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

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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 TGFβ 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

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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] culumus 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= Blatocyst; 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 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 µg/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 l [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
TGFβ	89	36	2	0
bFGF	72	39	11	4
TGFβ+ 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 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 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 TGFβ 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.

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