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Cleavage ability of in vitro embryos of bali cattle based on different reproductive status of ovary at 48 hours after fertilization process

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Abstract. This study aims to determine the ability of in vitro embryo cleavage of Bali cattle 48 hours after the fertilization process based on different ovarian reproductive status. Ovaries from abattoirs are grouped into four categories: ovaries without corpus luteum and dominant follicles (-CL and -FD), without corpus luteum and with dominant follicles (-CL and + FD), with corpus luteum and without dominant follicle (+ CL and -FD), and with corpus luteum and dominant follicles (+ CL and + FD). After that, oocytes are collected by slicing and only oocytes with a cytoplasmic are homongent and have compact cumulus cells used in the study. The selected oocytes are then matured in an incubator of 5% CO₂, a temperature of 38.5 °C for 24 hours. After maturation, in vitro fertilization was carried out using fertilization media with a final concentration of spermatozoa 1.5 x 10⁶ spermatozoa/ml. Oocytes 5-6 after fertilization were washed 3 times using CR1aa media then cultured in 5% CO₂ incubator, temperature 38.5 °C for 48 hours. After 48 hours the culture was evaluated for the ability of embryo cleavage. The results showed that the number of embryos capable of cleave to stage 2 cells were not significantly different (P>0.05) in all treatments (1.64 ± 0.38% vs 8.00 ± 0.90% vs. 4.85 ± 0.96% vs. 8.22 ± 1.86%), while the number of embryos capable of cleave reached stage 4 cells (11.48 ± 0.82% vs 24.00 ± 2.64% vs. 24.27 ± 2.23% vs. 10.96 ± 0.69%), 8 cells (11.48 ± 1.15% vs 8.00 ± 1.07% vs. 26.21 ± 2.79 % vs 15.07 ± 1.40%), 16 cells (0.00 ± 0.00% vs 1.33 ± 0.38% vs. 4.85 ± 0.76% vs. 1.37 ± 0.38%), and the ability of embryos cleavage (24.59 ± 11.60% vs 41.33 ± 24.32% vs. 60.19 ± 22.45 % vs. 35.62 ± 10.83%) was significantly higher (P<0.05) in the ovaries with corpus luteum and without dominant follicles (+ CL and – FD). Conclusion, the ability of embryo division to reach 4 cells, 8 cells, and 16 cells after 48 hours culture was higher in the ovaries with corpus luteum and without dominant follicles (+ CL and – FD).

1. Introduction

Bali cattle are germplasm of Indonesia that needs to be maintained. Bali cattle have several advantages including being of good reproductive and carcass quality, resistant to tropical environmental conditions and poor feed, and having high fertility [1]. Therefore, to maintain its existence it is



necessary to apply technology, especially in vitro embryo production technology (IVEP) consisting of in vitro maturation (IVM), in vitro fertilization (IVF) and in vitro culture (IVC) [2].

The successful application of IVEP technology is influenced by several factors, one of which is the media used. The media used influences the process of oocyte nucleus maturation and for the development of the embryo to the stage of morula and blastosis. In vitro culture (IVC) is the final stage in the application of IVEP technology after IVF and IVM [3, 2]. After the in vitro fertilization process, the proportion of zygotes that can develop to the blastocyst stage during culture is only around 30-40% [4]. The quality of oocytes and culture media used after the fertilization process are the main factors that greatly influence the ability to develop to the blastocyst stage [5].

Embryogenesis is a complex process characterized by the use of maternal and transcription proteins to support the development of the embryo until the genome activation process leads to the synthesis and transcription of new proteins at the right amount and in accordance with the stages of their development [6]. Deviations during the initial embryogenesis process can lead to developmental and survival disorders of the embryo [5]. Currently, information on the application of IVEP technology and the fission ability of Bali cattle embryos after the fertilization process is still limited. Therefore, this study aims to determine the fission ability of the embryos in vitro of Bali cattle based on different ovarian reproductive status at 48 hours after the fertilization process.

2. Material and methods

2.1. Material

The Bali cattle ovaries are obtained from Tamangapa slaughterhouse, Makassar City, South Sulawesi Province. Oocytes are selected based on a homogeneous cytoplasmic state and compact cumulus cells. Freezing semen was obtained from the Livestock and Animal Health agency of South Sulawesi Province.

2.2. Methods.

2.2.1. Collection and in vitro oocytes maturation. The Bali cattle ovaries from slaughterhouse brought to the laboratory in a solution of 0.9% NaCl plus 100 IU/ml penicillin and 100 µg/ml streptomycin sulfates. Ovaries are classified according to ovarian reproductive status: ovaries without corpus luteum and dominant follicles (-CL and -FD), without corpus luteum, but with dominant follicles (-CL and + FD), with corpus luteum, but without dominant follicle (+ CL and + -FD), and with corpus luteum and dominant follicles (+ CL and + FD). Furthermore, oocytes collected in collection medium (phosphate buffered saline (PBS)) (Gibco by life technologies, USA) plus 0.2% bovine serum albumin (BSA) (Sigma-Aldrich, USA) by slicing technique. Collected oocytes are selected and only oocytes with grade A and B were used. The selected oocytes were washed three times in the maturation medium and then matured in a M199 maturation medium (Gibco by life technologies, USA) added 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 BV Boxmeer-Holland), and 50 µg/ml gentamycin (Sigma, USA). Oocytes maturation is carried out on petri dish with 35 mm Ø (Nunclon, Denmark) in drop of 100 µl for 10-15 oocytes and covered with mineral oil (Sigma-Aldrich, USA) in 5% CO₂ incubator and 38.5 °C for 24 hours [7].

2.2.2. In vitro fertilization. Freezing semen from one male is used every time in in vitro fertilization. The frozen semen is thawed using fertilization medium at 37 °C for 20 seconds then centrifuged at 700 g for 5 minutes [8]. Spermatozoa deposits are added the fertilization medium to a final concentration of spermatozoa 1.5×10^6 cells/ml. Fertilization was carried out in a drop (80 µl for 10-15 oocytes) and covered with mineral oil (Sigma-Aldrich, USA) and then incubated in 5% CO₂ at 38.5 °C for 5-6 hours [9].

2.2.3. *In vitro culture.* After 5-6 hours IVF, the oocytes were washed four times on the Charles Rosenkrans 1aa culture medium (CR1aa) then were transferred to the culture medium. The culture was carried out in a drop of 80 μ l and covered with mineral oil (Sigma-Aldrich, USA) and then placed in incubator of 5% CO₂ 38.5°C [10]. Dividing ability of embryos is evaluated after 48 hours of culture, that are divided into 2 cells, 4 cells, 8 cells, and 16 cells stages.

3. Result and discussion

The ability to divide of in vitro embryos of Bali cattle based on different ovarian reproductive status at 48 hours after the fertilization process can be seen in fig. 1, the result of this study presented in table 1.

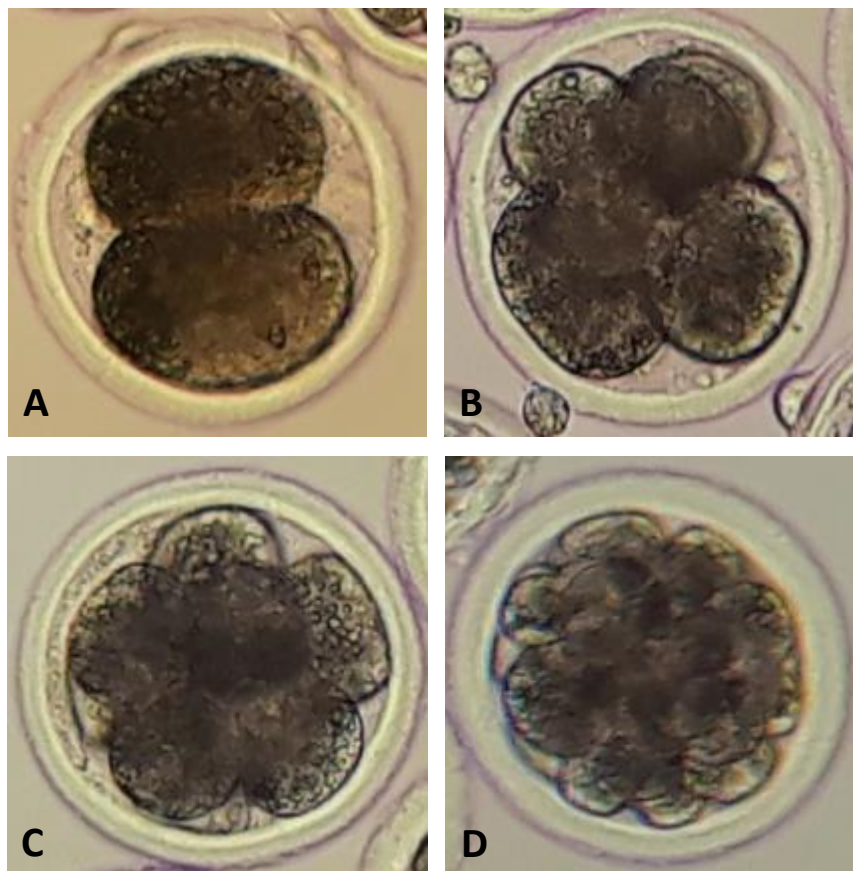


Figure 1. Cleavage ability of in vitro embryos of Bali cattle on different ovarian reproductive status at 48 hours after fertilization process. A: 2 cells, B: 4 cells, C: 8 cells, D: 16 cells (Zeiss Axio Imager A2 with a Zeiss AxioCam HRc, Germany). 10 x 20 magnification.

Table 1. Cleavage ability of in vitro embryos of Bali cattle on different ovarian reproductive status at 48 hours after fertilization process

Ovarian Reproductive Status	Number of oocytes	Cleavage ability (% mean \pm SD)			
		2 cells	4 cells	8 cells	16 cells
-CL and -FD	61	1 (1.64 \pm 0.38) ^a	7 (11.48 \pm 0.82) ^a	7 (11.48 \pm 1.15) ^a	0 (0.00 \pm 0.00) ^a
-CL and + FD	75	6 (8.00 \pm 0.90) ^a	18 (24.00 \pm 2.64) ^{ab}	6 (8.00 \pm 1.07) ^a	1 (1.33 \pm 0.38) ^a
+ CL and -FD	103	5 (4.85 \pm 0.96) ^a	25 (24.27 \pm 2.23) ^b	27 (26.21 \pm 2.79) ^b	5 (4.85 \pm 0.76) ^b
+ CL and + FD	73	6 (8.22 \pm 1.86) ^a	8 (10.96 \pm 0.69) ^a	11 (15.07 \pm 1.40) ^a	1 (1.37 \pm 0.38) ^a

Different superscripts in the same column show significant differences ($P < 0.05$). Ovaries without corpus luteum and dominant follicles (-CL and -FD), Ovaries without corpus luteum and with dominant follicles (-CL and + FD), Ovaries with corpus luteum and without dominant follicle (+ CL and -FD), Ovaries with corpus luteum and dominant follicles (+ CL and + FD).

Table 1 shows that the in vitro division ability of bovine embryos 48 hours after fertilization was significantly higher ($P < 0.05$) in ovarian + CL and -FD of reproductive status at 4, 8 and 16 cells stage, whereas in 2 cells stage the ability of division the embryo is same in all ovarian reproductive status. This shows that the presence of corpus luteum and the absence of dominant follicles influence the ability of embryo to divide up to stage 16 cells. McGee and Hsueh (2000) reported that presence of corpus luteum in the ovary which produces progesterone does not inhibit the growth of subordinate follicles but inhibits the growth of dominant follicles resulting concentration decreasing of estrogen and inhibin. Low concentrations of estrogen and inhibin will increase the secretion of follicle stimulating hormone (FSH) which causes the growth and development of subordinate follicles to produce better quality oocytes [12]. Furthermore, Boediono et al. (2006) explains that the presence of dominant follicles inhibits follicle development and affects the quality of oocytes produced.

The results of this study also showed that the ability of the bovine embryo dividing 48 hours after fertilization process was 14.29% for 2 cells stage, 43.95% for 4 cells, 37.97% for 8 cells, and 3.79% for 16 cells. This shows that 81.92% in vitro embryos of Bali cattle are able to divide up to 4-8 cells 48 hours after fertilization process. Gordon (2003) explained bovine embryos reach stage 4 cells take 2-3 days and stage 8 cells 3-5 days after estrus. Furthermore, Senger (2005) reported that bovine embryos reach stage 4 cells takes 1.5 days and 8 cells 3 days.

The ability of early embryonic development after fertilization is also greatly influenced by the follicular status which oocytes are obtained [16]. Most of the early embryos cannot reach the blastocyst stage due to developmental obstacles that are affected by maternal sygotic transition (MZT) stage which generally occur at 8 cells stage in cattle [17]. The occurrence of developmental obstacles in the embryo is closely related to the quality of oocyte cytoplasm and the ability of oocyte to activate the embryonic genome [16, 18]. Embryo is unsuccessful to transcribe its genome causes fail to develop [18]. Barnes and First (1991) reported that in early embryo development, the accumulation of protein and RNA in oocytes was important for the activation of the embryonic genome. In addition, it is necessary to activate the transcription of new embryo genomes outside the MZT to be able to develop further [17]. The activation of the embryonic genome may depend on activation or translation of several maternal transcription factors in oocytes [19]. To be able to develop normally, the embryo must begin the formation of zygote nucleus from the spermatozoa genomes through the demethylation active process of cytosine methylation which is strongly influenced by oocyte cytoplasm function [20].

4. Conclusion

The ability of embryo division to reach 4 cells, 8 cells, and 16 cells after 48 hours culture was higher in the ovaries with corpus luteum but without dominant follicles (+ CL and - FD).

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