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Usage of Rabbit uterine as natural incubator of Goat embryonic growth process originated from slaughter house

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Abstract

Embryo transfer is a new method that developed in Indonesia and has been applied to cows in west java, central java, and east java. The principle of embryo transfer is to transfer the embryos produced by superior female animals (donors) to the local animal as recipient. Embryo transfer techniques can not be separated from super ovulation measures to increase the number of ovum in a single period of estrus in the donors genital on in-vitro fertilization.

The aim of this research to find out how far the level of embryo development as the results of in vivo flushing after transferred to the female rabbit genital tract.

The hypothesis proposed of this research is the occurrence of embryo growth of *kacang*goat in rabbit genital tract at various cell level.

The research used a complete randomized design that use 20 adult female rabbits divided into 4 treatment groups consisting of : Group I was a control group consisting of 5 rabbits and treated with 2 cells embryo transfer of *kacang*goat. Group II was a treatment group consisting of 5 rabbit and treated with 4 cells embryo transfer of *kacang*goat. Group III was a treatment group consisting of 5 rabbit and treated with 8 cells embryo transfer of *kacang*goat. Group IV was a treatment group consisting of 5 rabbit and treated with 16 cells embryo transfer of *kacang*goat. The embryo transfer development result from cell level were tabulated, then to know the difference ANOVA test was performed and followed by BNT test.

The obtained result showed that average percentage of 2 cells embryo development of *kacang*beans are 9.80 ± 2.244, 4 cells embryo are : 12,46 ± 1,414, 8 cells embryo are : 23,49 ± 4,394 and 16 cells embryo are : 51,01 ± 2,124. After the statistical test was done the obtained result are : there is a significant difference ($P>0.05$) between 2 cells and 4 cells embryo group with 8 cells and 16 cells embryo groups, as well as 8 cells embryo group with 16 cells embryo group, meanwhile there were no significant difference between 2 cells embryo group with 4 cells embryo group ($P<0.05$).

Keywords: *Embryo Transfer, Rabbit Uterine, Embryonic Growth, Goat Embryo*

1. Introduction

Livestock development is aimed to increase farmers' income, encouraging food diversification and improving the quality of people's nutrition, as well as developing livestock and meat exports. To date, the efforts made to meet these needs largely revolve around increasing quantitative animal production.The challenges of livestock problems must be faced; efforts must be made to increase the production of livestock and meat.

In addition, goats have a small role in contributing to the provision of meat in Indonesia other than beef cattle. It is because goats include animals that have the ability to conceive and give birth to second or more children (Ludgate, 1989). Generally in Indonesia, the livestock's mainly give birth to one

calf per pregnancy. Therefore it can be understood if the population increase is very slow per year (Hardijanto, 1990). The slow increase of goat population is due to the low level of reproduction efficiency (Hardjopranjoto, 1987) and also because the processing done by traditional farmers, among others, has not applied knowledge and advanced technology.

One effort to improve the reproductive capacity of goats, among others, can be done through embryo transfer techniques, which will be very useful especially in efforts to increase the population and efficiency of livestock reproduction in order to meet the needs of food in the form of meat, milk and other goat products.

Embryo transfer is a new way developed in Indonesia and has been applied to cattle in West Java, Central Java, and East Java. Transfer embryos are principally the removal of embryos produced by superior female (donor) animals in local animal recipients. The embryo transfer technique cannot be separated from the superovulation action to increase the number of eggs in an estrus cycle of the donor's genital in vivo fertilization.

One of the factors that influence the success of the embryo transfer technique is the embryo's age and the level of embryo development that will greatly affect the quality of the embryo itself. Trouson (1978) reported that embryonic age had an effect on embryo survival at the time of preservation and re-dilution. Early morula (early morula) has a lower life force than late morula (late morula) and blastocyst.

Hafez (1987) embryonic cell development is highly variable and time-dependent from the onset of fertilization. If determined the peak time (embryo recovery) on goats on the fourth day means the development of embryonic cells can be shaped early morula. Compact morula only made up of eight cells. This uncertainty depends on the fertility of spermatozoa and ovum cells that meet in the fallopian tube or the chance of sperm to penetrate the mature egg.

However, any attempt to make use of embryos from slaughterhouses is common, but often the embryo is of a very young developmental type, so it is very unusual for transfers on the parent recipient to admit that young embryos have a poor survival when transferring to the recipient. Therefore, the researcher is able to utilize*kacang*goat embryo to be transplant into the rabbit's uterine tube as a temporary place (Natural Incubator) in order to get a chance to grow into a more mature level, making it possible to live in the uterus of the *kacang*goat.

Prior research by the Fukushima NLBS team (1987) has used a rabbit's uterus as a temporary storage space for young embryos for 4-5 days. The study of several possible rates of development of *kacang* goat embryo in vivo on the fourth day until the sixth day after insemination is intended to be the initial action of transfer embryo implementation skills that can be performed on the *kacang*goat. However, by knowing the right time about the level of post-insemination embryo development means it will make it easier to peak of the embryo on certain days according to the number of cells needed. So that known level of embryo development, will be determined how many cells that develop in embryo or the same level (stage of embryo) is feasible to transfer. This will make it easier for us to embryo the transfer process. Even this effort can be commercial in nature we can determine the level of development required, with later thinking for storage (embryo banks) in the form of frozen embryo.

2. Materials and Methods

This study were used some materials, including 20 adult female rabbits Uterus / female genital goat limbs of the corpus luteum in ovaries, TCM – 199 (Tissue Culture Medium -199), physiologic NaCl, sterilized aquades, alcohol 70 %, cat gut, penicilin – streptomicin, sulfanilamide, sterilized cotton, tissue paper, bandage, microscope dissection, a set of surgical instrument: scissor , scaple, tweezers, intragastric tube, sewing needle, disposable sterilized petri dish 36 mm and glass petri dish 3 cm, disposable syringe, various glassware and eye drops of various sizes, water bath, and operating table.

In this study, there were two method in research procedure. First method was goat embryo flushing. Flushing is intended to obtain goat embryo by inserting physiological fluid into the tube uterine junction through syringe as much as 5 – 10 cc then the result is accommodated in petri dish, and separated embryo from the tissue around by using NaCl. The second method was for the embryo transfer. Before embryo transfer from goat to rabbits was done, synchronization of rabbit estrous cycle should be done first, using PGF2α hormone, and repeated 10 days later after first injection, and then the surgery was done for about 2-3cm in the middle of the abdomen, and found the cornua using spay hook or hand and then the embryo was inserted into the cornua. The surgery incition were sutured and antibiotic (penicilin and streptomicin) were given on the surgery site. On the third days after Embryo transfer inside the cornua, the second surgery on the rabbit were done. Embryo was observed using dissecting microscope to examine the embryonic growth. The fluid from flushing result were identified using dissecting microscope to differentiate cells level. Grouping of each according

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cell level (2, 4, 8 and 16 cell) by way of taking the embryo through a small pipette.

The research procedure for this study was used a complete randomized design, 20 adult female rabbits divided into 4 treatment group that consists of :

Group I : Control group consists of 5 rabbits and treated with 2 cells embryo transfer of *kacang*goat.

Group II: Treatment group consists of 5 rabbits and treated with 4 cells embryo transfer of *kacang*goat.

Group III : Treatment group consists of 5 rabbits and treated with 8 cells embryo transfer of *kacang*goat.

Group IV : Treatment group consists of 5 rabbits and treated with 16 cells embryo transfer of *kacang*goat.

The observed variables were the percentage of embryonic development of goats within the rabbits uterus at the rate of cell development.

Result data from each treatment group were tabulated, and anova test was performed (Sarmanu 1989) and continued using BNT.

3. Results and Discussion

After transfer embryo goats in each group of rabbits using embryos 2, 4, 8 and 16 cells, then performed surgery on day 3, then the results can be seen at Table 1.

The mean percentage of development in 2 cell embryos was: 9.80 ± 2.244, the 4 cell embryo was 12.46 ± 1.414, the 8 cell embryo was 23.49 ± 4.394 and the embryo 16 cells was 51.01 ± 2.124. The highest percentage was obtained in the development of 16 cell embryo that is 54,16%, while the lowest number in 2 cell embryo development is 6.60%. after embryo transfer, then the embryo of 2 cells will develop into 4 cells up to 16 cells. Likewise the 4 cell embryo will develop into 8 to 16 cells, while for embryos of 8 and 16 cells will develop highest until morula.

From the statistical test using Anova One Way (One Way Anova) and followed by BNT test results showed that there was a significant difference ($p < 0.05$) between embryonic groups 2 and 4 cells with 8 and 16 cells, and between groups of 8 cells with 16 cell (look at table 2), whereas between group 2 cells with 4 cells there was no significant difference ($p > 0,05$).

From these results it appears that the largest percentage is in the embryo group of 16 cells (15.01%), while the smallest in group 2 cells (9.96%). The decrease in the percentage of cleavage in the embryo is due to the presence of obstacles primarily by glucose there is a rabbit uterine fluid. According to Seshogiri and Bavister (1989) states that at the time of early development embryo does not require glucose-containing media for its development

Table 1: Percentage of Goat Embryo Development 3 Days After Transferred On Rabbit Uterus

Rabbit sample Number	Number of embryo cells in transfer			
	2	4	8	16
1.	10,71	14,29	25,20	51,71
2.	12,50	12,50	20,83	54,16
3.	8,67	13,33	30,10	50,60
4.	6,60	10,77	22,60	48,40
5.	10,52	11,44	18,72	50,20
€ X	49,00	62,33	117,45	255,07
X	9,80	12,46	23,49	51,01
SD	2.244	1,414	4,394	2,124
N	5	5	5	5
€ X^2	500.35	785,009	2836,13	13030,19

The presence of glucose in the media at the beginning of development. The presence of glucose in the media at the beginning of the embryo development will spur the process of glycolysis in the cell, thereby resulting in the release of mitochondrial respiration in the embryo and subsequent rerpon to split will be disturbed (obstructed), but if given on the development of 16 cells then glucose will spur the development of the embryo towards the compact morula and blastosis. This is in accordance with what is reported by Sukra et al. (1992) that the embryos of cattle and goats of the 2 -4 cell stage are difficult to split if transferred to other animals, Parrish and First (1993) further suggest that embryo transfer will experience developmental resistance in stage 4 cells (goats) and 8 cell (cow).

The occurrence of early embryonic developmental inhibition in this study is another possibility caused by the media used (TCM -199) which requires the addition of animal serum estrus so as to achieve a perfect maturity level. Rao and Hart (1988) suggest that the addition of serum in culture has a biphasic effect, meaning that the addition of serum at the

beginning of the cleavage will suppress the cell division, but the addition of serum 48-72 hours after embryonic trasnfer or stage 4-8 cells will stimulate progression to the stage of morula and blastocyst. As it is known that the 8 cell embryo for its development requires an environment in which it contains a source of energy, protein and other factors essential to the further development of the embryo. It appears that the addition of estrus goat serum on TCM-199 media provides a positive response to the development of stage cell embryos (Hafez 1993). Estrus goat serum in addition to many estrogen and gonadotropin (FSH and FSH) hormones also contain amino acids, glucose, minerals, fatty acids and growth factors required by embryos for future embryonic development. This is consistent with that reported by Yang et al (1990). That the addition of serum in TCM-199 media can increase the development of embryos from 4 to 8 cells to the compact stage of morula and blastocyst.

Table 2: Average percentage of Goat Embryo Development 3 Days After Embryo Transfer On Rabbit Uterus

	2	4	8	16
Average	9,80 [a]	12,46 [a]	23,49 [b]	51,01 [c]
Standard deviation	2,244	1,414	4,394	2,124
Repeat	5	5	5	5

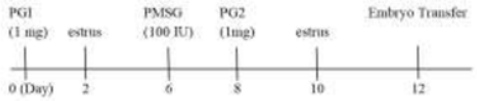

Fig. 1 Work Program Scheme.

6. Conclusions

The percentage of goat embryo development after transfer on the largest rabbit's uterus was found at the embryo level of 16 cells, while the smallest was found at the embryo level of 2 cells.

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