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Preface

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PREFACE

On behalf of the committee, I would like to welcome the conference participants. The 2nd ICAST 2019 International Conference is a forum for bringing together leading experts to discuss various issues in the field of animal science and the environment. Various research topics and latest discoveries will be discussed in this forum. The main topics discussed are related to problems in the world of animal science and the environment such as: physiology and reproduction of livestock, nutrition and feed technology, waste management, environment and by-products of livestock, breeding, animal welfare, poultry production, socio-economics and agriculture extension.

At this international conference, approximately 200 papers will be presented orally and posters (including papers from keynote speakers). The paper came from several universities and research institutions within the scope of ASEAN countries and outside ASEAN. The 2nd ICAST 2019 International Conference raised the theme "Sustainable Animal Production and Welfare in The Tropical Environment". I would like to thank the keynote speakers and participants who have contributed their thoughts in advancing the world of animal science and the environment.

In particular, I would like to thank the Government of South Sulawesi Province, the Rector of Hasanuddin University, Dean of Animal Science Faculty, Hasanuddin University, the sponsors of the activities, the committee of The 2nd ICAST 2019 and all those who have supported this conference. Without your participation, this conference will not be as successful as it is today.

On behalf of the committee, we wish you all the best, the best health and prosperity. Let's enjoy the city of Makassar together for mutual success. Thank you for your attention and cooperation.

With my best wishes!.

Muhammad Irfan Said
Chair of Organizing Committee The 2nd ICAST-2019



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Conference Committee

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Strategies to increase growth early embryo stages of bovine in achieving blastocysts in vitro: A Review

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Abstract. The growth of bovine embryos in vitro begins with fertilization is the unification of male and female gametes in the fertilization media into zygote. After unification, the embryo will cleavage from two cells until they reach a blastocyst which can be transferred to the recipient. One of indicator the success of bovine embryo culture in vitro is the ability of the embryo from an early stage of development to reach blastocyst. To achieve this aim an embryo enhancement strategy is needed to reach the blastocyst stage. Various strategies that can be carried out for the improvement include the use of an appropriate maturation media, removed cumulus cells and the addition of hormones to the culture media. The use of CR1aa media in culture media can reach 27.0% of embryos that reach blastosis of total fertilized oocytes. Removal of cumulus cells after 48 hours of fertilization can increase the development of embryos reaching the blastocyst stage. In addition, by increasing the TGF β hormone 1 ng/ml + bFGF 50 ng/ml can increase the levels of 2 cells by 314 reaching the blastocyst level of 30 embryos.

1. Introduction

Bali Cattle can be developed through embryo transfer, in support of embryo transfer is needed quality cattles embryos. Cattle embryos can be produced in vivo by superovulation in the cow donor and through the use of ovaries from slaughterhouses that are produced in vitro. Production embryo in vitro can be improve potentially to the quality Bali cattle seedstock [1]. Embryo production process in vitro that is slicing ovary, oocyte collection, oocyte selection, oocyte maturation, fertilization and embryo culture. Embryo development begins with the union of oocyte and sperm cell. The result of fertilization will develop into a zygote and be cultured in a culture media. The embryo will develop to morula stage or blastocyst and can be transferred directly to the resipein parent or frozen. The stages of the development of morula, then develop into blastocys the division has produced more than 100 cells [2]. The blastocyst usually resembles a layer of ball cells surrounding a fluid-filled cavity. In the blastula, the inner cells will form a fetus or embryoblast, while the outside forms the trophoblast.

Various factors that can influence the development of a bovines embryos in vitro are modification time and hormones [3], fertilization time and incubation system [4], oocyte cumulus cells [5], and media [6]. The success of an production embryo in vitro in reaching the blastocyst stage is an indicator of success in embryo production in vitro. To achieve this success, it needed a strategy in the production of embryos in vitro so that the embryos produced can develop into quality blastocysts and



worth the transfer. Therefore this paper will discuss strategies to increase the growth early stage embryos in cattle in vitro into the blastocyst stage.

2. Discussion

Production Embryo in vitro consists of 3 stages is in vitro maturation, in vitro fertilization and culture. Oocyte maturation is a complex process that depends on the quality of the oocyte [7]. Generally oocytes that are continued to be matured are oocytes that have compact cumulus cells. Development of cattle embryos begins with the meeting of sperm cells with oocytes called zygotes. Research Zhang et al [5] cumulus is needed early in the development of a bovine embryo in vitro because it is considered helpful in the initial process of cleavage to avoid blocks in 4 to 8 cells. embryonic development in the beginning is a critical point, embryos that develop earlier have more potential to develop to the next stage [8]. The effects of cumulus cells removal and cumulus removal time on oocytes before and after fertilization are in table 1.

Table 1. Development of embryo bovine in vitro of the effects of cumulus removal and time various (Zhang et al. [5])

Cumulus cells removed	No. of Oocyte	% of oocytes developed to ^b					
		MII ^b	Fertilization	Cleaved	Morulae	BL	HBL
Before IVM	219	26	9	5	1	1	0
Before IVF	192	93	53	47	19	6	3
7 hour after IVF	177	93	71	54	27	12	6
48 hour after IVF	202	96	92	78	48	19	12

BL= Blastocyst; HBL= Hatching Blastocyst

Table 1 showed that cumulus cells are needed for the early stages of bovine embryo cleavage. Fertilization rates also show success in cleavage. After 42-44 hours of fertilization, the embryo should reach the stage of division of 4 to 8 cells with the same number of blastomers [9]. The dynamics of early cleavage have been carried out as a tool to predict the potential for embryonic development and also the quality of embryos [10–12]; fast-grow embryo appear to have the good ability and potential for reaching the stages of morula and blastocyst. The degeneration process at the beginning of embryonic development may be caused by a dramatic change in embryo protein synthesis, an internal signal that turns off maternal control in the process of cleavage and control of embryo growth, so that the embryo can not develop or experience death [13]. Combination intracytoplasmic sperm injection (ICSI) and activation with strontium 20 mM reach the level of development 2-4 cells (50.5%), 8-16 cells (43.73%), and Blastocyst (15.63%) [14].

The developments of an early embryo in vitro can influence the embryo to reach blastocyst. In vitro, the development of embryos carried out 2 cells reaching the blastocyst is needed by the outside environment to support its development. The success rate of the development of embryos produced using a culture media is variously, especially embryos obtained through in vitro fertilization or from one cell. The number and survival of the resulting embryo is still low can be caused by one of them are a suboptimum culture condition [15]. The use of different culture media can be seen in table 2.

Table 2. Comparison of 4 different media for embryonic development of in vitro fertilized bovine oocytes (Lee et.al [6])

Media	No. of oocytes	cleavage (1 day)	8 cells (3 day)	Morulae (6 day)	Blastocysts (8 day)
TLP	184	127 (69.0)	73 (39.7)	57 (31.0)	32 (17.4)
TCM-199	189	141(74.6)	95 (50.3)	87 (46.0)	44 (23.3)
CR1aa	200	154 (77.0)	102 (51.0)	82 (41.0)	54 (27.0)
CR1BSA	165	112 (67.9)	81 (49.1)	39 (23.6)	21 (12.7)

Based on Table 2 that the use of CR1aa media in culture media has the potential to increase the stage of embryonic cell cleavage reaching the blastocyst stage. The results of other studies [6] showed that the rate of development of bovine embryo with addition of α -aminatin in culture media 1 and 10 ng increases the division of stage 2 cells and embryos in which reach the blastocyst stage compared to no control reaches blastocysts.

Components needed for early stage embryonic development such as protein and growth factors. If there is a failure of the transcription process, the cleavage of the embryo will stop and a blockage will occur [15]. The rate of embryonic development in vitro can be increased by the addition of growth hormones to the medium. Like research Pranatasari et.al [16] conducting research with the addition of the gonadotropin hormone in vitro maturation media of Bligon goat 25 IU/ml can be increase the development of stage 4 cell embryos by 16.67%.

Research by Ruan et al [17] that with the addition of 0.1 μ g/ml of the follicle stimulating hormone (FSH) in buffalo oocyte maturation media from 103 fermented oocytes reaches a cleavage rate of 67% and reaches 34% of the blastocyst stage. Another hormone used to increase cell cleavage is insulin. Addition of insulin 10 ng to the culture media increases cell division but has no effect on the amount reached blastocyst [18].

Insulin growth factor (IGF-1) can increase the survival of bovine embryos [19] and functions as an anti apoptotic when maturing bovine oocytes and embryo development during culture [20, 21]. IGF-1 can also protect cattle embryos during preimplantation against heat stress [22]. The addition of epidermal growth factor (EGF) influences the amount of blastocyst produced, but may not be an anti apoptotic factor [20]. Stem cell factor (SCF) can increase embryos to blastosis and [23] and improve the quality of embryos cultured by adding 500 ng/mL Fas-L [24]. Supplementation on TCM-199 culture media with 10 IU PMSG hormone can increase the number of cell cleavage in goats in vitro by 41% and embryo reach the blastocyst stage by 14% from amount of fertilized oocyte [25]. The rate of development of a bovine embryos with the addition of growth hormone are seen in table 3.

Table 3. Embryonic development reaches blastocysts with different hormones and combinations (Larson et.al [26])

Treatment	2 Cell	8 to 16- Cell	Beyond 8- to 16- Cell	Blastocysts
Kontrol	154	84	0	0
TGF β	89	36	2	0
bFGF	72	39	11	4
TGF β + bFGF	314	160	122	30

The rate of development of a cattle embryo with the addition of growth hormone is seen in table 3. TGF- β can be inhibit leukocyte and epithelial proliferation, but can be stimulation the proliferation of skin fibroblasts, stroma and smooth muscle cells. Biologically, TGF is secreted in a latent form and in vivo activated by induction with a complex process that is proteolytic activation dissociated latency protein subunit [27]. Research Kuhn et. al [28] that bFGF-2 (basic Fibroblast growth factor) is a powerful mitogen for cells that can be derived from bone in humans and mice that are produced in vitro FGF can accelerate the renovation of bone fractures and increase bone formation. To produce optimum blastocyst formation, it is necessary to activate FGF receptors and by adding FGF2 with a concentration of 500 ng/ml can increase the formation of blastocysts in cattle [29].

3. Conclusion

Based on the discussion, it can be concluded that the strategies that can be used to increase the early embryo cleavage of cattle to reach the blastocyst stage are the use of medium and removal of cumulus cells at the right time and addition of growth hormone. The use of CR1aa media in culture media can reach 27.0% of oocytes which reach blastocyst of total fertilized oocytes. Cumulus denudation after 48 hours of fertilization can support the enhancement of the blastocyst oocyte stage. The addition of the

hormone TGF β 1 ng/ml plus bFGF 50 ng/ml can increase embryo stage 2 cells reaching the blastocysts stage. The level of early development of cattle can be a reference for further development is achieving blastocyst.

References

- [1] Sonjaya H and Hasbi H 2019 Potential of embryo production techniques in vitro for improving bali cattle seedstock *IOP Conf. Ser. Earth Environ. Sci.* **247** 12001
- [2] Afriani T, Hellyward J, Purwanti E, Jaswandi, Lyzmanto F and Mundana M 2018 *Manipulasi Embrio pada Sapi* (Padang: Andalas University Press)
- [3] Triwulaningsih E, Toelihere M R, Rutledge J J, Yusuf T L, Purwantara B and Diwyanto K 2001 In vitro embryo production through modification of time and gonadotropin hormone during oocytes maturation *J. Ilmu Ternak dan Vet.* **6** 179–88
- [4] Syaiful F L, Saladin R, Jaswandi J and Udin Z 2011 Pengaruh waktu fertilisasi dan sistem inkubasi yang berbeda terhadap tingkat fertilisasi sapi lokal secara in vitro *J. Peternak. Indones.* **13** 27–35
- [5] Zhang L I, Jiang S, Wozniak P J, Yang X and Godke R A 1995 Cumulus cell function during bovine oocyte maturation, fertilization, and embryo development in vitro *Mol. Reprod. Dev.* **40** 338–44
- [6] Lee J H, Park J H, Choi K M, Im K S and Jin D I 2001 Improvement of in vitro development of bovine embryos in medium containing selenium. *Asian-Aust. J. Anim. Sci.* **14** (2):170-73
- [7] Diógenes M N, Guimarães A L S, Leme L O and Dode M A N 2017 Bovine in vitro embryo production: the effects of fibroblast growth factor 10 (FGF10) *J. Assist. Reprod. Genet.* **34** 383–90
- [8] Nedambale T L, Du F, Yang X and Tian X C 2006 Higher survival rate of vitrified and thawed in vitro produced bovine blastocysts following culture in defined medium supplemented with β -mercaptoethanol *Anim. Reprod. Sci.* **93** 61–75
- [9] Betteridge K J and Fléchon J-E 1988 The anatomy and physiology of pre-attachment bovine embryos *Theriogenology* **29** 155–87
- [10] Van Soom A, Van Vlaenderen I, Mahmoudzadeh A R, Deluyker H and de Kruif A 1992 Compaction rate of in vitro fertilized bovine embryos related to the interval from insemination to first cleavage *Theriogenology* **38** 905–19
- [11] Kubisch H M, Larson M A and Roberts R M 1998 Relationship between age of blastocyst formation and interferon-tau secretion by in vitro-derived bovine embryos *Mol. Reprod. Dev.* **49** 254–60
- [12] Lonergan P, Khatir H, Piumi F, Rieger D, Humblot P and Boland M P 1999 Effect of time interval from insemination to first cleavage on the developmental characteristics, sex ratio and pregnancy rate after transfer of bovine embryos *Reproduction* **117** 159–67
- [13] Bagis H, Keskinetepe L, Mercan H O and Sagirkaya H 2001 Effect of CZB medium on the two cell block of preimplantation mouse embryos *Turkish J. Vet. Anim. Sci.* **25** 725–9
- [14] Gunawan M, Fahrudin M and Boediono A 2014 Perkembangan embrio sapi setelah fertilisasi menggunakan metode Intracytoplasmic Sperm Injection (ICSI) dan aktivasi dengan strontium *J. Kedokt. Hewan* **8** 154–7
- [15] Gordon I R 1994 *Laboratory Production of Cattle Embryos: Wallingford Oxon, UK CAB Int.*
- [16] Pranatasari D, Kustono K and Widayati D T Suplementasi Hormon Gonadotropin Pada Medium Maturasi In Vitro Untuk Meningkatkan Perkembangan Embrio Stadium 4 Sel Kambing Bligon *Bul. Peternak.* **40** 83–91
- [17] Ruan Z, Zhao X, Li Z, Qin X, Shao Q, Ruan Q, Deng Y, Jiang J, Huang B and Lu F 2019 Effect of sex differences in donor foetal fibroblast on the early development and DNA methylation status of buffalo (*Bubalus bubalis*) nuclear transfer embryos *Reprod. Domest. Anim.* **54** 11–22
- [18] Laskowski D 2017 *Insulin and the early bovine embryo* (Uppsala, Swedish: Swedish

- University of Agricultural Sciences)
- [19] Block J, Wrenzycki C, Niemann H, Herrmann D and Hansen P J 2008 Effects of insulin-like growth factor-1 on cellular and molecular characteristics of bovine blastocysts produced in vitro *Mol. Reprod. Dev.* **75**(5) 895–903
 - [20] Sirisathien S and Brackett B G 2003 TUNEL analyses of bovine blastocysts after culture with EGF and IGF-I *Mol. Reprod. Dev.* **65**(1) 51–6
 - [21] Wasielak M and Bogacki M 2007 Apoptosis inhibition by insulin-like growth factor (IGF)-I during in vitro maturation of bovine oocytes *J. Reprod. Dev.* **53** 419–26
 - [22] Jousan F D and Hansen P J 2007 Insulin-like growth factor-I promotes resistance of bovine preimplantation embryos to heat shock through actions independent of its anti-apoptotic actions requiring PI3K signaling *Mol. Reprod. Dev.* **74**(2) 189–96
 - [23] Taniguchi F, Harada T, Nara M, Deura I, Mitsunari M and Terakawa N 2004 Coculture with a human granulosa cell line enhanced the development of murine preimplantation embryos via SCF/c-kit system *J. Assist. Reprod. Genet.* **21** 223–8
 - [24] Głabowski W 2005 The protective effect of stem cell factor (SCF) on in vitro development of preimplantation mouse embryos *Ann. Acad. Med. Stetin.* **51** 83–93
 - [25] Restiadi T I 2001 Pengaruh Pregnant Mare Serum Gonadotropin (PMSG) pada Maturasi dan fertilisasi in vitro Oosit Kambing Lokal *Media Kedokt. Hewan* **16** 25–30
 - [26] Larson R C, Ignatz G G and Currie W B 1992 Transforming growth factor β and basic fibroblast growth factor synergistically promote early bovine embryo development during the fourth cell cycle *Mol. Reprod. Dev.* **33**(4) 432–5
 - [27] Song Q H, Klepeis V E, Nugent M A and Trinkaus-Randall V 2002 TGF- β 1 regulates TGF- β 1 and FGF-2 mRNA expression during fibroblast wound healing *Mol. Pathol.* **55** 164–76
 - [28] Kuhn L T, Ou G, Charles L, Hurley M M, Rodner C M and Gronowicz G 2013 Fibroblast growth factor-2 and bone morphogenetic protein-2 have a synergistic stimulatory effect on bone formation in cell cultures from elderly mouse and human bone *J. Gerontol A Biol. Sci. Med. Sci.* **68** 1170–80
 - [29] Fields S D, Hansen P J and Ealy A D 2011 Fibroblast growth factor requirements for in vitro development of bovine embryos *Theriogenology* **75** 1466–75