



Effects of maternal Selenium-supplementation at varying stages of periconception period on murine blastocyst morphology and implantation status

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Abstract: Selenium is an important trace element for normal reproductive health, but there is a dearth of scientific information about the best stage within the periconception period for selenium-supplementation to promote successful pregnancy outcome. Hence, this study aims to assess the effects of maternal Selenium-supplementation at varying stages of periconception period on murine blastocyst morphometric measurements, percent occurrence of good quality blastocysts, and implantation status. ICR female mice were randomly assigned into the unsupplemented group (Group I) receiving basal diet without selenium, and treatment groups given with 3.0µg selenium supplement per day during pregestation only (Group II), pregestation-throughout-gestation (Group III) and gestation only (Group IV). Both blastocyst morphology and implantation status were assessed. Results show that the morphometric measurements of blastocysts appeared to be unaffected by selenium-supplementation at different periconception stages. Selenium-supplementation at pregestation only (Group II) and gestation only (Group IV) produced higher percent occurrence of good quality blastocysts and lower percent pre-implantation loss relative to Group III. Among treatment groups, selenium-supplementation during pregestation-to-gestation (Group III) yielded low quality blastocysts and high percent pre-implantation loss. In conclusion, maternal Selenium-supplementation during pregestation only and gestation only are the best stages to yield high percent occurrence of good quality blastocysts and pre-implantation success.

Key Words: Selenium-supplementation; Periconception period; Blastocyst; Morphometric parameters; and Implantation loss

1. INTRODUCTION

According to the 2009 report of the National Epidemiology Center, Department of Health (DOH) on Philippine Health Statistics, the total registered maternal deaths reached 1,599 due to pregnancy complications whereas total registered fetal deaths reached 8,043 due to low birth weight and premature birth. As of 2010 to 2011, fetal death in the Philippines increased by 1% based on the records of the Philippine Statistics Authority (de Guzman et al., 2015). This alarming state calls the formulation of possible health measures to improve

pregnancy outcome, which is one of the United Nations Millennium Development Goals.

There are reports suggesting that selenium is essential for normal female reproductive health, and its deficiency leads to pregnancy complications. This element is a trace mineral involved in the metabolic processes of thyroid hormone, antioxidative protection, DNA synthesis, and immune system physiology (Mehdi et al., 2013). Among these functions, the antioxidative protection is the most important, wherein selenium is a component of selenocysteine incorporated in diverse array of antioxidative selenoenzymes that reduce



reactive oxygen species (ROS) production (Kumar and Priyadarsini, 2014).

Currently, there are only limited research trials of selenium supplementation during pregnancy, and more intervention trials are recommended to determine the beneficial effects of selenium to pregnancy outcomes. According to the report of Mistry et al. (2012), one of the identified gaps among studies regarding selenium and reproductive health is finding out the best timing of selenium administration during pregnancy, which refers to the different stages of periconception period. Hence, this study aims to evaluate the effects of maternal Selenium-supplementation at varying stages of periconception period on murine blastocyst morphometric parameters, percent occurrence of good quality blastocysts, and implantation status.

2. METHODOLOGY

2.1 Test animals and maintenance

There were 56 seven-week old ICR female mice and twenty-eight 15 - 20 week old ICR male mice, approximately 30g of weight, were obtained from the stock bred of the MSI – Natural Products Laboratory, University of the Philippines, Diliman. All mice were kept individually in standard - sized cages in the animal house of DLSU – Manila, where they were acclimatized for two weeks maintaining at 12 h light: 12 h dark cycle with 28 - 30°C ambient temperature. All mice were given 6 grams of food pellets per day, and they had access to purified drinking water *ad libitum*. The handling and maintenance of laboratory test animals adhered to the Guiding Principles of Philippine Association for Laboratory Animal Science and Philippine Veterinary Medical Association.

2.2 The experimental design

After the acclimatization period, at which time the females were already sexually mature, these were randomly assigned into four groups with 14 members each.

For the unsupplemented group (Group I), all were given with basal food pellets (6.0g food pellet/day) without selenium supplement. For the treatment groups, the timing for the administration

of 3.0µg selenium supplement were as follows: pre-gestation only (Group II), pre-gestation-to-gestation (Group III), and gestation only (Group IV),

Mating was allowed to take place at the end of the third week. Females positive for plugs were placed in separate cages and their embryos were considered as 0.5 day post coitum (dpc).

2.3 Treatment administration

Selenium, in a form of selenium yeast, was administered everyday via dietary route. About 3.0µg selenium, prepared as 0.15ml solution, was coated into 2.0g food pellets, which was given as initial consumption for the day. After the initial consumption, the remaining 4.0g food pellets was supplied in full

2.4 Blastocyst morphology assessment

Two female mice per group were sacrificed by cervical dislocation on 4.5 dpc. Blastocysts were recovered by flushing the uterus with M2 medium. The images of blastocysts at 600x magnification taken under an inverted microscope were analyzed using the Image J software. The morphometric parameters that were measured using 'line and oval selection tools' are embryo and zona pellucida diameters, area, and perimeters (Matos et al., 2014).

Blastocyst quality was scored and evaluated based on the Manual of the International Embryo Transfer Society (IETS). The three classifications of blastocyst quality were excellent or good (score 1), fair (score 2), and poor (score 3) (Bo and Mapletoft, 2013). The descriptions of embryo images taken at 400x magnification were determined using Image J.

2.5 Implantation status assessment

On 16.5 dpc, twelve female mice per group were sacrificed through cervical dislocation. The percent pre-implantation and post-implantation loss per female was calculated using these formulas (Yeh et al., 2011).

$$\% \text{ Pre-implantation loss} = [(TCL - TIS)/TCL] \times 100$$

$$\% \text{ Post-implantation loss} = [(TIS - NVF)/TIS] \times 100$$

where: *TCL* = total number of corpora lutea
TIS = total number of implantation sites
NVF = number of viable fetuses

2.6 Data analysis

Data on blastocyst morphology among groups were analyzed by ANOVA followed by post-hoc Tukey HSD test. Percent pre- and post-implantation losses were analyzed using non-parametric Kruskal-Wallis test, followed by Mann Whitney test. Data were presented as mean±SEM (standard error on the mean). All statistical tests were conducted using SPSS Version 22. Effects of the treatment were considered significant at *P*-values < 0.05.

3. RESULTS AND DISCUSSION

3.1 Blastocyst morphology assessment

Morphometric measurements of blastocysts from all groups showed that blastocysts of Group III exhibited the lowest values in all the morphometric parameters. These were significantly lower than those of Group I, the unsupplemented group, in most of the morphometric parameters. Comparing all the treatment groups, Groups II, III, and IV, Group III was not significantly different. Figure 1 shows blastocysts from all groups measured by the 'straight line' selection tool of the Image J software.

This result may indicate that selenium-supplementation at varying stages of periconception have not strongly influenced these morphological parameters. It appears however, that a longer period of supplementation (25 days for Group III) may have a tendency towards yielding smaller sized blastocysts

Percent occurrences of good, fair, and poor quality blastocysts are shown in Table 1. Group I showed the lowest percent occurrence of good quality blastocysts, which was comparable with that of Group III. Group IV showed the highest percent occurrence of good quality blastocysts followed by Group II.

Selenium-supplementation either during pregestaion only or gestaion only may have similar influence on yielding good quality blastocysts. Probably, the antioxidant system during these stages of periconception could have been enhanced by selenium-supplementation. Moreover, it could have embryoprotective capacity on the deleterious impacts and influences of ROS, thus preventing abnormal and delayed embryo development.

The low percent occurrence of good quality blastocyst in Group III possibly shows the effects of long-term selenium supplementation in promoting type 2 diabetes mellitus (Streinbrenner et al., 2011) during pregnancy, which results into an increase in ROS production that can adversely affect the development of blastocysts. Moreover, long-term selenium supplementation and the type of selenium supplement administered, which was selenium yeast highly rich in selenomethionine, could have lowered the metabolic clearance rate of selenium.

Table 1. Percent occurrence of three classification of blastocyst quality

Groups	<i>n</i>	Actual blastocyst count (Percent Occurrence %)		
		Good (1)	Fair (2)	Poor (3)
Group I	12	4 (33.3)	6 (50)	2 (16.67)
Group II	15	11 (73.3)	3 (20)	1 (6.7)
Group III	13	5 (38.5)	4 (30.8)	4 (30.8)
Group IV	20	19 (95)	0	1 (5)

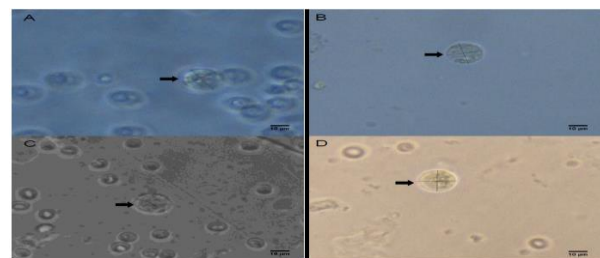


Figure 1. Photomicrographs of blastocysts (arrows) at 600x magnification measured through the Image J software (A) Unsupplemented group, (B) Pregestaion only group, (C) Pregestaion-to-gestaion group, (D) Gestaion only group

3.2 Implantation losses

Figure 2 shows the percent pre-implantation loss from all groups. Group II significantly exhibited the lowest percent pre-implantation loss followed by Group IV, which were both significantly lower than Group I. Group III had the highest percent pre-implantation loss among treatment groups. It was significantly higher than Group II, but not from Group IV.

Figure 3 shows the percent post-implantation loss from all groups. Group I (Unsupplemented group) and Groups II, III, and IV (supplemented groups) were not significantly different from each other. Figure 4 shows the

implantation sites in the uteri that have viable fetuses and resorbed embryos

The result in pre-implantation status of Groups II and IV are supported by the incurred high percent occurrence of good quality blastocysts (Table 1) in these two periconception stages. Blastocysts with good quality have higher competency for successful implantation (Zhang et al., 2013).

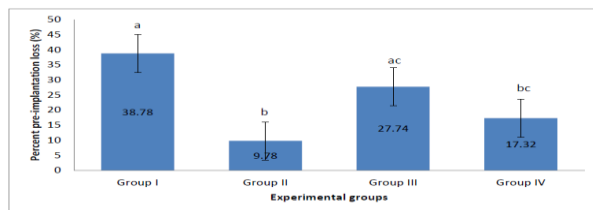


Figure 2. Percent pre-implantation loss among groups

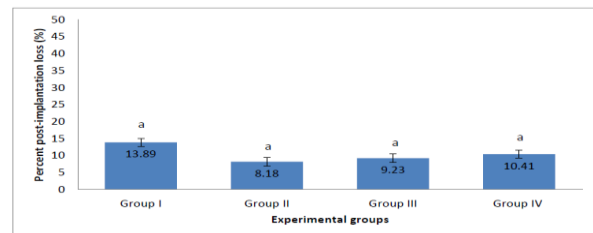


Figure 3. Percent post-implantation loss among groups

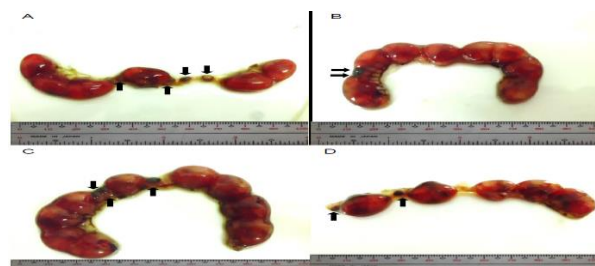


Figure 4. Photographs of 16.5 dpc murine uterine horns showing viable fetuses and resorptions (arrows) from all groups
 (A) Unsupplemented group, (B) Pregestation only group,
 (C) Pregestation-to-gestation group, (D) Gestation only group

4. CONCLUSIONS

The overall findings of the present study indicate that pregestation only and gestation only are the best periconception stages for selenium-

supplementation to yield high percent occurrence of good quality blastocysts and pre-implantation success.

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