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The Outcome of in Vitro Embryo Transfer on Bali Cattle by Utilizing Fresh and Frozen Embryos

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Abstract

The purpose of this research to find out the morphological differences of fresh and frozen embryos which is produced *in vitro* and the success rate of pregnancy embryo transfer in Bali cattle. The method used in embryos produced were oocyte collection, *in vitro maturation*, *in vitro fertilization*, *in vitro culture*, there are two embryos produced, fresh embryos and frozen embryos. Embryos that have been produced *in vitro* will be transferred to the recipient Bali cattle that have been estrus synchronized. Results revealed that morphology of fresh embryos have symmetrical shapes whereas frozen embryos shrink, then the pregnancy rate was higher in the used of fresh embryos (41%), compared to the used of frozen embryos (12,5%).

Keywords: Embryo transfer; *in vitro*; morphology; pregnancy.

1. Introduction

One of the reproduction technologies that can be applied in upgrading the quality and the population of livestock is Embryo Transfer (ET), a reproduction technology post artificial insemination. The ET technology can be applied by using *in vivo* and *in vitro* produced embryos.

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The transfer of embryos by *in vitro* has been widely used, however, from economical perspective, it requires more cost to fabricate hormones in the process, moreover, the estrus responses from the recipient are still varies [1]. The high cost and the varied estrus responses in the process of the ET by using in vivo produced embryos require to the use of *in vitro* produced embryos, not only lower in cost, but also utilizes the use of ovary waste Bali cattle from slaughterhouse (RPH). The applications of the *in vitro* produced embryos are rarely used, particularly in South Sulawesi. The outcome of ET by using in vitro embryos in the form of fresh and frozen has experienced fluctuation as a result of the used of variety medium and techniques [15]. Generally, the process of embryo production goes through main stages: oocyte maturation (*in vitro* maturation), oocyte fertilization (*in vitro* fertilization), and the growing of fertilized oocyte to the stage of morula or blastocyst (*in vitro* culture). One of the deficiency of the *in vitro* embryo transfer is the perishability of the fresh embryos. It is then been frost in order to have longer lifespan. The information of the application of ET for Bali cattle is still very limited, therefore, the research on ET application by using fresh and frozen embryos should be conducted and developed to accelerate the growth of Bali cattle. Based on the background in applying the ET by using in vitro method, this research is conducted to recognize the differences between fresh and frozen Bali cattle embryos produced morphologically by *in vitro* and the effects on the outcome of the ET, marked by the bred of the ET recipients.

2. Materials and Methods

2.1 Oocyte Collection And In Vitro Maturation

Bali cattle ovary which from abattoir waste is used. It then stored in container filled with NaCI physiologist. The collected ovarium is brought and re-cleaned by using NaCI physiologist in laboratories. The scalpel is used as the slicing technique to obtain oocyte [6]. Under stereomicroscopic, the *Phosphate buffered saline is used to collect the oocyte* (PBS, Gibco, USA) and add with 0.2% bovine serum albumin (BSA) (Sigma, USA). The collected oocytes are then selected based on their morphologies to obtain oocytes with cytoplasm which homogenic with cumulus cell more than three layers. The selected oocytes are cleaned for three times in maturation medium, then they are maturated. Maturation medium refers to research [8] medium 199 (Gibco, USA) added with 0.3% BSA, 10 IU/ml *pregnant mare serum gonadotrophin* (PMSG) (Intergonan, Intervet Deutschland GmbH), 10 IU/ml *human chorionic gonadotrophin* (hCG) (Chorulon, Intervet International B.V. Boxmeer-Holland), 50 µg/ml *gentamycin* (Sigma, USA). The oocyte maturation is conducted on *petri dish* with diameter of 35 mm (Nunclon, Denmark) in the form of drops each at 100 µl for 10-15 Oocytes and covered with mineral *oil* (Sigma, USA) in an incubator of 5% CO2, temperature 38.5^{o}C for 24 hours.

2.2 In Vitro Fertilization

The 24 hour maturated oocytes are then fertilized. The frozen semen used is from a male Bali cattle which obtained from the regional artificial insemination Office, Bureau of Husbandry and Animal Health, Province of South Sulawesi. The frozen semen is thawing at 37^{o}C for 20 seconds, next, it is centrifugated at the speed of 700 G for 5 minutes in fertilization media. The fertilization media used refers to [8, 15]. Post the centrifugation, spermatozoa sediment is liquified with fertilization media to the point of last concentrate 1.5×10^{6}

spermatozoa/ml. Fertilization is conducted in the form of drops (10-15 oocytes in 100 µl Fertilization medium) and covered with mineral *oil* (Sigma, USA), it is the incubated for 5-6 hours in incubators of 5% CO2 at the temperature of 38.5^{o}C.

2.3 In Vitro Culture

After 5-6 hours of IVF, oocytes are cleaned for four times at culture media (CR1aa) [2]. The culture is conducted in the form of drops each 100 µl for 10-15 oocytes and covered with mineral *oil* (Sigma, USA) then placed in incubator 5% CO2, with temperature of 38,5^{o}C for 6 to 8 days of modification [12].

2.4 Cryopreservation

Post the process of *In Vitro Culture*, Embryos reached the stage of blastocyst, they are then frosted /cryopreserved, the media for cryopreservation used is *Dymetil Sulfoxide* in *phosphate buffered saline* with the addition of sucrose (DMSO 15% + PBS + 0,5M) [19], it then filled into straw and cryopreserved in a container of liquid Nitrogen. The embryos are stored and been observed and evaluated to see the viability of embryos cell fission.

2.5 Embryo Transfer

The selection of female cows which will be used as recipients is conducted by examine the reproduction parts. The qualified cows are then used as recipients. Next, the libido is synchronized by injecting PGF2α (Estron) with the dosage of 2ml/one cow by intramuscular. The transfer of embryos is conducted after 6-7 days estrous cycle. The pregnancy examination is performed 2 months post the ET.

2.6 Data Analysis

The outcome of the data of the research will be analyzed by using SPSS with t-examination and presented in percentage.

3. Result and Discussion

3.1 Morphology of Embryonic Fresh and Frozen Bali Cattle Produced In Vitro

Morphology embryos fresh and frozen Bali cattle produced *in vitro* can be seen in Figure 1

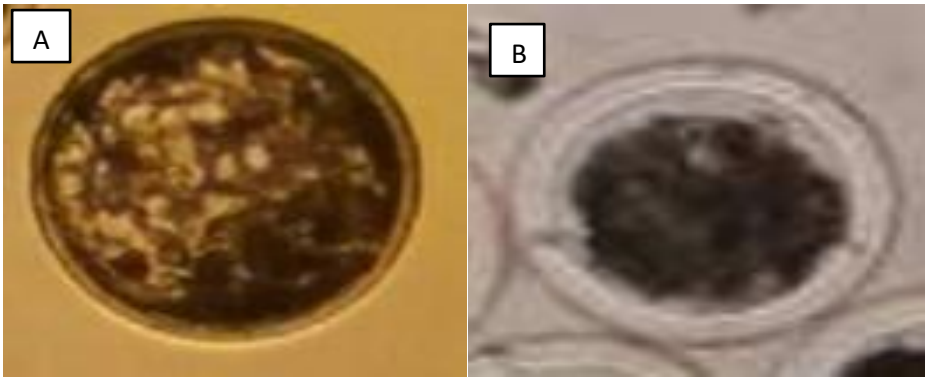

Figure 1: A: Fresh embryo, B: frozen embryo

The morphology of fresh and frozen embryos is different, the difference can be seen in (Figure 1.A) morphologically fresh embryos have a symmetrical shape, showing clearer barriers in each blastomere, compact blastomeric bonds and in fresh embryos also show cavities. Clearly this result is in accordance with the results of the study [1] which states that morphologically, the good quality of embryos at the blastocyst stage shows a circular and symmetrical shape, is not deformed, while the blastomere bond is tight and compact. The morphological quality of the embryo can affect the success of the embryo transfer, Febretrisiana and Pamungkas [4] stated that the quality of the embryo transfer is one way of projecting the success of embryo transfer. Embryos in frozen form or have gone through a cryopreservation stage are then thawed again before being transferred (Figure 1.B) morphologically the size of the embryo has diminished, showing no boundaries in each blastomeric bond, each blastomere appears to shrink, the cavity of *perivitelline widens* due to the osmotic pressure that occurs during the freezing process. The results is in line with the results of research obtained by Tao [18] who stated that the morphological changes in the embryo after the embryo is frozen. Changes in embryo morphology during the frozen process might occur due to several factors, one of them is the type of cryoprotectant used in the freezing process, this is in line with the results of research obtained by Cadre [10] which states that the use of cryoprotectant types or a combination of cryoprotectant use affects and will risk the osmotic pressure against cells.

3.2 The Success Rates of Embryo Transfer (TE) Using Fresh and Frozen Embryos of Balinese Cattle Produced In Vitro

The success rate of embryo transfer using fresh and frozen embryos of Bali cattle produced *in vitro* can be seen in Table 1.

Table 1: Results of the Success of Fresh and Free Embryo Transfer frozen

Type embryos	**Number of embryos**	**Successful Transfer embryos (TE)**	
		Pregnant	**Not Pregnant**
embryos Fresh	12	5 (41%)[a]	7 (59%)[a]
embryo frozen	8	1 (12.5%)[b]	7 (87.5%)[b]

Note: Superscript different there the same column showed a significant difference (P <0.05).

The results of the study showed that the recipient pregnancy rate of Bali cattle using fresh embryos was higher compare to the frozen embryos. While the quantity of embryos in fresh form successfully transferred to the recipient is 12, while frozen embryos is 8. The success rate of embryo transfer is characterized by whether the recipient has transferred the embryo in both fresh and frozen forms. The data obtained shows that the embryos in fresh form that have been transferred to each recipient are as many as 12 embryos, and after pregnancy examination 5 positive recipients are pregnant and the remaining are negatives. Whereas, successfully transferred frozen embryos, only 1 out of 8 is pregnant. Data from the percentage of embryo transfer shows that from 100% of fresh embryos that were successfully transferred, the success rate of transfer of fresh embryos in Bali cattle *in vitro* was 41%, 59% failed. Whereas, frozen embryos pregnancy results is 12.5% and the remains 87.5% failed. The results are in line with the results of studies of Febretrisiana and Pamungkas [4] states that 85% of the embryos transferred experience implantation failure and only 10% -15% have successfully implanted. Thus, from the two types of embryos, fresh embryos having a high success rate of 41% compared to frozen embryo blastocysts at 12.5%, this result is in line with the results of research by Huang and his colleagues [9] and Ferraz and his colleagues [5], which stated that the success rate of fresh embryo transfer is higher compared to embryos that have gone through a freezing process. This occurs due to the breakdown of cell occurs during the cryopreservation of the embryo, crystal ice are formed in both extracellular and intracellular resulting the accumulated electrolytes which damage the cell wall and at the thawing process the plasma membrane permeability is decreasing and cells will die. This is in accordance with Sutarjo's opinion [16] stated that the damage to embryonic cells when frozen occurs due to the formation of crystal ice.

The use of cryoprotectants can protect cells during the cryopreservation process. The degree of protection of cryoprotectants against crystallization during freezing depends on the type and concentration of cryoprotectants used and the duration of exposure, similar results obtained by [13] that the tendency to decrease the life span of the embryo after cryopreservation can also be caused by physical damage due to physical damage. the formation of ice crystals during solidification and the toxic effects of cryoprotectants and osmotic stress during cryoprotectant is released. Thawing process before embryo transfer is one of the factors causing the low pregnancy rate of ET using frozen embryos. The process of thawing can cause embryonic cells to become stressed, this is in line with the results of the study of Mori and his colleagues [11] which states that there is damage and decreased viability of embryo cells in the thawing process and shows the expression of stress markers on embryonic cells increases when thawing is completed. One of the challenges in conducting this research is the limitation of time in obtaining Cryoprotectant material for embryo crypto preservation. In addition, the number of recipient livestock is still very limited due to its insufficient availability at the breeders.

4. Conclusion

The morphology of frozen embryos has symmetric form, while frozen embryos experienced wrinkling or looks wrinkled. The fresh embryo has better results compare to the cryopreserved embryos which experiences cell damage due to the present of icy crystal.

5. Suggestion

Further research needs to be done with by using a type of cryoprotectant which is easier to get and increasing the number of recipient samples used in the study

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