The effect of feeding serum on bovine blastocyst development

E.Triwulanningsih[1], M.R.Toelihere[2], J.J.Rutledge[3], T.L.Yusuf[2], B.Purwantara[2], K.Diwyanto[1]

[1]Research Institute for Animal Production, PO BOX 221, Bogor Indonesia
[2]Department of Clinic, Reproduction, and Pathology, Faculty of Veterinary Medicine, Bogor Agricultural University, Darmaga, Bogor Indonesia
[3]University of Wisconsin, USA

The main objective of this experiment is to obtain information on proper technique for in *vitro* production of Holstein x Bali cattle hybrid (F_1) embryos using USA Holstein oocytes collected from the slaughterhouse and fertilized with spermatozoa of Bali or *Bos sondaicus* bulls. Holstein x Brahman cattle hybrid embryos using spermatozoa of USA Brahman bulls and purebred Holstein embryo production. All viable embryos were frozen and brought to Indonesia and some of them had been transferred to FH recipients in Indonesia.

These embryos may be used for improving the genetic value of the Indonesian dairy cattle. There is transportation constraint in importing oocytes from USA. It takes more than 24 hours to bring it to Indonesia. In fact, maturation of oocytes and readiness to be fertilized normally requires only 24 hours in 5% CO_2 incubator at 38.5°C. Therefore, this research is needed to study the effect of gonadotropin hormone treatmens and time for oocytes maturation and readiness to be fertilized at a period more than 24 hours. If this problem could be solved then the importation of oocytes would be cheaper and easier than importation of live animals or embryos.

Ovaries were collected from the slaughterhouse in Wisconsin, USA. The oocytes were matured in TCM-199 medium enriched with FSH 10 µl/ml, oestradiol 17 β 1µl/ml and 10 % FCS for 20 hours in CO_2 incubator at 39 °C and RH 90%. The oocytes were fertilized *in vitro* with motile sperm selected by 90% and 45% of Percoll gradient. The sperm pellet was obtained after seven minutes of the first centrifugation. The sperm pellet was washed with 1 ml TL Hepes and were centrifugatated again for two minutes, the supernatant was discarded and spermatozoa in the pellet were directly counted using haemacytometer. The semen with 1 to 2.5 x 10^6 sperm concentration was used for fertilization of matured oocytes. Sperm and oocytes were incubated in fertilization medium (TALP) for 20 hours. All zygotes were cultured in CR1aa medium and fed on day 4, 5 and 6 (as treatment A, B and C, respectively) with 5 µl serum /50 µl culture up to blastocyst. Counting of morula, blastocyst, expanded blastocyst, degenerated embryo and cleavage followed the method of Wosik and Stubbings (1994).

Results of this experiments showed that the percentages of cleavage, blastocysts and unfertilized ova were not different among treatments ($p>0.05$), but morula, degenerated embryos and expanded blastocysts were significantly different among treatment A vs C ($p<0.01$) but A vs B and C vs B were not different ($p>0.05$) (Table 1 and Figure 1). Based on results of this study, it is concluded that the best method for IVP *(in vitro* production) of embryos is feeding serum on day 6 (early blastocyst).

Some of the embryos have been transferred to FH recipients in Indonesia. Twenty two cows were used as recipients, after estrous synchronization using CIDR (Controlled Internal Drug Release) implanted for 12 days, but only seven cows which had good quality of corpora lutea were transplanted with the IVF embryos (three embryos per recipient) and resulted in pregnancy of three recipients (pregnancy rate 42.86 %). Unfortunately two of them aborted and only one (female) was born alive with 45 kg birth weight (hybrid FH x Brahman). In the second trial, 32 cows were prepared as recipients after synchronization with CIDR implanted for 12 days, but only nine cows had good quality of corpora lutea. Three recipients were transplanted with IVF embryos and two of them became pregnant (pregnancy rate 66.7%) but one of them was aborted at seven months of pregnancy and one (male purebred FH) was born alive. Six other recipients

were transplanted with the in *vivo* embryos(two embryos per cow) and three of them became pregnant (pregnancy rate 50%). Three days after they were born. however. the calves died due to mastitis problem of the dams.

Table 1. The effect of feeding serum on *in vitro* embryo development

Feeding serum Treatment	Development of oocytes (%)				Undeveloped oocytes (%)	
	Cleavage	Morula	Blastocyst	Expanded blastocyst	Unfertilized ova	Degenerated embryo
Day4 (n=524)	445 (85 3)[a]	319 (62 5)[b]	235 (47.8)[a]	64 (13.2)[b]	79 (14 8)[a]	126 (22 8)[a]
Day-5 (n=472)	434 (91.8)[a]	329 (70 0)[ab]	252 (53.8)[a]	91 (19 8)[ab]	38 (8 2)[a]	105 (21.8)[ab]
Day-6 (n= 494)	447 (90 9)[a]	363 (74 9)[a]	273 (57 5)[a]	121 (27 3)[a]	47 (9 0)[a]	84 (16 0)[b]

Note Values within rows with different supercripts differ (p<0 01)

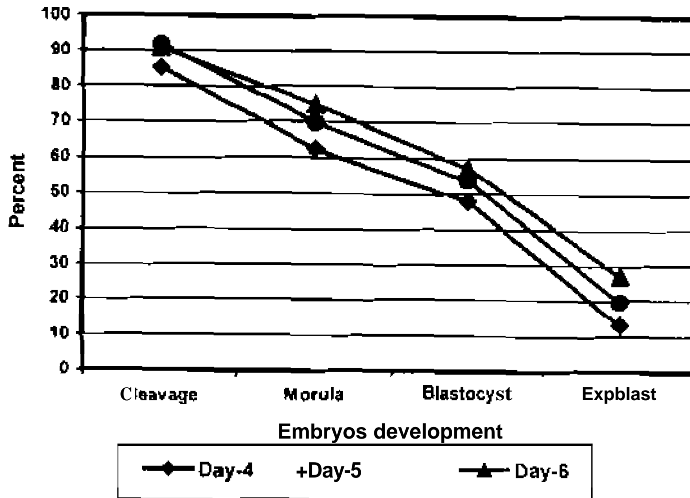

Figure 1. The effect of feeding serum on *in vitro* embryos development

It is generally concluded that USA originated oocytes could be preserved, matured and fertilized *in vitro* after a period of more than 14 hours up to 36 hours with or without hormone supplement in of its medium. Embryos produced by this process could reach the blastocyst and expanded blastocyst stages and will be transferred to recipients in Indonesia.

It would be appropriate to recommend that further cooperarcd action research and implementation in this reproduction biotechnology (IVF) should be conducted using genetically superior oocytes of Holstein cows from USA and fertilized with spermatozoa from developed countries in the effort to increase production and improve the dairy industry in Indonesia.

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Alternative method for gonad maturation of mu (*Scylla olivaceous*) broodstock

Y. Fujaya, D. D. Trijuno, J. Tresnaty, Irmawati, Nadiar

Department of Fisheries, Faculty of Marine and Fisheries Science, Hasanuddi
Kampus UNHAS Tamalanrea Makassar Indonesia

Gonad maturation of mud crab can be stimulated through hormonal and en
tion (Lockwood 1967). Recently, eyestalk ablation as hormonal manipulati
hatcheries. Through this method, X-organ, the organ that produces Gona
(GIH) which is located in the eyestalk is removed. GIH has a role in inhi
ment. According to Adiyodi and Adiyodi (1970), when GIH content in th
Gonad Stimulating Hormone (GSH) will be sinthesized by central nervous
gonad maturation occurs. Unfortunately, in the eyestalk also contains si
duces various hormones that important in metabolism. Sinus gland also re
perglycemic Hormone (CHH), Molt Inhibiting Hormone (MIH), and Pig
mone (PEH). Consequently, when eyestalk is removed, not only GIH is v
various hormones includes other reproductive hormones. CHH also has
mobilization from extra-ovarian such as hepatopancreas, besides its hyperg
(Rao 1990). Such was the case, Huberman (2000) has reported a negative
lation in *Penaeus monodon*. Unilateral eyestalk ablation in the prawn resu
duce of the total important biochemical substances in the circulation. This
ber of nauplii per spawning and further effect survival rate of post larvae.
tion have an effect on abnormal vitellogenesis as a result of lower amount
Carcinus maenas.

Based on that phenomenon, searching for alternative methods in stimulatin
urgently needed. Fujaya (2004) has used thorax ganglion extract (TGE
crabs like *Neoepisesarma lafondi*, *Varuna litterata*, and *Metopograpsus la*
velopment stimulant in the *in vitro* research scale. The result indicated t
plementation of TGE has bigger diameter than ovarium without TGE. Thi
tent in the thorax ganglion of the crab. GSH, a peptide secreted from the b
glia, while not yet sequenced, has been reported in several studies as stim
in vitro (Eastman-Reks and Fingerman 1984; Sarojini *et al* 1995; Fingerma
This year, *In vivo* assay for gonad maturation by TGE injection has been c
assays were carried out during April 2004 to July 2005. TGE was prep
(2004). All of the female crabs used was immature. Crabs were injecte
pereiopod, by means of a 1-mL syringe provided with a 27-gauge needle.
was always 100 µL with different concentration of TGE (0.25, 0.50, 0.75,
mg body weight of crab). Each crab was injected once, twice, and three
Before being injected, crabs were feed ad libitum on trash fish or non-ec
the assay, the animals were kept in to bamboo cage that replacement in *ta*
matic index (GSI) was calculated as fresh weight of ovary/whole crab x 10
Result study showed, administering TGE yielded significant results, wi
Three times of injection were effectively increase the GSI. The GSI was
with the concentration of TGE, but more of 0.75 µL TGE per mg body w