Effect of DMSO concentration on the quality of goat fetus fibroblast cell cultured in vitro

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Abstract. Dimethyl sulfoxide (DMSO) is one of the cryoprotectants that have been used in cell freezing. Cryoprotective agents are necessary to deal with damage in cells associated with slow freezing. Goat foetuses are obtained from local slaughterhouse in Sukun, Malang, East Java.

Using various DMSO concentration that were 0% control, 5%, 10%, and 15%. The cell was free with slow freeze methods using Mr. Frosty in -20°C. After 24 days, the samples were analyzed for their viability, size, and abnormality. The result shows the cell size ranged from 3 µm until 5 µm in all samples, categorized as small cell. In viability is significantly influential on 10% DMSO concentration of 92%, while at 0% concentration is 0%, 5% concentration is 56%, and 15% concentration is 89.67%. The abnormality in DMSO 0% all cells are dead and the tissue isn't developing, in DMSO 5% the abnormality is 3.17%, DMSO 10% is 2.5%, and DMSO 15% 5.67. From the results, we can conclude that the concentration of 10% of DMSO is the best concentration to be used as a freezing method for goat fetus cells.

Keywords: DMSO, Goat, Fetus, Mr.Frosty

1. Introduction
Livestock biodiversity needs serious attention. Overall the existence of a number of domestic livestock has been threatened, and is ready to experience extinction. Out of 6379 domestic animals 9% of them are in critical condition and 39% of them are endangered [1]. Notter [2] adds that the extinction of a cattle nation because beneficial genes are always in a low number of types of production so a systematic program of genetic resource conservation needs to be done.

There are many methods that can be chosen for the conservation of genetic sources, including cryopreservation of germplasm (ex situ). Germopreservation of germplasm is useful for storing the nation's livestock germplasm that is as limited as possible. Cryopreservation of germplasm is usually done through tissue storage in cold temperatures or freezing it [3]. The cryopreservation process is a very long process, including the equilibration process at -20°C, cooling at -80°C, and finally freezing on liquid nitrogen -196°C. So many processes must be passed, the most important thing to do first is to pay attention to the initial handling process, namely the handling process at the equilibration temperature of -20°C because the initial process will determine the success of the subsequent cryopreservation process.
At temperatures of -20°C there are a number of cells that have not frozen completely, this means there are still a number of cell components that are still in the form of water even though in very small amounts, if this is left it will poison cells and cause cell death resulting in low cell viability. Low cell viability is also caused by the occurrence of dehydration in cells, cold temperatures (-20°C) outside the cell cause the discharge of fluid in the cell and therefore a cryoprotectant material is needed to replace the cell fluid loss. Storage in freezing temperatures requires the addition of cryoprotectant material to maintain optimal cell conditions [4]. There are many types of cryoprotectant materials that can be used in the cell freezing process. DMSO or Dimethyl sulfoxide is one of many types of cryoprotectants that are currently widely used in somatic cell freezing [5]. The advantage of DMSO compared to glycerol is that DMSO has a lower molecular weight than glycerol and DMSO can better prevent problems. Changes in osmosis pressure after thawing compared to glycerol.

DMSO as cryoprotectant can be added to the freezing medium with a certain concentration and this must be considered because it involves changes in osmosis pressure in cells caused by differences in the concentration of DMSO and cell fluid. It is necessary to find the DMSO concentration that is at equilibrium temperature -20°C fetal skin goat fibroblast tissue so that cells with high viability will be obtained.

2. Materials dan methods
The sample of this study was using fetus fibroblast tissue which is collected from slaughterhouse. Each tissue was cut into pieces approximately 1 mm in size, and then freezing with TCM 199 media with 50 U/mL penicillin, 50 g/mL streptomycin, 20% FBS which added with various DMSO concentration 0% (as a control), 5%, 10%, 15% after that the tissue in cryovial was frozen in Mr frosty during 24 hours.

Tissue in the cryovial was thawing in waterbath 38 °C and then cultured in TCM 199 media, 50U/mL penicillin, 50 g/mL streptomycin, 1% FBS and incubate at 38 °C, 5% CO₂. Cell culture media were change twice a week. Upon reaching confluence cells were trypsinized with 0.25% trypsin EDTA solution in phosphate buffer saline (PBS).

Cells then analyzed their quality. For viability test, Hoescht 33342 – PI dye were used, after cell were trypsinized, living cells shows in blue colour and died cells shows in red colour. For cell concentrations, using haemocytometer to calculate the red blood cells using formula:

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\text{Cell concentration} = \frac{N \text{ total cells}}{\text{Volume}} \] [6]. For cell abnormality, cells morphology was taken analyzed comparing for normal cells.

3. Result and Discussion
3.1. DMSO concentration on cell viability evaluation
The cell viability value is calculated, shows in percentage, from the sum of cells that are blue color. The percentage data on cell viability as found in Table 1. The results of the observation show that in the control (0% DMSO) there is no data that can be obtained because there is no cell expands, which is can be conclude as dead cell. Seeing the tendency above, it is increasingly proving that the need to add cryoprotectant to avoid cell death caused by the freezing process in this case is DMSO.

Cells or tissues are stored at low temperatures and cells will lose fluid and will become more dehydrated. Continuous loss of cell fluid causing intracellular cell freezing [7]. Cold temperatures and thawing processes dangerous for membrane conditions. Rupture of plasma membranes is one indication of cell death which is currently the most easily recognized. Freezing method also interferes with biological activities in the membrane [8]. The presence of cryoprotectant material in membranes greatly contributes to the osmotic pressure in the membrane which will affect the chemical of water molecules. Adding 5% to 15% DMSO the intracellular ice formation process can be avoided. DMSO is one of the cryoprotectant ingredients commonly used in cell freezing [9]. The DMSO work system is to replace the position of water in cells so that polar water molecules can cooperate with polar constituents in cells.
Table 1. The cell viability percentage data were based on different DMSO concentrations and the freezing time at equilibration temperatures -20 °C.

| DMSO’s Concentration | Freezing (hour) | Repetition (%) |
|-----------------------|-----------------|----------------|
|                       |                 | 1  | 2  | 3  |
| 5%                    | 1               | 57.7 | 45.5 | 61.5 |
|                       | 8               | 47.5 | 0  | 0  |
| 10%                   | 1               | 65 | 52.5 | 67.5 |
|                       | 8               | 63 | 43 | 47.5 |
| 15%                   | 1               | 63 | 58.5 | 61.5 |
|                       | 8               | 27.5 | 25.5 | 57.5 |

Figure 1. The percentage of cell viability based on the effect of different DMSO concentration

Based on the graph in figure 1, 10% DMSO concentration showed the best effect even though there was no statistically significant difference (P> 0.05). The addition of DMSO cryoprotectant material will affect the osmotic pressure in the cell. Cryoprotectant materials that have low molecular weight and increased concentration in the membrane system will reduce indications of incidents associated with the danger of dehydration, besides high concentrations also help reduce the high temperature of membrane transitions and reduce the occurrence of non-lamellar phase [10]. The percentage of viability in the 15% DMSO treatment which tended to decrease compared to the 10% DMSO treatment showed that an increase in DMSO concentration of 5% (from 10% to 15%) resulted in fatal and reduced cell viability. This decrease in cell viability is probably caused by high osmotic pressure in the cell so that the cell will bulge and then the cell membrane breaks, then the rupture of the cell membrane will cause cell death.

3.2. DMSO concentration on cell size

The trypsinized cells and the number of small size cells based on DMSO concentration were presented in Figure 2 and Table 2, respectively. Based on the result from table 2, it shows that at a concentration of 10% and 15% DMSO, the number of small-sized cells is likely to be the same. Whereas based on freezing time at equilibration temperature stated the number of small size cells is not significantly different (P> 0.05). Cell size can describe the phase in the cell. There are several factors that influence the efficiency of core transfer, one of which is the cycle phase of the donor cell when transferred [11]. According to research conducted by [12] in cattle somatic cells small cell size
in general can divide better when transferred to recipient cells and usually cells with small size will be in phase G1. In goat somatic cells Cell sizes are grouped into 2 categories: small cells (3 pm-5 pm) and large cells (7 pm-9pm).

![Figure 2. Trypsinized cells condition (1 bar = 1 µm)](image)

| DMSO’s concentration | Mean (%) | Notation |
|----------------------|----------|----------|
| 5%                   | 56.00    | a        |
| 15%                  | 89.67    | b        |
| 10%                  | 92.83    | b        |

Note: Different notations indicate the influence between treatment

3.3. DMSO concentration on cell abnormality

Cell abnormality is a condition of cell abnormalities seen from the morphological side, this condition can be a wrinkle seen in cell membranes and oval shaped cells like those seen in Figure 3. Based on the results of the analyzed, it was shown that there was a significant difference in the treatment of DMSO concentrations of cell abnormalities (P <0.05) such as Table 3.

![Figure 3. The abnormality of cell based on the morphology (1 bar = 1 µm)](image)
Table 3. BNT test results (5%) of Cell abnormalities based on different DMSO concentrations.

| DMSO’s concentration | Mean (%) | Notation |
|-----------------------|----------|----------|
| 10%                   | 2.50     | a        |
| 5%                    | 3.17     | a        |
| 15%                   | 5.67     | b        |

Note: Different notations show differences between treatments (p <0.05)

The high abnormalities of cell morphology are probably due to the high concentration of DMSO can be toxic, and it is estimated that toxic effects affect the cell system and eventually appear as morphological abnormalities [13].

3.4. DMSO concentration on total cell

The results from the observation showed that there were significant differences in the number of cells based on the effect of different DMSO concentrations (p <0.05). The results of further tests after Kruskal Wallis are shown in Table 4. The results based on the non-parametric test of Kruskal Wallis state that the highest number of cells is produced at 10% DMSO concentration. The high number of cells is also influenced by the high value of cell viability at 10% DMSO concentrations as discussed previously. Cell tissue that is able to grow and proliferate is tissue that has viable cells in it. Cell proliferation is needed because cells will multiply until finally confluence.

Table 4. Kruskal Wallis further test results for total cells based on DMSO concentration.

| DMSO’s concentration | Total cells (Rank) | Notation |
|-----------------------|--------------------|----------|
| 5%                    | 7.0                | b        |
| 15%                   | 17.0               | a        |
| 10%                   | 21.0               | b        |

Note: Different notations indicate the influence between treatment

4. Conclusion

The concentration of 10% of DMSO as the addition of cryoprotectant material to maintain optimal cell conditions is the best concentration to be used as a freezing method for goat fetus cells. To support the next cryopreservation process, it is necessary to observe cell chromosomes to ensure that the high cell viability produced must be followed by a low value of cell abnormalities.

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