ที่ ศธ 0514.7.1.5/ว 140

ฝ่ายวิชาการ คณะแพทยศาสตร์
มหาวิทยาลัยขอนแก่น
อ. เมือง จ.ขอนแก่น 40002

วันที่ 30 มีนาคม 2560

เรื่อง ขอเชิญชวนส่งผลงานทางวิชาการเข้าร่วมนำเสนอในงานประชุมวิชาการประจำปี 2560

เรียน นายแพทย์สาธารณสุขจังหวัด

สิ่งที่ส่งมาด้วย 1. แบบฟอร์มส่งผลงาน
2. ตัวอย่างการเขียนผลงาน

ตามที่ คณะแพทยศาสตร์ จะจัดให้มีการประชุมวิชาการประจำปี พ.ศ. 2560 ครั้งที่ 33 ระหว่างวันที่ 2-4 สิงหาคม 2560 เพื่อถ่ายทอดความก้าวหน้าในองค์ความรู้ต่าง ๆ ที่เกี่ยวกับการดูแลรักษาสุขภาพ รวมทั้งการทำวิจัยเพื่อพัฒนาการดูแลรักษาผู้ป่วยให้ดียิ่งขึ้น ความละเอียดดังแจ้งแล้วนั้น ในการนี้ ฝ่ายวิชาการ ใคร่ขอเชิญชวนท่านและบุคลากรที่สนใจส่งผลงานทางวิชาการเข้าร่วมประกวด และ/หรือ นำเสนอในงานประชุมวิชาการดังกล่าว โดยส่งมาที่ สมปอง จันทะคราม งานบริการการศึกษา ฝ่ายวิชาการ อาคารเรียนรวม ชั้น 6 หรือที่ **smj_supple@kku.ac.th** ภายในวันที่ 30 มิถุนายน 2560 โดยผลงานแต่ละเรื่องจะเข้าสู่ขั้นตอนการ peer review จากผู้เชี่ยวชาญที่เกี่ยวข้อง หากผู้เชี่ยวชาญพิจารณาแล้วผลงานดังกล่าวไม่เหมาะสมจะไม่ได้รับการนำเสนอในงานประชุมวิชาการประจำปี พร้อมนี้ได้แนบแบบฟอร์มการส่งผลงาน และตัวอย่างการเขียนบทคัดย่อ และ proceeding มาด้วยแล้ว

จึงเรียนมาเพื่อโปรดพิจารณา และแจ้งผู้เกี่ยวข้องทราบ

ขอแสดงความนับถือ

(รองศาสตราจารย์สุรพล วีระศิริ)
รองคณบดีฝ่ายวิชาการ
ปฏิบัติราชการแทนคณบดีคณะแพทยศาสตร์

ฝ่ายวิชาการ
โทร. 043-363385

แบบฟอร์มการส่งต้นฉบับเพื่อพิจารณานำลงศรีนครินทร์เวชสาร (ฉบับประชุมวิชาการประจำปี)
คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

วันที่_____เดือน_____พ.ศ._____

ข้าพเจ้า (นาย/นาง/น.ส.) _____

<u>สถานะ</u> ○ เจ้าหน้าที่ ○ พยาบาล ○ แพทย์ใช้ทุน/แพทย์ประจำบ้าน
○ แพทย์โรงพยาบาลชุมชน

<u>**ขอส่ง**</u> (เลือกได้ 1 ข้อเท่านั้น) ○ Abstract ○ Proceeding

เรื่อง _____

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<u>โดยจะนำเสนอในรูปแบบ</u> ○ **Oral presentation** ○ **Poster presentation**
☐ ประกวด ☐ ประกวด
☐ ไม่ประกวด ☐ ไม่ประกวด

เพื่อเข้าสู่ขั้นตอนของการพิจารณาของคณะกรรมการจัดประชุมวิชาการ เมื่อผ่านขั้นตอนดังกล่าวแล้ว
มีความยินดีที่จะ ○ **ตีพิมพ์เผยแพร่ในศรีนครินทร์เวชสาร ฉบับประชุมวิชาการ**
○ **ไม่ตีพิมพ์เผยแพร่ในศรีนครินทร์เวชสาร ฉบับประชุมวิชาการ**

ที่อยู่ที่สามารถติดต่อได้สะดวก _____

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โทรศัพท์_____โทรศัพท์มือถือ_____

โทรสาร_____ E-mail_____

สิ่งที่ส่งมาด้วย ○ แผ่นดิสก์ข้อมูลต้นฉบับ ชื่อแฟ้มข้อมูล_____
○ เอกสารพิมพ์ต้นฉบับ จำนวน 1 ชุด

ลงนาม.....**ผู้นิพนธ์**

ตัวอย่างการเขียน abstract

The Protocatechuic Acid Attenuates Vascular Complication in Streptozotocin-induced Chronic Diabetic Rats

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Background and objective: Free radical-induced vascular dysfunction plays a key role in pathogenesis of vascular diseases found in chronic diabetic patients. Protocatechuic acid (PCA) is a phenolic compound, found in many plant such as *Hibicus sabdariffa* Linn. Interestingly, antidiabetic and antioxidant activities of PCA have been reported in experimental animals. Thus, the objective of this study was to investigate the effect of PCA on vascular dysfunction in chronic diabetic rats.

Methods: Male Sprague-Dawley rats were induced to be diabetes by a single intraperitoneal injection of 50 mg/kg streptozotocin. The diabetic rats were kept in hyperglycemic condition for 12 weeks, and then were orally administered PCA (50 and 100 mg/kg/day) or subcutaneously injected with insulin (4 U/kg/day) for 6 weeks. After each treatment, fasting blood glucose, blood pressure, vascular responses to vasoactive agents and aortic weight/body weight ratio were examined.

Results: PCA reduced blood glucose (16-31%), as well as decreased the high blood pressure (22-24mmHg) significantly in diabetic rats. Vascular responses of the chronic diabetic rats to vasoactive agents, acetylcholine (3-30nmol/kg) and sodium nitroprusside (1-10nmol/kg) were significantly suppressed by 37-46% and 35-44% respectively, phenylephrine (0.01-0.1μmol/kg) were significantly increased by 23-124% as compared to normal rats. Interestingly, the administration of PCA or insulin significantly restored the vascular reactivity of diabetic rats. Moreover, 18 weeks of diabetes resulted in the increasing of aortic weight/body weight ratio and PCA treatment significantly lessened the increase.

Conclusions: These results provide the first evidence for the efficacy of PCA in restoring the vascular reactivity of diabetic rats.

Keywords: Protocatechuic acid; Diabetes mellitus; Vascular reactivity

The Effect of Folic Acid on Post-thaw Quality of Human Spermatozoa

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ABSTRACT

Background: Cryopreservation provides a useful and effective method in fertility preservation for men desiring to conceive later in life such as cancer patients about to obtain radiation or chemotherapy treatments. Freezing and thawing methods, however, can lead to increase in the concentration of reactive oxygen species (ROS). Exposure to high ROS concentrations can result in impairment of semen quality as assessed by sperm concentration, percentage of motility and percentage of progressive motile sperm. Antioxidants are scavengers of ROS and their use have been studied as a treatment to reverse the adverse impact of high ROS concentrations on semen parameters. Folate in an water soluble vitamin and shows antioxidant activity. It was shown that folic acid can efficiently scavenge free radicals and inhibit lipid peroxidation.

Objective: To determine the effects of supplementation of cryopreservation medium with folic acid on the improvement of post-thaw semen quality.

Methods: This study investigated the effect of folic acid supplementation in a total of 30 semen samples prior to cryopreservation process. After one week of cryopreservation, the semen samples were thawed and assessed the quality using Computer Aided Sperm Analysis (CASA).

Results: The results showed that after supplementation of folic acid in semen samples prior to cryopreservation significantly improve semen quality after thawing in comparision with control group in terms of percentage of motile sperm, percentage of progressively motile sperm, and percentage of viable sperm (29.13% VS 18.60%, $p<0.001$; 1.43% VS 0.70%, $p=0.019$; 73.05% VS 63.90%, $p<0.001$, respectively).

Conclusion: These results suggest that the supplementation of folic acid to cryopreservation medium improves post-thaw semen quality.

Key Words: Spermatozoa, folic acid, cryopreservation

Introduction

Sperm cryopreservation provides a useful and effective method in infertility management for many men. Cancer patients can use cryopreservation to preserve their fertility prior to treatments by radiation or chemotherapy. Also, men undergoing vasectomy procedures can use this method to maintain their fertility. Freezing and thawing methods, however, expose spermatozoa to much physical and chemical damage, and thus several methods have been proposed to improve semen quality after cryopreservation and thawing [1]. Cryopreservation can induce an increased rate of lipid peroxidation in the sperm plasma membrane causing an overall increase in the concentration of radical oxygen species (ROS) [2]. Exposure to high ROS concentrations can result in the decrease of sperm parameters as assessed by sperm concentration, percent motility and quality of motility [3].

Antioxidants (such as vitamin C and E, folate, zinc, selenium, carnitine and carotenoids) are scavengers of ROS and their use has been studied as a treatment to reverse the adverse impact of high ROS concentrations on semen parameters [4].

Folate in an water soluble vitamin and shows antioxidant activity. It was shown that folic acid can efficiently scavenge free radicals [5] and inhibit lipid peroxidation [6]. Folic acid is required for DNA synthesis and thus important for spermatogenesis [7].

Objective

To determine if the supplementation of cryopreservation medium with folic acid could improve the post-thaw quality of cryopreserved human spermatozoa.

Methods

Written approval for this study was granted by Khon Kaen University Ethics Committee in Human Research, Khon Kaen University, Thailand (Reference No. HE561348)

This study utilized semen remaining after routine semen analysis at Andrology Laboratory, Srinagarind Hospital, Khon Kaen University, Khon Kaen, Thailand.

In a total of 30 semen samples from patients were collected by masturbation into sterile containers. Patients consent to use surplus semen was obtained prior to use.

Total of 30 semen samples were initially assessed using World Health Organization (WHO, 1999) standards[8]. After initially assessment semen samples were processed by Discontinuous density gradient centrifugation technique. The remaining semen were divided in to two groups. The first semen fraction was mixed with an equal volume (1:1) of semen cryopreservation medium (Sperm CryoProtec™II, Sweden). The same batch of cryopreservation medium was used for all samples assessed in this study. The second semen fraction was mixed with an equal volume of cryopreservation medium containing 50

nmol/L of folate. The samples were cooled immediately after addition of the cryopreservation medium in a freezer at 23-25 °C for 15 minutes prior to rapid plunging into liquid nitrogen (-196 °C). The samples were then stored in liquid nitrogen for one week until post-thaw analysis.

To assess post-thaw sperm parameters, straws were removed from liquid nitrogen and allowed to thaw at room temperature for 5 minutes and incubated at 37 °C for 15 minutes. The post-thaw analysis was carried out by one embryologist, who was blinded with regard to the experimental group.

Several semen parameters including sperm concentration, sperm motility and sperm morphology were assessed by Computer Aided Sperm Analysis (CASA) (Hamilton Thorne IVOS version 14.0, Hamilton Thorne, Inc, USA).

To assess the post-thaw vitality, the eosin–nigrosin dye exclusion staining assay was used as previously described[9]. Briefly, one drop of the thawed spermatozoa was mixed with two drops of 1% eosin stain. After 30 seconds, three drops of 10% nigrosin were added to each solution. A drop of each fraction was smeared onto glass microscope slides and allowed to air-dry. The smears were assessed by light microscopy at x100 magnification. Live spermatozoa appear white whilst dead spermatozoa with disrupted membranes have taken up the eosin stain and appear red. Vitality was quantified by counting a minimum of 200 spermatozoa on each slide and the proportion of live spermatozoa expressed as a percentage.

Results

Demographic data of the recruited subject as well as the semen parameters of the investigated samples are presented in Table 1.

Among the 30 subjects recruited to this study, the mean age was 35.13 ± 1.18 years. The mean abstinence period before semen collection was 4.63 ± 0.34 days. With regards to initial semen analysis prior to cryopreservation, the mean volume of investigated semen samples was 3.72 ± 0.26 ml, the mean sperm concentration was 139.88 ± 23.00x10^6/ml, the mean percentage of semen motility was 68.40 ± 3.33, the mean percentage of progressive motile sperm was 18.70±2.03, the mean percentage of sperm viability was 86.66 ± 2.06, and the mean percentage of morphologically normal sperm was 12.63 ± 1.65.

The result of post-thaw semen analysis was demonstrated in Table 2. These result revealed that all of the observed semen parameters (sperm concentration, percentage of motile sperm, percentage of progressive motile sperm and percentage of viable sperm) were compromised after the cryopreservation and thawing processes.

The results of this study, however, pointed out interestingly that supplementation of 50 nmol/L of folate in the cryopreservation medium prior to undertaking the cryopreservation process significantly

improve the post-thaw percentage of motile sperm, percentage of progressively motile sperm, and percentage of viable sperm when compared to those parameters of the control group (29.13 ± 2.45% VS 18.60 ± 1.49%, $p<0.001$; 1.43 ± 0.24% VS 0.70 ± 0.14%, $p=0.019$; 73.05 ± 1.78% VS 63.90 ± 1.76%, $p<0.001$, respectively).

Table 1 Demographic data of the recruited 30 subjects and their semen parameters.

Parameters	Mean ± SEM
Age (years)	35.13 ± 1.18
Abstinence period (days)	4.63 ± 0.34
Volume (ml)	3.72 ± 0.26
Sperm concentration ($x10^6$/ml)	139.88 ± 23.00
% Motility	68.40 ± 3.33
% Progressive motile	18.70 ± 2.03
% Viability	86.66 ± 2.06
% Morphology	
- Normal	12.63 ± 1.65
- Abnormal	87.37 ± 1.65
pH	8.00 ± 0.03
WBC ($x10^6$)	0.10 ± 0.07

Values are presented as mean ± standard error of mean (S.E.M.)

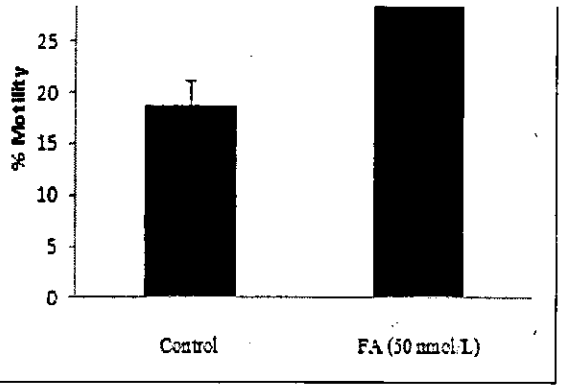

Figure 1 The effect of folic acid supplementation on post-thaw motility of sperms.

*$p<0.001$ compared with control group.

Table 2 Results of post-thaw semen analysis.

Parameters	Control group	Folic acid Supplementation group	p-value
Sperm concentration ($x10^6$/ml)	111.46±19.04	121.74±20.04	
% Motility	18.60±1.49	29.13±2.45[a]	<0.001
% Progressive motile	0.70±0.14	1.43±0.24[b]	0.019
% Viability	63.90±1.76	73.05±1.78[a]	<0.001

The results are presented as mean ± standard error of mean (S.E.M)

[a] statistical significant by independent *t*-test is reached at $p < 0.001$

[b] statistical significant by independent *t*-test is reached at $p < 0.050$

Discussion

This study revealed that supplementation of folic acid at a concentration of 50 nmol/L in cryopreservation medium before the process of semen cryopreservation significantly improved semen quality after thawing while compared with the control group in terms of percentage of motile sperm, percentage of progressively motile sperm, and percentage of viable sperm. To date, there has been no studies investigating the direct impact of folate supplementation on semen cryopreservation. Folate is an essential water soluble vitamin that occurs naturally in a wide variety of foods. Supplementation of biotin (vitamin B7) to sperm preparation medium was reported to increase the motility and longevity of cryopreserved human spermatozoa[10]. These results were comparable to the finding of our study.

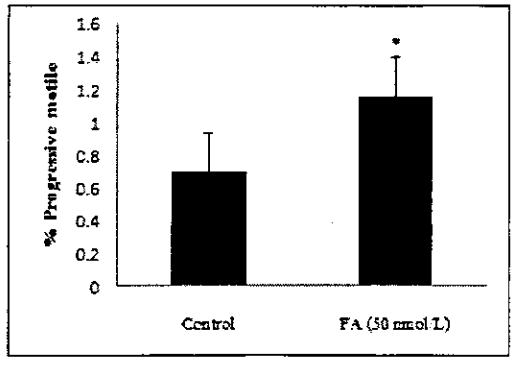

Figure 2 The effect of folic acid (FA) supplementation on post-thaw percentage of progressive motile. *$p<0.050$

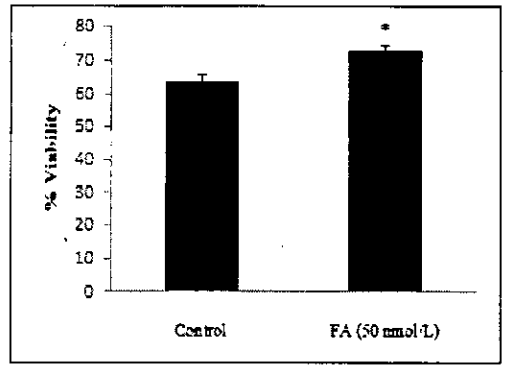

Figure 3 The effect of folic acid (FA) supplementation on post-thaw percentage of sperm viability. *$p<0.001$

Cryopreservation can induce an increased rate of lipid peroxidation in the sperm plasma membrane causing an overall increase in the concentration of oxygen radicals in the sample. Exposure to high reactive oxygen species (ROS) concentrations can result in the disruption of mitochondrial and plasma membranes, cause chromosomal and DNA fragmentation and bring about a reduction in sperm motility[11].

Studies have revealed that apoptosis markers tend to increase in spermatozoa following cryopreservation and thawing. In an animal model, cryopreservation increased manifestations including mitochondrial membrane potential, caspase activation, membrane permeability and phosphatidylserine externalization[12].

This study, however, did not determine the precise mechanisms underlying the positive impact of folic acid supplementation on the quality of frozen-thawed semen. Further studies are required to uncover the possible mechanisms responsible for such beneficial impact of folic acid supplementation.

Conclusion

Supplementation of 50 nmol/L of folate in the cryopreservation medium prior to the process of semen cryopreservation results in a significant improvement in post-thaw semen parameters including sperm motility and sperm viability. The precise mechanism underlying this improvement, however, required further investigations.

Acknowledgements

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