The SJR indicator measures the scientific influence of the average article in a journal. It expresses how central the journal is to the global scientific discussion. An average article of the journal is. Cites per Doc. (2y) measures the scientific impact of an average article published in the journal. It is computed using the same formula that journal impact factor™ (Thomson Reuters).
The SJR indicator measures the scientific influence of the average article in a journal, it expresses how central to the global scientific discussion an average article of the journal is. Cites per Doc. (2y) measures the scientific impact of an average article published in the journal, it is computed using the same formula that journal impact factor™ (Thomson Reuters).
Scope of the journal:

International Journal of Pharmaceutical and Clinical Research is a monthly international journal publishing the finest peer-reviewed research in all fields of Pharmaceutical, Chemical, Medical and Clinical Sciences like Biochemistry, Analytical Chemistry, Phytochemistry, Pharmaceutics, Pharmaceutical & Medicinal Chemistry, Pharmacology, Pharmacognosy, Pharmaceutical Analysis, Computational Chemistry & Molecular Modeling/Drug Design including Pharmacokinetics, Pharmacodynamics, Pharmacoinformatics, Pharmacovigilance, Chemoinformatics and Pharmacogenomics etc. on the basis of its originality, importance, disciplinary interest, timeliness, accessibility, elegance and surprising conclusions.

Editorial Policy:

Any technical queries will be referred back to the author, although the Editors reserve the right to make alterations in the text without altering the technical content. Manuscripts submitted under multiple authorship are reviewed on the assumption that all listed authors concur with the submission and that a copy of the final manuscript has been approved by all authors and tacitly or explicitly by the responsible authorities in the laboratories where the work was carried out.

If accepted, the manuscript shall not be published elsewhere in the same form, in either the same or another language, without the consent of the Editors. Authors must state in a covering letter when submitting papers for publication the novelty embodied in their work or in the approach taken in their research.

IJPCR insists on ethical practices in both human and animal experimentation. Evidence for approval by a local Ethics Committee (for both human as well as animal studies) must be supplied by the authors on demand. Animal experimental procedures should be as humane as possible and the details of anaesthetics and analgesics used should be clearly stated.

The ethical standards of experiments must be in accordance with the guidelines provided by the CPCSEA (animal) and ICMR (human). The journal will not consider any paper which is ethically unacceptable. A statement on ethics committee permission and ethical practices must be included in all research articles under the ‘Materials and Methods’ section.

Authors must be careful when they reproduce text, tables or illustrations from other sources. Plagiarism will be viewed seriously. If any ethical concerns are raised against published manuscript along with proofs, then the editorial board will investigate the matter and can take decisions accordingly, the decision may include removal of manuscript as well as blacklisting of authors for future publication in the journal. The authors must understand the seriousness of above statement before submitting papers in IJPCR.

All rights are reserved to editor-in-chief, IJPCR. IJPCR will be published monthly
Editorial Boards

Editor in Chief

Dr. Anantha Naik Naga ppa
Manipal College of Pharmaceutical Sciences
Manipal University, Madhava Nagar Manipal 576 104, Karnataka State, INDIA

Members:

- Dr. D. N. Mishra
  Department of Pharmaceutical Sciences,
  Guru Jambheshwar University of Science and Technology, Haryana, INDIA
- Dr. Shailender Singh
  Department of Pharmaceutical Sciences,
  Guru Jambheshwar University of Science and Technology, Haryana, INDIA
- Dr. Amit K. Tiwari
  A304 Patterson Hall, Department of Biomedical Sciences, College of Veterinary Medicine,
  Nursing and Allied Heath, Tuskegee University, Tuskegee, AL 36088, USA
- Dr. Rakesh Gollen
  Novartis Institutes for Biomedical Research, Drug Metabolism and Pharmacokinetics,
  NPKPD, USEH, 436 3203, One Health Plaza, East Hanover, NJ 07936-1080, USA
- Dr. Kalpesh Gaur
  Geetanjali Institute of Pharmacy,
  Geetanjali University Udaipur INDIA
- Dr. M.M. Gupta
  School of Pharmacy, Faculty of Medical Sciences
  The University of the West Indies, St. Augustine, Trinidad & Tobago, West Indies
- Dr. Akram Ahmad
  Department of Clinical pharmacy,
  UCSI University, Kuala Lumpur, Malaysia
- Dr. Soheir El Sayed El Sayed Kotob
  Ph.D, Researcher on Hormones Department,
  Medical Research Division, National Research Centre, Egypt
- Dr. Jongwha Chang
  Department of Social & Administrative Sciences
  800 Lakeshore Dr, Birmingham, Alabama 35229, United State
- Dr. Abdul Rohman
  Department of Pharmaceutical chemistry, Faculty of Pharmacy
  Kaliurang KM 4.5 Sekip Utara, Yogyakarta, Indonesia 55281
- Dr. Zullies Ikawati
  Gadjah Mada University, Faculty of Pharmacy
  Jl. Kaliurang Km 6.7 Gg Sumatera E-117 Yogyakarta, Indonesia
- Dr. Agung Endro Nugroho
  Gadjah Mada University, Faculty of Pharmacy
  Kaliurang KM 4.5 Sekip Utara, Yogyakarta, Indonesia 55281
- Dr. Nobuyuki Wakui
  2-4-41 Ebara, Shinagawa-ku,
  Tokyo 142-8501, Japan
- Dr. Asim Ahmed Elnour
  Department of Pharmacology, Faculty of Medicine and Health Sciences
  United Arab Emirates University, UAE
- Dr. Consolacion Y Ragasa
  Chemistry Department,
  De La Salle University, Philippines
1. The Effect of Vitrification of Oocytes Cumulus Complex Apoptosis of Mice (Mus musculus) to Apoptosis, Rate of Fertilization and Embryo Quality
   Widjianti Widjianti, Aulanni’am Aulanni’am, Viski Fitri Hendrawan
   179-182

2. Evaluation of Gloriosa superba for Yield Attributing Characters and Quantification of Colchicine Originated from Different Agro Climatic Zones of Tamil Nadu and Andhra Pradesh
   Arun Kumar P, Elangaimannan R
   183-192

3. Comparative Study to Evaluate the Surface Detail Reproduction of Dental Stone after Immersion in Various Different Disinfectant Solutions, Under Stereomicroscope 10 X Magnification –An In-Vitro Study
   Rathika Rai, M A Easwaran, K T Dhivya
   193-196

4. Effect of Jatropha curcas Latex on L3 Haemonchus contortus Larval Motility
   Noorzaid Muhamed, Syahirah Saizale, Resni Mona, Jannathul Firdous
   197-200

5. Development of Brucellosis Vaccine Based on Determinant Antigenic of Outer Membrane Protein (OMP) 36 KDA From Brucella abortus Local Isolate Aulanni’am Aulanni’am, Wwiwek Tyasingsih, Dyah Kinasih Wuragil, Fedik Abdul Rantam
   201-204

6. Assessment of Anticholinesterase Toxicity, Oxidative Stress and Antioxidant Status in Carbamate and Organophosphorus Pesticides-Exposed Agricultural Workers
   Magda M Noshy, Amal Saad-Hussein, Eman M Shahy, Haidan M El-Shorbagy, Mona M Taha, Ebtesam A Abdel-Shafy
   205-209

7. The Utilization of Fitr (Fourier Transform Infra-Red) Method Combined with Chemometrics For Authentication of Indonesian Coffee Powder
   Firman Syah A, Winingsih W, Soebara Y S
   210-213

8. Advancement of Riluzole in Neurodegenerative Disease
   Vidy Viswanad, Anand P, Shammika P
   214-217

9. Triterpenes and Sterols from Premna nasseosa Blanco
   Phelan G Apostol, Mark Anthony G Fran, Chien-Chang Shen, Consolacion Y Ragasa
   218-220

10. Management of Out of Specification (OOS) for Finished Product
    Ravi Kiran S N, Gowrav M P, Gangadhara Prapa H V, G Ravi
    221-232

11. Microencapsulation and Nanoencapsulation: A Review
    V Suganya, V Anuradha
    233-239

12. The Effectiveness of Video Education How to Use Diskus® Dry-Powder inhaler on Out-Patients Copd in Mojokerto, Indonesia
    Rifaatul Laila Mahmudah, Zullies Ikawati, Djoko Wahyono
    240-246

13. Prevalence of Human Intestinal Parasitic Nematode Among Out- Patients Attending Wudil General Hospital, Kano State, Nigeria
    Lurwan Muazu, Yahaya Abdullahi, Zaharaddeen Umar
    247-251

14. Phytochemical Screening and In Vitro Antioxidant Activity of Ethanolic Extract of Cassia occidentalis
    S Srividya, G Sridevi, A G Manimegalai
    252-256

15. Successful Conservative Management of Recurrent Focal Placenta Accreta, A Case Study
    AliSaif Batool, Aljarrash Majeda
    257-259

16. Sucrose and Facilitated Tucking for Pain Among Neonates Receiving Vaccination, in Puducherry
    Sujatha S, Rebecca Samson, Christopher Amalraj, Sundaresan
    260-263
The Effect of Vitrification of Oocytes Cumulus Complex Apoptosis of Mice (*Mus musculus*) to Apoptosis, Rate of Fertilization and Embryo Quality

Widjiati Widjiati1*, Aulanni’am Aulanni’am2, Viski Fitri Hendrawan2

1Faculty of Veterinary Medicine – Airlangga University 2Faculty of Veterinary Medicine - Brawijaya University

Available Online: 25th March, 2017

ABSTRACT

The purpose of this study was to determine the quality of cumulus oocyte complex (COC) after vitrification process toward apoptosis of COC, fertilization rate and embryo quality. Frozen occurs during the cold shock that can lead to changes in the molecular level COC. These changes will affect the quality of frozen thawed oocytes after COC. The study used two groups, There are COC not frozen and frozen COC. Parameter observed were apoptosis of blastomere cells, fertilization rate and embryo quality. Apoptosis of COC were observed with tunnel apoptec staining, fertilization rates were measured based on number of zygotes and embryo quality were observed through number of blastocyst. The data of apoptosis of blastomere cells were analyzed by Kruscal Wallis. The result showed that the apoptosis number, fertilization rate, and morula number between are significantly (p<0.05) between groups. The administration of frozen COC increase number of apoptotic blastomer cells, decreased fertilization rate up to 51.1% and embryo quality up to 69.2 %. In conclusion, Frozen on COC increased apoptosis of COC, decreased both of fertilization rate and embryo quality. The embryo Frozen of vitrification is required in the ART technology necessary to add an antioxidant to improve the fertilization rate and embryo quality.

Keywords: COC, apoptosis, fertilization rate, embryo quality.

INTRODUCTION

The aid of reproductive technology (ART) is nowadays developing to support the success of test tube baby program. One of the supporting methods is Frozen tissues, embryos, or oocytes. Frozen is conducted by storing tissues, excessive embryos, or oocytes so that the embryos can be utilized if they are needed someday. However, storing embryos or oocytes are not allowed in some countries. In vitro fertilization (IVF) usually produce many embryos, but not all embryos are transferred to the patient, and the excess embryos are stored in a frozen state. The number of embryos transferred to the patient depends on patient’s age, and maximally 3 embryos can be transferred to the patient. The remaining embryos that are not transferred to the patient can be saved through the Frozen process. However, until recently it was reported that the quality of frozen embryos is still very low. Many researchers reported poor quality of the embryo, followed by the low percentage of successful pregnancies, because of the occurrence of a failure of implantation. In recent years, it has developed a method of Frozen either COC or ovaries of patients suffering from cancer1, 2, 3. Many methods have been developed to freeze the COC, which is critical of this method is the ability to maintain the level of maturity of oocytes after in vitro

*Author for Correspondence: widjiati1962@gmail.com

maturation1. This time the results of Frozen oocytes have a low level of maturation and will be followed by difficulties in reaching the metaphase II stage2. Low oocyte maturity level after thawing is supposedly caused by cell death or blastomer cell apoptosis. Blastomere cell death caused by the temperature, the change from Frozen conditions to room temperature. Oocyte quality highly influences fertilization rate and embryo quality produced. A number of blastomeres cells undergoing apoptosis will result in lower fertilization rate, and directly reduce the quality of the blastocyst in vitro. Therefore, research is needed to determine the effect on the number of oocytes vitrified oocytes result of apoptosis, the rate of fertilization and embryo quality.
MATERIALS AND METHODS

The study used female mice (Mus musculus), age of 8 weeks. The used animal in this research was approved by Committee Ethics of Animal, Airlangga University (500 KEP).

Superovulation and Egg Collection

Female mice were injected by hormones of Pregnant Mare Serum Gonodotropin (PMSG Foligon) with a dosage of 5 IU. 48 h later they were also injected by hormones of Human Chorionic Gonadotropin (Chorulon) and directly were mated with male mice which were castrated monomattingly. Vagina plug examination was carried out after 17 h after mated, followed by collecting eggs. The eggs were washed and frozen using vitrification method.

Vitrification and Oocyte Thawing

This vitrification used the method with slight modification.

Table 1: Number apoptosis cumulus oocyte complex.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Rank</th>
<th>Asymp. Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Not Frozen)</td>
<td>8.50</td>
<td>0.00</td>
</tr>
<tr>
<td>Treatment (Frozen)</td>
<td>24.50</td>
<td></td>
</tr>
</tbody>
</table>

The brown color indicates a positive expression of apoptosis on COC

In vitro Fertilization

Cumulus oocyte complex resulted from thawing was washed 3 times in PBS and MEM media respectively. Cumulus oocyte complex washed was moved into fertilization medium. Spermatozoa was taken from cauda epididymis of male mice, then soaked in fertilization medium containing eggs with a dosage of 200.000. Cumulus oocyte complex was incubated in CO2 5% with the temperature of 37°C for 7 h and zygotes was observed, next it was moved into a new culture medium to prevent it from being contaminated by granulose cell fall off.

In vitro Embryo Culture

Fertilized embryo was moved into culture medium and incubated in incubator of CO2 5% at the temperature of 37°C. Embryo culture was changed every two days until embryo reached blastocyst stage.

Examination of Apoptosis

Microslides were deparaffinized in xylol and alcohol series. Put PBS on the slide for 10 min, absorbed the liquid with a tissue. Then it were covered with 50 mL proteinase K for 15-30 min, washed twice with diionized water (dH2O) each for 2 min. Put drops on the samples with sequencing solution for 5 min, washed the slides by using PBS for 1 min. Put drops of TdT labeling buffer to the samples for 2 reactions: TdTdNTP Mix (Cat #4828-30-04) 2 mL, TdT Enzyme (Cat #4810-30-05) 2 mL, 1 X TdT labeling Buffer 100 mL, incubated for 1 h at 37°C in a humid place. Washed slides with 1xTdT stop buffer for 5 min, washed the slides by using PBS for 1 min. Put drops of DAB solution for 2-7 min, washed slides with dH2O each for 2 min. Put drops of 1% methylgreen on the slides for 10 min, soaked and washed slides10 times with dH2O. Then microslides were dried overnight and covered with entellan.

Figure 1: Expression of COC apoptotic in Frozen and no Frozen group (Nikon microscope H600L, camera DS Fi2 300 megapixel, magnification of 100x)
RESULT AND DISCUSSION
The results of the analysis oocyte cumulus complex apoptosis showed significant (p<0.05) between groups.

![Embryos of in Vitro fertilization.](image)

**Figure 2:** Embryos of in Vitro fertilization.

(A) No Frozen COC
(B) Frozen COC

<table>
<thead>
<tr>
<th>Groups</th>
<th>Zygotes (%)</th>
<th>2 cells</th>
<th>4 cells</th>
<th>8 cells</th>
<th>Morula</th>
<th>blastocyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Frozen</td>
<td>99.6</td>
<td>99.3</td>
<td>98.9</td>
<td>99.6</td>
<td>99.6</td>
<td>99.3</td>
</tr>
<tr>
<td>Frozen</td>
<td>51.03</td>
<td>74.5</td>
<td>79.3</td>
<td>70.5</td>
<td>62.9</td>
<td>69.2</td>
</tr>
</tbody>
</table>

The number of apoptotic cumulus oocyte complexes (COC) from Frozen group was higher than the group without Frozen COC observed with immunocytochemistry (Table 1, Figure 1).

The COC vitrification process more difficult than embryos, vitrification on intact cumulus cell hinder cryoprotectants enters the cell, so that the COC is not well protected by cryoprotectants extra-cellular or intracellular. Cold shock will cause cell damage. The previous report stated on vitrification of COC cyoprotectant difficult to penetrate the cell membrane, thus forming intra-cellular ice crystals in the oocyte, which will cause damage organell intra-cellular, followed by damage to the spindle or mitochondria.

The results showed that the number of frozen COC higher than the group with no frozen COC. The results are consistent with previous research which states that the COC is vitrified cause spindle damage and mitochondria, and further causes the COC apoptosis.

Macroscopically, after vitrification process in group of no frozen COC confirmed there are no cell damage, and cumulus cells, oocytes and cytoplasm look clear. However, in Frozen COC group induced oxidative stress, apoptosis, low fertilization rate. Frozen COC group normally exposed by cryoprotectant. Intra-cellular damage takes place when cryoprotectant is not able to protect COC from cold shock. The previous research reported that on in vitro vitrification condition, spindel of microtubuli can depolarized and followed by fragmentation of DNA. The no Frozen group determined that fertilized COC result developed into embryo on second stage cells, or even reached fourth stage cells, but frozen COC group still in the form of zygotes and have low fertilization rates. The development of the embryo reaches the blastocyst stage at COC group that is not frozen reaches as high as 99.3%, while the frozen COC reached 69.2%. Frozen OCC affect the ability of embryos pass through a block of cells, in rats this happens in embryonic development stages of one cell into two cells. In this study generated the number of embryos progressed to stage 2 cells reached 74.5% and 99.3% in no frozen and frozen group, respectively. The COC quality conditions determine the quality of the embryos that developed to the blastocyst stage. Stress caused by differences in temperature will affect the quality of COC. Quality of blastomeres in the group no lower than frozen COC.

CONCLUSION
The frozen process increase apoptosis of COC, decrease both of fertilization rate and embryo quality. The embryo Frozen of vitrification is required in the ART technology necessary to add an antioxidant to improve the fertilization rate and embryo quality.

REFERENCES