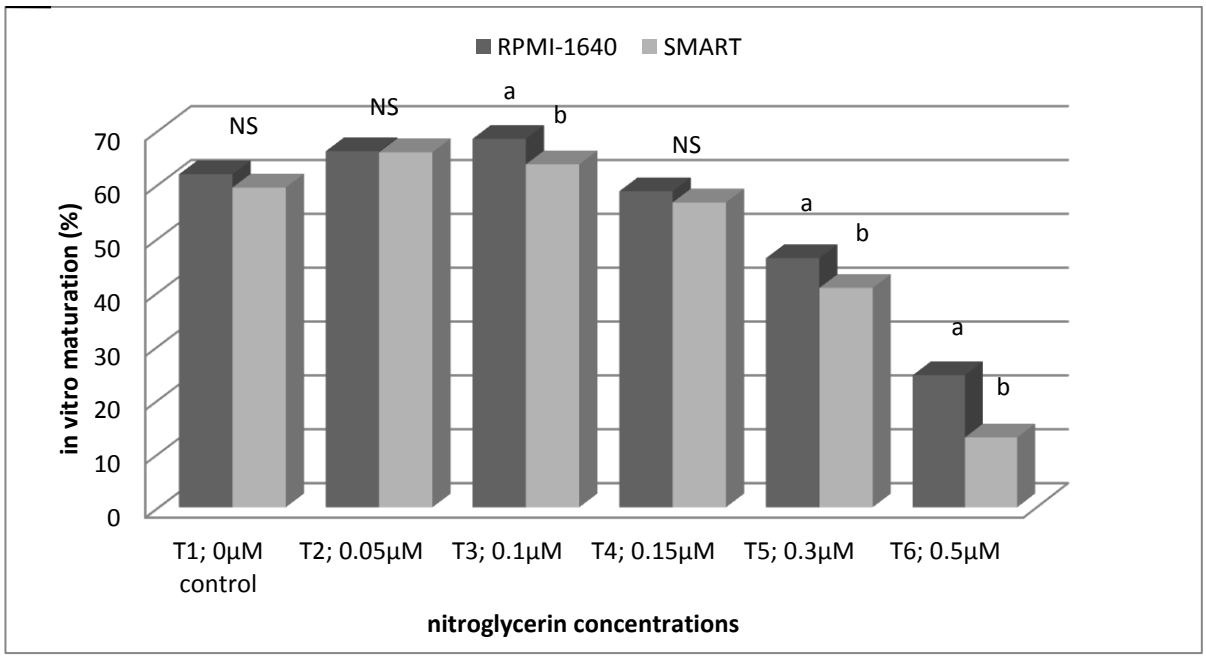

Figure 1. percentages of IVM using RPMI-1640 and SMART media supplied with different concentrations of NTG.

* Means with different superscripts within each columns are significantly different (P<0.05).

** NS: non significant differences.

Redundant NO in the cell might react with another free radical (O_2^-) and produce a more toxic radical peroxynitrite ($ONOO^-$) (13). There are many mechanisms through which NO acts either intracellularly or in a paracrine fashion, diffusing through cell membranes (14). In several somatic cell systems, the effects of NO are mediated via activation of soluble guanylyl cyclase (sGC) and induction of cGMP synthesis.This intracellular transduction pathway is known to mediate the effects of NO, for instance, in vascular smooth muscle cell relaxation, platelet aggregation and neurotransmission (15). NO is synthesized by the ovary and hypothesized to play a role in steroid genesis, ovulation and luteolysis (16). A growing amount of experimental data indicates that NO can induce its biological effects even via cGMP-dependent pathways (binding to heme-containing proteins other than sGC) (10). However, It is documented that cGMP has an important role in maintaining the meiotic arrest of oocytes (8). The exact mechanisms through which NO influences oocyte maturation have not been reported by Amidi *et al*. (12) found that a complete prevention of GVBD was only obtained after exposure to high concentration of SNP for 1–5 hours. This effect is very similar to that of forskolin, a stimulator of adenylate cyclase

(AC), which can stimulate cumulus cells to produce a positive signal(13).

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