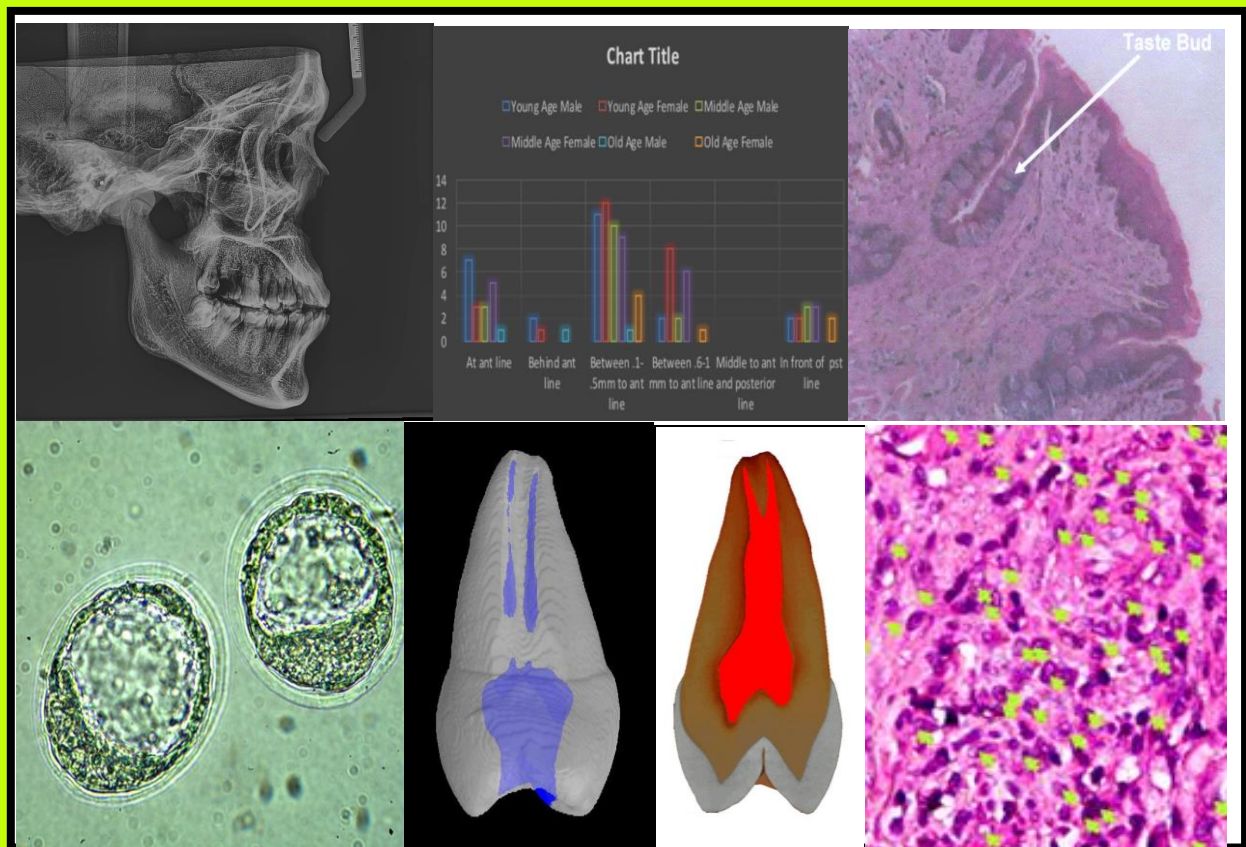


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DENTISTRY

- CLINICAL ARTICLE**
1. **Modification of Dental Age Estimation Technique among Children from Transcarpathian Region**
Myroslav Goncharuk-Khomyn
Pages 851-855
- CLINICAL ARTICLE**
2. **Oral Health Status, Malocclusions and S. Mutans Counts in Children with Down's Syndrome**
Agim Begzati, Kastriot Meqa, Blerta Xhemali-Latifi, Teuta Kutllovci, Merita Berisha
Pages 856-861
- CLINICAL ARTICLE**
3. **Prevalence and Evaluation of Bone Loss Pattern among Patient with Aggressive Periodontitis**
Mohd Faizal Hafez bin Hidayat, Fouad Hussain AL-Bayaty, Ihsan Bin Maidin, Mohammad Azrin Bin Abd Samad
Pages 862-867
- CLINICAL ARTICLE**
4. **Prevalence of Oral Mucosal Lesions in Geriatric Patients Living in Lower Northern Thailand: A 10 Years Retrospective Study**
Chaidan Intapa, Chalatip Chompunud Na Ayudhya, Anawat Puangsombat, Bundit Boonmoon, Thida Janyasurin, Ubonwan Tonum
Pages 868-871
- CLINICAL ARTICLE**
5. **Awareness and Demand of Prosthodontic Treatment for Tooth Loss Replacement**
Saraventi Mursid, Candrika Kusuma Pujnadati, Lindawati S. Kusdhany
Pages 872-876
- CLINICAL ARTICLE**
6. **Association between Tooth Loss and Oral Awareness Amongst Dentate and Partially Dentate Subjects of Pakistani population**
Huma Sajid, Yousaf Athar, Aamina Sagheer, Nazia Yazdanie, Anam Arshad, Fazal Shahid
Pages 877-882
- CLINICAL ARTICLE**
7. **Assessment of location of fovea palatine in relation to vibrating line in South Indian population**
Aslin Sanofer A, Revathy Gounder
Pages 883-886
- CLINICAL ARTICLE**
8. **sCD14 Protein Analysis in Children with Very High and Low pufa Index**
Dudi Aripin, Inne Suherna Sasmita, Anne Agustina Suwargiani
Pages 887-890
- CLINICAL ARTICLE**
9. **Microbiological and cytological response to dental implant healing abutment**
Wifaq M.Ali Al-Wattar, Warkaa M.Al-Wattar, Afya Sahib Diab Al-Radha
Pages 891-898
- CLINICAL ARTICLE**
10. **Tooth Mortality in Concurrent Cigarettes Smoking and Khat Chewing in Yemeni Population**
Fouad Hussain Al- Bayaty, Nidhal Wahid Ali, Aqil Daher, Saba F.Hussain, Mohd Masood
Pages 899-904

- CLINICAL ARTICLE**
- 11. The Correlation between Mother's Knowledge and Parenting Toward Childhood Caries in the Remote Area**
Leny Marlina A. Pinat, Darmawan Setijanto, Taufan Bramantoro
Pages 905-908
- CLINICAL ARTICLE**
- 12. Gingival Recession and Dentine Hypersensitivity in Periodontal Patients: is It Affecting Their Oral Health Related Quality of Life?**
Masud M, Al-Bayaty FH, Muhamed NAH, Alwi AS, Takiyudin Z, Hidayat MFH
Pages 909-914
- CLINICAL ARTICLE**
- 13. Prevalence of Medically Compromised Children Regarding Dental Caries and Treatment Needs in Wahidin Sudirohusodo Hospital**
Harun Achmad, M. Hendra Chandha, Sri Harun, Imam Sudjarwo, Muliaty Yunus, Rahmah K. Rusdi, Putri Khairunnisa
Pages 915-920
- CLINICAL ARTICLE**
- 14. Relationship between Oral Health Status with Knowledge, Attitude, And Behavior of Elementary School Children**
Fuad Husain Akbar, Rini Pratiwi, Reagan Cendikiawan
Pages 921-926
- CLINICAL ARTICLE**
- 15. The Oral Health of Elderly Residents in a State Institution in Jakarta: A Preliminary Study**
Dwi Ariani, Febrina Rahmayanti, Harum Sasanti, Masita Mandasari
Pages 927-932
- CLINICAL ARTICLE**
- 16. Pandan Leaves (Pandanus Amaryllifolius) Aromatherapy and Relaxation Music to Reduce Dental Anxiety of Pediatric Patients**
Seno Pradopo, Betadion Rizki Sinaredi, Bernadeth Vindi Januarisca
Pages 933-937
- CLINICAL ARTICLE**
- 17. The Relation of Follicle Stimulating Hormone and Estrogen to Mandibular Alveolar Bone Resorption in Postmenopausal Women**
Susi R Puspitadewi, Pitu wulandari, Sri Lelyati C Masulili, Elza I Auerkari , Hanna Bachtiar Iskandar, Izzet Yavuz, Lindawati S Kusdhany
Pages 938-944
- CLINICAL ARTICLE**
- 18. Cross-Cultural Adaptation and Psychometric Properties of The Indonesian Version of Servqual For Assessing Oral Health Service Quality**
Yohanes Tebai, Diah Ayu Maharani, Anton Rahardjo
Pages 945-951
- EXPERIMENTAL ARTICLE**
- 19. Effect of Endodontic Instrumentation Technique on Root Canal Geometry**
Miranda Stavileci, Veton Hoxha, Mehmet Ömer Görduysus, Kjell Laperre, Ilkan Tatar, Rina Hoxha
Pages 952-957

- EXPERIMENTAL ARTICLE
- 20. No Recombinant EGF and bFGF is Required on HUVECs Culture Supplemented with Human Platelet Lysate**
Lisa Rinanda Amir, Ria Puspitawati, Hazriani R, Shafira Imanina, Harvi Damayanti, Nadira Dwiyan, Afridayanti Nurwulan, Mindya Yuniastuti, Erik Idrus
Pages 958-963
- EXPERIMENTAL ARTICLE
- 21. The Effect of Light and Dual Cured Resin Cement to the Color of Porcelain Laminate Veneer**
Melissa Delania, Ira Tanti, Roselani W. Odang, Leonard C. Nelwan
Pages 964-969
- EXPERIMENTAL ARTICLE
- 22. Loss of Taste Buds in The Circumvallate Papillae of Rat Tongue after Ovariectomy**
Ervin Rizali, Widurini Djaja Suminta, Budiharto Sudiroatmodjo, Nadhira Haifa Prabowo, Elza Ibrahim Auerkari
Pages 970-974
- EXPERIMENTAL ARTICLE
- 23. The Effect of Brotowali Stem Extract (Tinospora Crispa) Towards Increasing Number of Lymphocytes in the Healing Process of Traumatic Ulcer on Diabetic Wistar Rat**
Ira Arundina, Indeswati Diyatri, Theresia Indah Budhy, Foo Yau Jit
Pages 975-980
- EXPERIMENTAL ARTICLE
- 24. Antibacterial Effects of Bioceramic and Mineral Trioxide Aggregate Sealers Against Enterococcus Faecalis Clinical Isolates**
Rusdiana, Munyati Usman, Ratna Meidyawati, Endang Suprastiwi, Dewa Ayu NPA
Pages 981-986
- EXPERIMENTAL ARTICLE
- 25. Different Food Hardness Affect Memory**
Wahyuning Ratih Irmalia, Jenny Sunariani, Christian Khoswanto
Pages 987-990
- EXPERIMENTAL ARTICLE
- 26. Brotowali Extract (Tinospora Crispa) for Oral Traumatic Ulcer in Diabetes Mellitus Wistar Rat**
Retno Indrawati Roestamadji, Ira Arundina, Indeswati Diyatri, Dewi Tamara Sambodo, Wahyuning Ratih Irmalia
Pages 991-996
- EXPERIMENTAL ARTICLE
- 27. Orthodontists Reproducibility and Accuracy in Linear and Angular Measurement on 2d Digital and 3d Cbct Radiographic Examination**
Dwita Pratiwi, Benny Mulyono Soegiharto, Krisnawati, Brama Kiswanjaya
Pages 997-1004
- CASE REPORT
- 28. Reconstruction with fibula transfer and implant supported overdenture for a mandibular defect: A multidisciplinary approach**
Tri Ardi Mahendra, Nina Ariani, Saraventi Mursid, Parintosa Atmodiwirjo, Kristaninta Bangun, Dwi Ariawan
Pages 1005-1009
- CASE REPORT
- 29. An Overjet Reduction of Class II, Division 1 Malocclusion in Twin Block Dentofacial Orthopedic and Fixed Orthodontic Treatment: Case Report**
Harun Achmad, Mardiana Adam, Sri Oktawati, Sri Ramadhany Karim, Hasanuddin Thahir, Rini Pratiwi, Annisa Wicita
Pages 1010-1016

TABLE OF CONTENTS / 2017; 10 (3)

- CASE REPORT
- 30. Impact of Delay on Diagnosis and Treatment of Oral Squamous Cell Carcinoma: Three Cases Report**
Hamdatun Rakhmania, Irna Sufiawati
Pages 1017-1020
- REVIEW
- 31. The Promising Clinical Applications of Growth Factors in Periodontal Regeneration: A Literature Review**
Ichaya Yiemwattana
Pages 1021-1028
- REVIEW
- 32. Role of Oral and Maxillofacial Surgeon in Detecting Domestic Violence**
Anand Deep Shukla, Abhay T Kamath, Chithra A
Pages 1029-1031
- REVIEW
- 33. A Review of Ribonucleotide Reductase and Cancer Therapies**
Khor Goot Heah, Nurul Ain Bt Khoruddin, Nur Rawaidah Bt Mohd Shobri, Syairah Nabila Bt Suhaimi, Tang Thean Hock, Mohd Yusmiadil Putera Mohd Yusof
Pages 1032-1037
- MEDICINE**
- CLINICAL ARTICLE
- 34. Digital Hematocrite Test, a New Breakthrough in the Medical Equipment for Non Invasive Hematocrite Level Measurement in Dengue Patients**
Prihartini Widiyanti, Nasronudin
Pages 1038-1041
- CLINICAL ARTICLE
- 35. Bone Quality, Biochemical and Blood Markers in High Power Electrical Workersf**
Cemil SERT, Pelin YAZGAN
Pages 1042-1047
- CLINICAL ARTICLE
- 36. The Relation of Reflux Finding Score and Reflux Symptom Index with Middle Ear Pepsin Level in Chronic Suppurative Otitis Media**
Ayu Astria Sriyana, Susyana Tamin, Syahrial M. Hutahuruk, Saptawati Bardosono, Ina S. Timan, Ratna D. Restuti
Pages 1048-1051
- CLINICAL ARTICLE
- 37. Occupational Risk Factors for Acute Fatigue Symptoms among Indonesian Beverage Industry Workers**
Baiduri Widanarko, Robiana Modjo
Pages 1052-1054
- CLINICAL ARTICLE
- 38. A Study of Readmission Rates and the Implementation of National Health Insurance**
Kurnia Sari, Pujiyanto, Atik Nurwahyuni, Atmiroseva
Pages 1055-1059
- EXPERIMENTAL ARTICLE
- 39. Protective Effect of Propolis Extract in Kidney Male Mice (Mus musculus) Induced by Lead Acetate**
Citrasari Henra, Wiwik Misaco, Hani Plumeriastuti
Pages 1060-1065

TABLE OF CONTENTS / 2017; 10 (3)

- EXPERIMENTAL ARTICLE
- 40. Maturity and Apoptosis Rate of Cumulus - Oocyte Complex in Aceh Cattle after in Vitro Maturation**
Hamny Hamny, Widjiati Widjiati, Aulanni'am Aulanni'am, Budianto Panjaitan
Pages 1066-1069
- EXPERIMENTAL ARTICLE
- 41. Hylocereus Polyrhizus Peel Ethanol Extract- The Potential Effect to Tumor Necrosis Factor-A, Macrophage, and Matrix Metalloproteinase-9 in Endometriosis Mice**
Anindya Hapsari, Hendy Hendarto, Widjiati
Pages 1070-1073
- EXPERIMENTAL ARTICLE
- 42. Effect of Combined Cryoprotectant of Ethylen Glicol and Propanodiol on Embryo Cryopreservation to Blastomere Cell Apoptosis and Blastocyst Quality**
Epy Muhammad Luqman, Widjiati, Suryo Kuncorojakti
Pages 1074-1079
- EXPERIMENTAL ARTICLE
- 43. Effectivity of Insulin Transferrin Selenium and Bovine Serum Albumin Addition on In Vitro Culture Medium on Fertilization and Blastocyst Rate of Mice (Mus musculus)**
Widjiati, Epy Muhammad Luqman, Benjamin Christoffel Tehupuring
Pages 1080-1083
- REVIEW
- 44. Effect of Radiofrequencies Emitted from Mobile Phones and Wi-Fi on Pregnancy**
Hava Bektas, Suleyman Dasdag
Pages 1084-1095

Effectivity of Insulin Transferrin Selenium and Bovine Serum Albumin Addition on In Vitro Culture Medium on Fertilization and Blastocyst Rate of Mice (*Mus musculus*)

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Abstract

The aim of this research was to know best composition of in vitro culture medium that can support the development of zygote and cleavage into blastocyst stage of embryo and ready to be transferred into recipient. Insulin Transferrin Selenium (ITS) and Bovine Serum Albumin (BSA) need to be added to optimize culture medium so it can produce embryo with high viability to support embryo transfer program.

The addition of Insulin Transferrin Selenium into in vitro culture medium bind free radical, trigger development of the cell, inhibit damage of the cell because of its antioxidant component so it can increase blastocyst viability. The addition of Bovine Serum Albumin can increase the competence of the embryo development to grow in the in vitro culture medium.

The research started with estrus synchronized, oocyte collection, in vitro fertilization, addition of Insulin Transferrin Selenium and Bovine Serum Albumin into culture medium and examine the number of fertilization and blastocyst. The result show combination of Insulin Transferrin Selenium and Bovine Serum Albumin supplementation increasing number of fertilization and blastocyst is better compare with group that added Insulin Transferrin Selenium only ($p>0,05$) but it is not different with group that added Bovine Serum Albumin only. The conclusion from this research is addition of Insulin Transferrin Selenium and Bovine Serum Albumin can increase the number of fertilization and support the development of embryo.

Experimental article (J Int Dent Med Res 2017; 10(3): pp. 1080-1083)

Keywords: Fertilization rate, blastosis rate, cultur medium, in vitro fertilization.

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Introduction

The successful of embryo transfer is depending its embryo quality that will be transferred and the condition of endometrium. Embryo stated as good quality if the embryo will develop and grow in the uterus of recipient. Good quality embryo can be obtained in vivo also in vitro. Embryo that obtained from in vitro has some advantages, the numerous of embryo produced and embryo produced is in the same stage¹.

Nowadays the provision of in vitro embryo as transfer embryo needs is not fulfilling the quality of embryo with high viability. It is based on the low number of pregnancy from the in vitro embryo recipient. It is necessary to review the

low number of pregnancy in molecular reproduction because there are many factor that affect in vitro embryo culture like source of nutrition and stress during embryo culture².

Modifying condition of in vitro culture is one of technique to increase the number of fertilization and blastocyst viability due to the need of embryo transfer. Some growth factor is added into culture medium like Insulin Transferrin and Bovine Serum Albumin as maturation and culture medium to increase oocyte ability into meiosis II stage³.

Insulin Transferrin Selenium is complex supplement medium that consist of insulin, transferrin and selenium if it is added into culture medium can decrease the binding of free radical^{4,5}. Insulin Transferrin Selenium is complex protein that can increase development of the cell and inhibit cell damage because of its antioxidant so that in can maintain the viability of embryo. Insulin Transferrin Selenium can increase the number of fertilization, quality and viability of blastocyst from the result of in vitro culture^{6,7}.

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Bovine Serum Albumin as protein sources is composed many essential amino acids. BSA supplementation into culture medium can increase the competence of embryo development to grow into the in vitro culture medium, accelerate cleavage stage of embryo so that the embryo can grow and develop and maximally produce excellent blastocyst with high viability. Low viability of embryo will affect the implantation process on its attachment with endometrium. The decreasing of quality and viability of embryo also caused by the numerous of apoptotic trophoblast cell so the implantation and pregnancy is not occurred. Besides, endometrium thickness from recipient must be ready^{8,9,10,11}.

Viability of blastocyst from in vitro culture is affecting the successful number of embryo implantation and pregnancy after the blastocyst is being transferred. Because of that, the study to optimize culture medium is needed, so in vitro blastocyst can be produced as embryo bank and fulfill the embryo transfer needed and increase the number of pregnancy.

Based on the background the research is needed to prove the Effectivity of Insulin Transferrin Selenium and Bovine Serum Albumin Addition on In vitro Culture Medium on Apoptotic Trophoblast Cell, Blastocyst Number and Successful of Embryo Transfer.

Materials and methods

This research were using mice oocyte and embryo as the sample. This research consist of 3 treatment groups, Treatment Group 1 (T1) : Minimum Essential Medium Eagle Minimum + Insulin Transferrin Selenium 5% + Bovine Serum Albumin 5%, Treatment Group 2 (T2): Minimum Essential Medium Eagle + Insulin Transferrin Selenium 5% and Treatment Group 3 (T3): Minimum Essential Medium Eagle + Bovine Serum Albumin 5%.

Research Materials and Equipments

Materials that used in this research include male mice aged 5 month, female mice aged 3 month, Insulin Transferrin Selenium (ITS), Bovine Serum Albumin (BSA), *Pregnant Mare Serum Gonodotropin* (PMSG) (Folligon®, Intervet, Boxmeer, Holland), *Human Chorionic Gonadotropin* (HCG) (Chorulon®, Intervet, Boxmeer, Holland), *Phosphate Buffer Saline* (PBS), Medium Eagle Minimum (Sigma®, St. Louis, USA), ethilen glikol (Sigma®, St. Louis,

USA), propanediol (Sigma®, St. Louis, USA), mineral oil (Sigma®, St. Louis, USA), CO₂ Equipments that used in this research include CO₂ incubator (Thermo), mikroskop inverted (Meiji), program image raster 3.0, syringe (Terumo), pipet pasteur (Thermo), Hemi straw, petridish disposable (Thermo), millipore Tthermo),

Research Variables

The independent variable of this research are Insulin Transferrin Selenium, Bovine Serum Albumin. The dependent variable of this research are the number of fertilization and blastocyst.

Research Method

1. Superovulation and oocyte collection

Female mice were being injected with Pregnant Mare Serum Gonadotropin (PMSG or Foligon) with dosage 5 IU. Ethical clearance received from Faculty of Veterinary Medicine Airlangga University with number 717-KE. Forty-eight hours later continued by injecting Human Chorionic Gonadotropin (HCG or Chorulon) and mated with vasectomy male mice used mono-mating method. Seventeen hours after mated then the vaginal plug was checked. The female mice with vaginal plug was decapitated and the fallopian tube was collected. The fallopian tube was washed in Phosphate Buffer Saline and moved into petri dish and rip fertilization sac under the inverted microscope. The oocyte collected was washed.

2. In Vitro Fertilization

The oocyte collected was washed three times using PBS and MEM medium then moved into the fertilization medium while waiting for the preparation of spermatozoa. Spermatozoa was collected from cauda of epididymis from male mice and after that the spermatozoa was put on the same fertilization medium with oocyte before. Oocyte with spermatozoa then incubated on CO₂ 5% incubator with temperature 37° C during 7 hours and the granulosa cell was fallen out to examine zygote or 2 cells.

3. Embryo Culture until Blastocyst Stage

The zygote was moved into culture medium and incubated on CO₂ 5% incubator with temperature 37°C. Culture medium was changed twice a day until reaching the blastocyst stage.

Results

Result of this research were the number of fertilization from in vitro fertilization and the

number of blastocyst obtained from in vitro culture. Supplementation of Insulin Transferrin and Bovine Serum Albumin show the number of fertilization increased better than fertilization medium that only added with Insulin Transferrin Selenium or Bovine Serum Albumin. Analytic result from the number of fertilization were showed in the table 1 and table 2 below.

Group	Mean ± SD	p
T1	98,3340± 3,72529	0,0036
T2	90,9100± 6,42760	
T3	93,3360± 3,72529	

Table 1. Mean and standard deviation of fertilization number from BSA, ITS and Combination of BSA and ITS on in vitro fertilization medium group.

Fertility number	Group	P
	T1-T2	0,032*
	T1-T3	0,072
	T2-T3	0,080

Table 2. Mann Whitney Test result for determining the difference between treatment group to fertility number.

*The difference between each treatment group.
 T1 : Minimum Essential Medium Eagle Minimum + Insulin Transferrin Selenium 5% + Bovine Serum Albumin 5%.
 T2: Minimum Essential Medium Eagle Minimum + Insulin Transferrin Selenium 5%.
 T3 : Minimum Essential Medium Eagle Minimum + Bovine Serum Albumin 5%.



Figure 1. Embryo that have been cleavage into 2 cells.

Analytic result from the development of embryo from zygote to 2 cells, 4 cells, 8 cells,

morula and blastocyst were showed in table 3 and table 4 below. Supplementation Insulin Transferrin Selenium and Bovine Serum Albumin into in vitro culture medium can increase the number of embryo that develop become blastocyst stage and has significant different with group that only added with Insulin Transferrin Selenium or Bovine Serum Albumin.

Group	Zygote-2 Cells (Mean ± SD)	P	2 Cells-4 Cells (Mean ± SD)	P	4 Cells -8 Cells (Mean ± SD)	P	8 Cells- Morula (Mean ± SD)	P	MORULA - Blastocyst (Mean ± SD)	p
T1	100,00 ± 0,0	0,008	74,55 ± 8,4	0,977	80,00 ± 11,7	0,041	100,00 ± 0,0	1,000	91,31 ± 8,1	0,037
T2	87,27 ± 5,0		77,11 ± 4,1		70,00 ± 7,4		100,00 ± 0,0		72,33 ± 11,8	
T3	91,21 ± 5,9		74,73 ± 7,6		84,28 ± 5,3		100,00 ± 0,0		87,14 ± 13,8	

Table 3. Mean and standard deviation of blastocyst number from BSA, ITS and Combination of BSA and ITS on in vitro culture medium group.

Embryo Development	Group	P
Zygote-2cells	T1-T2	0,005*
	T1-T3	0,017*
	T2-T3	0,238
4cells-8cells	T1-T2	0,160
	T1-T3	0,242
	T2-T3	0,014*
Morula-Blastocyst	T1-T2	0,014*
	T1-T3	0,588
	T2-T3	0,070

Table 4. Mann Whitney Test result to determine the difference between each treatment group to blastocyst number.



Figure 2. Mice embryo that have been cleavage into blastocyst stage from in vitro fertilization.

Discussion

Culture medium is one of the most important component on producing in vitro embryo, many culture medium have been

develop for embryo culture importance. Generally, culture medium is containing serum or BSA. Medium that has serum or BSA will increase the rate of in vitro embryo development. BSA can bind free radical, metal, toxin, regulate redox potential, pH and osmolality and finally increase embryo development¹².

Insulin is polypeptide hormone that can affect glucose absorption and amino acid and also has mitogenic effect¹³. Addition of Insulin and Insulin Growth Factor on IVC and IVM medium can increase oocyte and embryo quality of pig. Selenium (Se) is trace element that important for some physiological activity¹⁴. Selenium on culture medium will create sodium selenite that has function to protect the cell from oxidative damage by decreasing the production of free radical and inhibit lipid peroxidation¹⁵. ITS is the best supplement to increase oocyte development and generally used in various in vitro culture¹⁶. ITS supplementation can support the development of follicle and in vitro oocyte maturation^{17,18}.

Conclusions

The conclusion from this research is supplementation using Insulin Transferrin and Bovine Selenium Albumin can increase the number of fertility compared with treatment group only added Insulin Transferrin or Bovine Selenium Albumin. The addition of Insulin Transferrin and Bovine Selenium Albumin can increase the number of embryo that developed into blastocyst compared with treatment group added with Insulin Transferrin but not with treatment group added Bovine Selenium Albumin.

Declaration of Interest

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