PROCEEDING

international seminar

STRATEGY TO MANAGE BIO-ECO-HEALTH SYSTEM FOR STABILIZING ANIMAL HEALTH & PRODUCTIVITY TO SUPPORT PUBLIC HEALTH

Surabaya-Indonesia, 19-20 June 2012
JW Marriott Hotel Surabaya

EDITORS:
Michael P. Ward (Australia)
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FACULTY OF VETERINARY MEDICINE - UNIVERSITAS AIRLANGGA
I-MHERE SUB-COMPONENT B.2.C PERFORMANCE BASED CONTRACT

H. Bambang Poernomo S
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Phone. +62 31 5992246, 5992247 Fax. +62 31 5992248
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I. Faouzi Kechrid
II. Montip Gettayacamin
III. Fedik Abdul Rantam
IV. Suzanita Utama

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ACKNOWLEDGEMENT

CORELATION BETWEEN DURATION TIMES OF CRYOPROTECTANT TOWARD MICE EMBRYO DEVELOPMENT

Bambang Poernomo S.,[1] Soeharsono,[1] Trianto Nur Abdullah[2]

Dept. Anatomy Veterinary, Fac. Veterinary Medicine, Universitas Airlangga
E-mail: hajibps2009@gmail.com
Student, Fac. Veterinary Medicine, Universitas Airlangga

ABSTRACT

1,2-Propanediol is considerably less toxic than ethylene glycol, moreover it is used as a cryoprotectant. Purpose of the research was find correlation between duration times of cryoprotectant toward mice embryos development. Zygote embryos were vitrified through plunging into the vitrification medium which it contained mixture propanediol 30% as cryoprotectant and phosphate buffer saline. Duration of vitrification times were 5, 10, and 15 minutes, respectively. Viability of post thaw embryos was assessment through inverted microscope everyday till die or damage. Analysis correlation between duration time of three groups toward embryos development were compared of both descriptive statistics and test of within subject contrast through estimated marginal means and estimated relative marginal means. Research shown correlation between duration times of cryoprotectant and mice embryos development has different form, it depend on facing the data. Linear correlation was assessed on the descriptive data. On the contrary, shown more quadratic than linear on the within subjects data. However, both duration times of cryoprotectant and mice embryo development has correlation.

Keywords: cryoprotectant, duration times, mice embryo

INTRODUCTION

Propylene glycol, also known as 1,2-propanediol is a colorless, odorless, slightly sweetish, viscous, highly hygroscopic liquid. It is fully miscible with water, methanol, ethanol, acetone, diethyl ether, and chloroform; bounded soluble in benzene. Propylene glycol forms azeotropic mixtures with aniline (bp 179.5°C; 43% wt of propylene glycol), o-xylene (135.8°C; 10.0% wt), toluene (110.5°C; 1.5% wt). Propylene glycol is a diatomic alcohol. It can form mono- and di- ethers and esters being treated with alcohols or acids respectively. It also reacts with alkali metals and alkalis to form corresponding salts (glycolates). 1,2-Propylene glycol dehydrates in the presence of acids or alkalis to form dimethyl-1,4-dioxanes (mixture of isomers). Catalytic dehydratation of 1,2-propanediol at 250°C results in propionic aldehyde. Propylene glycol reacts with propylene oxide to give the mixture of di-, tri-, tetra- and polypropylene glycols. The yield of products depends on the ratio of reagents and reaction conditions. 1,2-Propanediol was considerably less toxic than ethylene glycol, moreover it was used as a cryoprotectant (Son and Tan, 2009; Anonymous, 2010).

Purpose of the research was find correlation between duration times of cryoprotectant toward mice embryos development.

MATERIALS AND METHODS

Zygote embryos were vitrified through plunging into the vitrification medium which it contained mixture propanediol 30% as cryoprotectant and phosphate buffer saline. Duration of vitrification times were 5, 10, and 15 minutes, respectively. Viability of post thaw embryos was assessment through inverted microscope everyday till die or damage.

Analysis correlation between duration time of three groups duration time toward embryos development were compared of both descriptive statistics and test of within subject contrast through estimated marginal means and estimated relative marginal means.

RESULT AND DISCUSSION

Table 1. Descriptive statistics of embryo development

Descriptive Statistics				
	Treatments	Mean	Std. Deviation	N
Cell 1	Pr-OH 30% 5 minutes	9.00	.894	6
	Pr-OH 30% 10 minutes	13.00	.894	6
	Pr-OH 30% 15 minutes	8.83	3.488	6
	Total	10.28	2.824	18
Thawing	Pr-OH 30% 5 minutes	8.50	1.225	6
	Pr-OH 30% 10 minutes	9.00	1.414	6
	Pr-OH 30% 15 minutes	8.17	1.722	6
	Total	8.56	1.423	18
Cell 2	Pr-OH 30% 5 minutes	7.83	1.329	6
	Pr-OH 30% 10 minutes	5.83	1.472	6
	Pr-OH 30% 15 minutes	5.17	1.472	6
	Total	6.28	1.776	18
Cell 4	Pr-OH 30% 5 minutes	6.50	1.225	6
	Pr-OH 30% 10 minutes	5.33	1.633	6
	Pr-OH 30% 15 minutes	3.83	2.483	6
	Total	5.22	2.074	18
Morulae	Pr-OH 30% 5 minutes	4.83	1.472	6
	Pr-OH 30% 10 minutes	.67	.816	6
	Pr-OH 30% 15 minutes	.00	.000	6
	Total	1.83	2.383	18
Blastulae	Pr-OH 30% 5 minutes	.33	.516	6
	Pr-OH 30% 10 minutes	.00	.000	6
	Pr-OH 30% 15 minutes	.00	.000	6
	Total	.11	.323	18

On the Table 1, embryos were assessed till day 4 where post thaw embryos reached blastulae. According to the descriptive data, correlation analysis was seen at Figure 1.

The most common fertility preservation technique is cryopreservation, which involves freezing cells and tissues at cryogenic temperatures. Cryopreserved cells and tissues can endure storage for centuries with almost no change in functionality or genetic information, making this storage method highly attractive. However, developing efficient cryopreservation techniques is challenging, as both freezing and thawing exposes cells to severe stresses, potentially causing cell death (Nakahara, *et al.*, 2010).

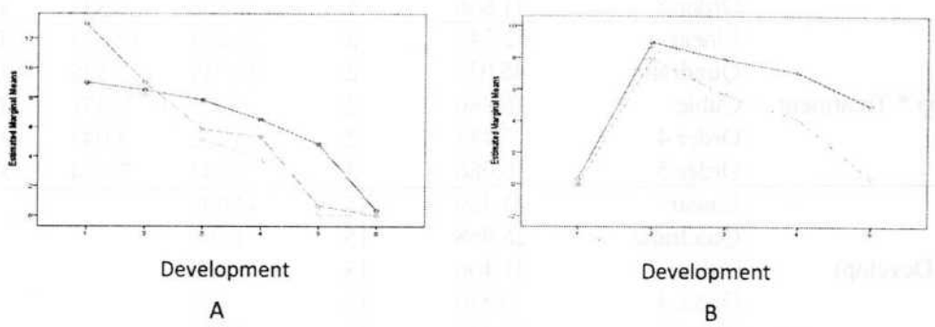

Figure 1. A. Estimated mice embryo development. B Estimated relative mice embryo development. Whether compact line was Pr-OH 30% 5 minutes, dash line was Pr-OH 30% 10 minutes, and rare dash line was Pr-OH 30% 15 minutes treatment, respectively.

Therefore, compared of the both data in the Table 1 and Table 2 were analysis through descriptive data and within subject contrast data. Data on the Table 1 was seen like development of the embryos was sharply decreased from the beginning to the zero level at the end of research. However, total among development embryos was seen at Figure 1 A, where estimated mice embryos development has linear correlation.

Table 1 was statistical analysis through estimated relative mice embryo development, where all data began at zero level. Sum of analysis was seen on Table 2 below. According to the data on Table 2, correlation between mice embryo development and duration time of cryoprotectant was more quadratic than linear lines. It was mean mice embryos development could reach the optimal condition at the first step of development before decreased caused of degradation or damage. However, total among development embryos was seen at Figure 1 B, where estimated relative mice embryos development has more quadratic than linear correlation.

There are two major techniques for cryopreservation: freeze–thaw processes and vitrification. The major difference between them is the total avoidance of ice formation in vitrification. The use of both theoretical models that describe cell response to freezing and thawing, and experimental investigations of freezing behavior, has led to the development of successful freeze–thaw and vitrification procedures for a number of cell types. Among reproductive cells, there exist efficient cryopreservation techniques for spermatozoa and embryos. Oocytes, however, present significant hurdles in achieving successful cryopreservation, primarily due to their sensitive microtubule structure. Recently, cryopreservation of ovarian and testicular tissues has been investigated with success reported. Ovarian cryopreservation can help circumvent many of the problems associated with oocyte cryopreservation, while testicular tissue preservation may be helpful when insufficient sperm counts are available for routine semen preservation (Bagchi, *et al.*, 2008).

Research shown correlation between duration times of cryoprotectant and embryos development has different form, it depend on facing the data. Linear correlation was assessed on the descriptive data. On the contrary, shown more quadratic than linear on the within subjects data. However, both duration times of cryoprotectant and mice embryo development has correlation.

Table 2. Test of within subject – contrasts

Source	Develop	Type III Sum of Squares	Df	Mean Square	F	Sig.
Develop	Linear	1335.087	1	1335.087	636.381	.000
	Quadratic	4.233	1	4.233	2.354	.146
	Cubic	.020	1	.020	.026	.874
	Order 4	3.175	1	3.175	3.448	.083
	Order 5	11.866	1	11.866	6.833	.020
Develop * Treatment	Linear	52.544	2	26.272	12.523	.001
	Quadratic	45.037	2	22.519	12.525	.001
	Cubic	16.980	2	8.490	11.171	.001
	Order 4	7.444	2	3.722	4.043	.039
	Order 5	10.086	2	5.043	2.904	.086
Error (Develop)	Linear	31.469	15	2.098		
	Quadratic	26.968	15	1.798		
	Cubic	11.400	15	.760		
	Order 4	13.810	15	.921		
	Order 5	26.048	15	1.737		

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