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QUALITY OF BALI CATTLE OOCYTES POST VITRIFICATION WITH THE ADDITION OF ANTIOXIDANT GLUTATHIONE SULFIHYDRYL (GSH) IN MATURATION MEDIA

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Abstract

The success of oocyte maturation in vitro depends on several factors, namely the type of supplement used in the in vitro maturation medium, the quality of the oocytes used, and the risk of contamination and culture conditions. In vitro culture can cause oxidative stress, generating free radicals that can damage cells, so antioxidants are often added to overcome this problem. This study aimed to determine the effect of adding GSH to the maturation medium on the oocyte quality of Bali cattle after vitrification. This research was conducted at the Emryo In vitro Production Laboratory, Institute for Research and Community Service (LPPM) Building, Hasanuddin University, 2023. The study consisted of 4 GSH treatments: 0 mM, 0.5 mM, 1 mM, and 1.5 mM, with 6 replications. The research variables were viability, fractures of the zona pellucida, cytoplasmic shrinkage, and cytoplasmic lysis in post-vitrification and thawing oocytes. The results showed that adding GSH significantly affected oocyte viability ($P<0.05$). Oocyte viability in control (without GSH) was significantly lower ($P<0.05$) compared to the 0.5 mM, 1 mM and 1.5 mm treatments, but between the 0.5 mM, 1 mM and 1.5 mm treatments were not significantly different. The effect of treatment on the fractures of the zona pellucida variables, cytoplasmic shrinkage, and cytoplasmic lysis had no significant effect ($P>0.05$). It can be concluded that there was an increase in oocyte viability in line with the increase in GSH dose.

Keywords: quality, oocyte Bali cattle, cryopreservation, antioxidants

KUALITAS OOSIT SAPI BALI PASCA VITRIFIKASI DENGAN PENAMBAHAN ANTIOKSIDAN GLUTHATIONE SULFIHIDRIL (GSH) PADA MEDIA MATURASI

Abstrak

Keberhasilan maturasi oosit secara in vitro sangat tergantung pada beberapa faktor yaitu jenis suplemen yang digunakan dalam media maturasi in vitro, kualitas oosit yang digunakan, dan resiko kontaminasi serta kondisi kultur. Kultur in vitro dapat menimbulkan stress oksidatif yang menimbulkan radikal bebas yang dapat merusak sel sehingga ditambahkan antioksidan untuk mengatasi masalah tersebut. Tujuan penelitian untuk mengetahui pengaruh penambahan GSH pada media maturasi terhadap kualitas oosit sapi Bali pasca vitrivikasi. Penelitian ini dilaksanakan di Laboratorium produksi Emryo In vitro, Gedung Lembaga Penelitian dan Pengabdian Masyarakat (LPPM), Universitas Hasanuddin 2023. Penelitian terdiri dari 4 perlakuan GSH, yaitu 0 mM, 0,5 mM, 1 mM, dan 1, 5 mM dengan 6 kali ulangan. Variabel penelitian adalah viabilitas, zona pelusida fraktur, penyusutan sitoplasma, dan sitoplasma lisis pada oosit pasca vitrifikasi dan thawing. Hasil penelitian menunjukkan bahwa penambahan GSH berpengaruh nyata terhadap viabilitas oosit ($P<0,05$) . Viabilitas oosit pada kontrol (tanpa GSH) nyata lebih rendah ($P<0,05$) dibanding dengan perlakuan 0,5mM, 1mM dan 1,5Mm tetapi antara perlakuan 0,5mM, 1mM dan 1,5Mm tidak berbeda nyata. Pengaruh perlakuan terhadap variabel zona pelusida fraktur, penyusutan sitoplasma, dan sitoplasma lisis tidak berpengaruh nyata ($P>0,05$). Dapat disimpulkan bahwa terjadi peningkatan viabilitas oosit sejalan dengan peningkatan dosis GSH.

Kata Kunci: kualitas, oosit sapi Bali, vitrivikasi, antioksidan

INTRODUCTION

An evident demonstration of rapid advancements in science and technology within the biotechnology field is the application of biotechnology in animal agriculture reproduction. This includes techniques such as artificial insemination, frozen semen and liquid semen production, sperm sexing, and embryo transfer. This embryo transfer program covers oocytes and embryo handling (Widyastuti et al., 2015). The vitrification method was often carried out to increase the added value of

oocytes. The oocyte vitrification technique is a method to store oocytes for a long time by freezing them for storage purposes. This method is often used to keep samples frozen to store, preserve, guarantee, and maintain the survivability of cell oocytes.

Vitrification is often used to preserve strains with high genetic value and endangered species (Hartoyo, 2019). The vitrification method has been done for a long time to preserve and store oocytes in adult females for long periods (Gook, 2011). Vitrified oocytes are not considered dead but only suspend their growth and metabolism for a specific duration. Oocytes can continue developing after the thawing process (Hartady et al., 2018). Before vitrified, oocytes have to be matured *in vitro*.

In vitro oocyte maturation is a culture and oocyte maturation outside the body. Through maturation, mature *in vitro* can be obtained in large amounts by culturing oocytes that have not been ovulated on maturation media (Shamsuddin, 2017). A maturation media that can effectively nurture immature oocytes into mature ones is crucial for enabling fertilization and subsequent development into zygotes and embryos. To achieve successful oocyte culture *in vitro* (Hammam et al., 2010) and protect the quality of the initial oocytes before the culture process (Lonergan et al., 2003; Anguita et al., 2007), several materials or supplements in the medium are needed. It's also important to note the possibility of contamination from the culture's environment (Sagirkaya et al., 2007). To prevent contamination, antibiotics, and antioxidants are often added to the medium, such as vitamin C, vitamin E, Insulin Transferrin Selenium (ITS), and GSH (Glutathione).

Antioxidants also play an important role in the oocyte maturation process. The oocyte maturation process includes several molecular events in the cell, such as biochemical component synthesis, protein phosphorylation, and activation of certain metabolic pathways. GSH antioxidants can ward off free radicals due to reactive oxygen species (ROS) in oocytes, guarding oocytes from damage (Quarterly et al., 2002). Studies show that activated oocytes increased after adding GSH to the culture medium (Hasbi et al., 2012).

There is no information regarding the Bali cattle oocyte quality post-vitrification. Therefore, this research assesses the Bali cattle oocyte quality post-vitrification after adding GSH in the media.

RESEARCH METHODS

This research was conducted in March-May 2023 at the In Vitro Embryo Production Laboratory, Institute for Research and Community Service (LPPM) Building, Hasanuddin University.

Materials used in this study were Bali cattle ovary, mineral oil (Sigma Chemical Co. St. *Louis MO*, USA), glutathione, NaCL, distilled water, pen-strip (100 IU/ml), phosphate-buffered saline (PBS), FBS /serum, TCM-199, PMSG, hCG, gentamycin, BSA, EG, DMSO, sucrose, 70% alcohol, and liquid nitrogen.

The tools we used were an incubator, Olympus SZ51 microscope, infusion hose, scalpel, petri dish, measuring cup, Erlenmeyer ash flask, freezer, water heater, analytical balance, object and cover glass, dropper pipette, micropipette, straw, measuring cup, stainless steel spatula, test tubes, centrifugation microtube, centrifuge tube, gloves, and tissue.

The medium for the oocyte was a transport medium consisting of 9 grams of NaCl (Natrium Chloride) + distilled water until it reached a final volume of 1 liter, then 500 µl of penicillin-streptomycin (pen-strep) (100 IU/ml) was added, a collection medium consisting of from 48.75 ml *phosphate-buffered saline (PBS)* + 1.25 ml FBS/ *serum* (2.5%) + µl streptomycin penicillin (100 IU/ml), and maturation medium consisting of 1800 µl TCM-199 solution +200 µI *serum* /FBS (10%)+ 20 µI FSH, 20 µI hCG + 4 µ I *gentamycin*.

Oocytes were collected with the slicing method (enumeration), and the oocytes were selected using an *Olympus SZ51* Japan microscope (only grade A and B oocytes were collected for maturation). Grade A and B oocytes are characterized by several layers of compact cumulus cells and homogeneous cytoplasm. Culture oocytes were done in drops (80 µl/drop) with the number of oocytes 10-15/drop, then coated with mineral oil. Maturation was carried out in a 5% CO_2 incubator at 38.5 ^oC for 24 hours (Hasbi et al., 2012).

Mature oocytes were vitrified. Oocyte vitrification was carried out using a closed straw system with a vitrification medium

containing 3% BSA + 15% DMSO + 15% EG + 0.5 M sucrose. Oocytes were equilibrated in an equilibration medium containing 3% BSA + 10% EG for 3-5 minutes. Then the oocytes were put into a 0.25 ml straw. Straw was filled with sucrose, followed by an air chamber, then sucrose, air chamber, vitrification medium, air chamber, then vitrified medium containing oocytes, followed by an air chamber, then sucrose. The open end of the straw was then sealed and pre-chilled on top of the container containing liquid N_2 for 10 seconds and stored in the container.

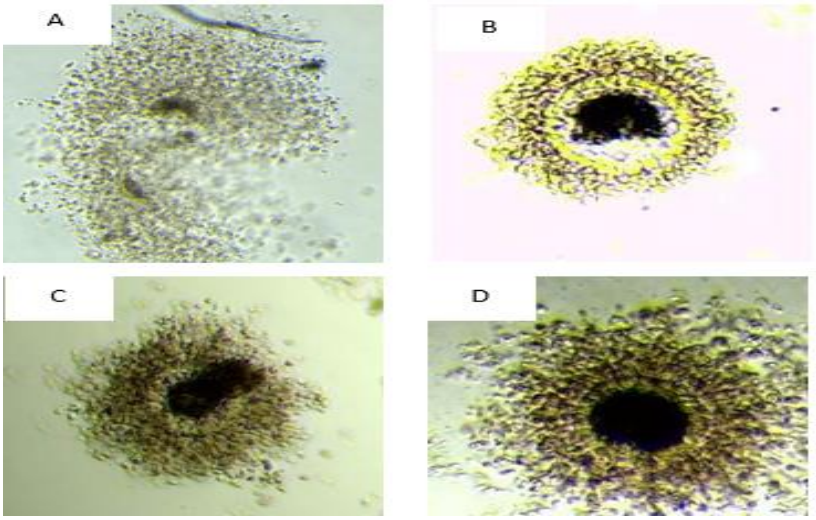
After the vitrification process, oocytes were thawed after storage in liquid nitrogen for 7 days. Straws taken from liquid nitrogen were allowed to sit at room temperature for 6 seconds and then immersed in warm water at 37 ˚C for 30 seconds. The straw was then flicked gently to mix the solution in the straw before being transferred to a thawing medium containing 3% BSA and 0.25 M sucrose. The oocytes were transferred to an adaptation medium containing 3% BSA and gentamicin. After 30 minutes of recovery in media adaptation, the oocytes were moved to PBS to assess their survivability. The surviving oocytes were those regular, round shaped that do not lyse, shrink, swell or blacken. The surviving oocytes were genetically activated and cultured in *vitro* (Zhou et al., 2016).

This research was an experimental laboratory study using a Completely Randomized Design (RAL) Anova consisting of 4 treatments and 6 replications. The treatments were adding different GSH levels: 0.5 mM, 1 mM, and 1.5 mM on the maturation medium. The observed variable was the oocyte morphology indicated by viability, zone of fluid fracture, cytoplasmic shrinkage, post-vitrification, and cytoplasmic lysis thawing. If there were a significant difference among the vitrification result data, the analysis would proceed with Duncan's test. The data was processed using the SPSS v16 program.

RESULTS AND DISCUSSION

The vitrification process was conducted to provide oocytes for *in vitro* fertilization (VIF) which were then used for embryo transfer programs. Research on GSH supplementation on maturation media was expected to provide quality Bali cattle oocytes judging from the conditions of oocyte morphology after cryopreservation and thawing processes.

The morphological condition of post-thaw oocytes can be seen from the zona pellucida and cytoplasm condition. Dead oocytes are characterized by fractures of the zona pellucida (A) and shrunken cytoplasm (B) or lysed cytoplasm (C). Viable oocytes (viability (D)) were characterized by intact zona pellucida and cytoplasm after thawing.



Description: A. Oocyte with fractures of the zona pellucida B. Oocyte with shrinkage of the cytoplasm C. Oocytes with lysed cytoplasm (AC dead oocytes). D. Surviving oocytes

Figure 1. Post Vitrification Oocytes

Table 1. Observations after vitrification of Bali cattle oocytes

Treatment	N	Parameters (%)			
		Life (Viability ity) N (%±SD)	**Fractures of the Zona Pellucida N (%±SD)**	**Cytoplasmic Shrinkage N (%±SD)**	**Cytoplasm lysis N (%±SD)**
P0	39	22 (53.86±10.78) [a]	1 (3.33±8.16) [a]	11 (26.13±15.82) [a]	5 (16.66±26.58) [a]
P1	33	20 (66.62±8.72) [b]	0 (0.00±0.00) [a]	9 (24.68±8.67) [a]	4 (8.69±11.16) [a]
P2	38	26 (69.30±9.86) [b]	0 (0.00±0.00) [a]	7 (17.64±17.38) [a]	5 (13.05±10.56) [a]
P3	44	32 (73.33±8.16) [b]	1 (1.38±3.40) [a]	8 (20.83±10.20) [a]	3 (4.44±7.57) [a]
Total	154	100 (65.78±11.52)	2 (1.18±4.35)	35 (22.32±13.06)	17 (10.71±15.47)

Note: SD = Standard Deviation
Different letters in the same column indicate a significant difference (P <.0,05).

After thawing the oocytes and analyzing the variable's viability, it was found that there was a significant (P<0.05) influence of GSH administration in the culture medium. The viability of oocytes in control (without GSH) was slightly higher (P<0.05) compared to other levels of GSH administration (0.5mM, 1mM, and 1.5mm). However, there was no significant difference between treatments 0.5 mM, 1 mM, and 1.5 mm. A GSH concentration of 1.5 mM showed the highest viability of 73.33%, as presented in Table 1. This was caused by higher GSH concentration in the medium.

Results obtained in this study are similar to Gunawan's (2021) research, which found that adding 1.5 mM concentration of GSH shows a higher oocyte viability percentage. The percentage of viable Garut sheep oocytes post vitrification and thawing increases along with the increasing GSH concentrations. The GSH acts as a natural antioxidant in cells, guarding its front against reactive oxygen species (ROS) effects of oxidative stress. Following Luberta's (2005) statement, *glutathione sulfhydryl* is a tripeptide thiol *(y-glutamylcysteinylglycine)* part of the non-protein *sulphhydryl* that has a vital function in the antioxidants detoxification process, such as maintaining/looking after intracellular redox condition and opposing the presence of oxidative stress in GSH to reduce and oxidize (GSSG). Yuniastuti (2016) agreed that GSH's molecule structure has antioxidant characteristics, capable of donating electrons to molecules that don't pair and cutting off free radical reaction chains.

Results also show that on fractures of the zona pellucida, cytoplasmic shrinkage and cytoplasmic lysis variables did not change significantly (P > 0.05). However, cytoplasm shrinkage and the highest cytoplasmic lysis contained in P0 are presented in Table 1. This shows that using GSH in the maturation medium results in more living oocytes than those without GSH on fractures of the zona pellucida, cytoplasmic shrinkage and cytoplasmic lysis variables. This was due to GSH's ability to stop free radical chain reactions by donating electrons and converting them into harmless forms. GSH can also regenerate other antioxidants, such as vitamin C and vitamin E, thereby increasing the efficiency of the antioxidant system (Luberda, 2005). However, each treatment had no difference (P>0,05).

CONCLUSION

The addition of the antioxidant *glutathione* to the maturation medium showed an increase in the Bali cattle oocyte quality after vitrification. It showed an increase in line with the increase in GSH concentration by 0.5 mM, 1 mM, and 1.5 mM (66.62%, 69.30%, and 73.33%, respectively).

SUGGESTION

Based on the study's results, supplementing 1.5 mM of GSH in the oocytes culture medium is suggested, hoping that more oocyte viability will be obtained after the vitrification process.

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