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EMBRYO TRANSFER TECHNIQUE FOR IMPROVEMENT OF CATTLE PRODUCTIVITY

การเพิ่มจำนวนและคุณภาพโคจากการถ่ายฝากตัวอ่อนที่ผลิตจากหลอดทดลอง

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ABSTRACT

The pregnancy rates and calve offspring in an embryo transfer program of bovine embryos which were produced in vitro *were investigated. The bovine embryos were produced by tissue culture method to mature oocytes (in TCM-199 medium) and fertilize* in vitro *with heparin-treated sperm (in TALP-glucose free medium). The oviductal epithelial cells were co-cultured* in vitro *(in TCM-199+10% HTFCS) and were capable of supporting normal growth*

of embryos to the stages at which non-surgical embryo transfer could be performed (compact morula, early blastocyst and blastocyst). It was found that the pregnancy rate following non-surgical of the same stage of two excellent embryos to the native recipient cows was higher in early blastocyst (75%) than compact morula (55%) and blastocyst (30%) stages and the total rate of pregnancy was 55% while the twin pregnancy rate was 28%. A total of 21 healthy calves were born from 17 recipients and mean birth weight was 21.2 kg. The result of the present study indicates that satisfactory pregnancy could be obtained when excellent embryos produced in vitro *were transferred to-1 day a synchronous native recipient cows and the early blastocysts were the best for transfer.*

บทคัดย่อ

ศึกษาอัตราการฝากติดและการตกลูกของโคโดยวิธีการถ่ายฝากตัวอ่อนที่ผลิตได้จากหลอดทดลอง ตัวอ่อนได้มาจากการนำเซลล์ไข่ของโคไปเพาะเลี้ยงให้เจริญจนเป็นไข่สุกในน้ำยาเพาะเลี้ยงตัวอ่อนสูตรTCM-199 แล้วจึงทำการปฏิสนธิกับเซลล์อสุจิซึ่งผ่านกระบวนการที่พร้อมสำหรับผสมกับเซลล์ไข่โดยใช้สารเฮฟปาริน (heparin) ในน้ำยาเพาะเลี้ยงสูตร TALP-Glucose free ตัวอ่อนที่ได้จะพัฒนาและแบ่งเซลล์หลังจากการปฏิสนธิ เมื่อเพาะเลี้ยงร่วมกับเซลล์บุท่อนำไข่ของโคในหลอดทดลองในน้ำยาเพาะเลี้ยงสูตร TCM-199+10%HTFCS จนกระทั่งได้ตัวอ่อนที่แบ่งตัวถึงระยะมอรูลาหนาแน่น (compact morula) ระยะบลาสโตซีสเริ่มต้น (early blastocyst) และระยะบลาสโตซีส (blastocyst) จึงสามารถที่จะถ่ายฝากให้กับแม่โคตัวรับได้ อัตราการฝากติดของการถ่ายฝากตัวอ่อนคุณภาพดีเยี่ยมในระยะเดียวกัน 2 ตัว โดยวิธีไม่ผ่าตัดให้กับแม่โคตัวรับพันธุ์พื้นเมือง พบว่า อัตราการฝากติดสูงสุดคือตัวอ่อนในระยะบลาสโตซีสเริ่มต้น สามารถฝากติด 75% มากกว่าตัวอ่อนระยะมอรูลาซึ่งมีอัตราฝากติดเพียง 55% เท่านั้น สำหรับตัวอ่อนระยะบลาสโตซีส จะมีอัตราการฝากติดต่ำสุด คือ 30% และผลรวมอัตราการฝากติดของตัวอ่อนทุกระยะคือ 55% โดยมีอัตราการฝากติดแฝด 28% และอัตราการแท้งลูก 5.5% การศึกษาครั้งนี้ได้ลูกโค 21 ตัว จากแม่โคตัวรับ 17 ตัว ลูกโคทุกตัวสมบูรณ์ดี มีน้ำหนักเฉลี่ยแรกเกิด 21.2 กก. ผลการทดลองแสดงให้เห็นว่าการถ่ายฝากตัวอ่อนคุณภาพดีเยี่ยมอายุ 7 วันที่ผลิตจากหลอดทดลองให้กับแม่โคพันธุ์พื้นเมืองตัวรับหลังการเป็นสัดของแม่โคตัวรับ 8 วันในระยะบลาสโตซีสเริ่มต้นของตัวอ่อนเป็นระยะที่เหมาะสมที่สุดในการถ่ายฝาก

INTRODUCTION

Infertility, especially in the older animal, is one of the most wasteful and economically devastating problems of the livestock industry. In the majority of cases,

infertile food-producing animals should be culled. There are, however, many genetically superior females which become infertile due to uterine or oviductal diseases or the old age, and in many instances embryo transfer technique can be used to obtain additional progeny from such animals.

Embryo transfer also offers new opportunities for reproduction of other domestic and nondomestic animals. The main objective of embryo transfer is the improvement of animal populations through increased utilization of superior females. The expansion of genetic pools provided by embryo transfer is especially important for monotocous species, such as the bovine and equine, which have long gestation periods and low rates of reproduction. In these species, the intensity of selection that can be applied and the natural rate of genetic improvement are limited by this relatively low reproductive efficiency. It has been estimated that the ovaries of a prepubertal heifer contain more than 100,000 oocytes. Even under ideal conditions of health and manageme highly productive cow may produce only 8-12 calves in her reproductive lifetime. Using current embryo transfer technology, it is feasible to obtain 30-40 calves from a single superior cow over a period of a year[4]. This could be acheived if the cows are well superovulated.

It is generally believed that the use of *in vitro* techniques for the study of early mammalian development is easier, more informative, and more controllable than the alternative of attempting to study events occurring in the natural environment. While this is essentially true, there is much to be gained from the latter approach. The risk of introducing artifacts into an experiment should be much less with the *in vivo* approach, and it avoids the whole problem of designing culture media suitable for gametes and embryos. The two most fundamental discoveries about mammalian fertilization that have made to date, namely the need for sperm to first undergo capacitation and then the acrosome reaction, are both accomplished by examination of eggs undergoing fertilization that are recovered from mated animals. By this means, Austin[2] and Chang[8] showed that a period of residence in the female reproductive tract was needed by sperm before they could effect fertilization (capacitation).

In Thailand, embryo transfer has expanded rapidly in the last few years but the retrospective summary may have been a little achieved in transferring bovine embryos during

the early years of its commercial application. Because the superovulation technique to produce embryo has many problems; for example, too expensive, produce a small number of embryos per flush, making it possible trauma to reproductive track[21] and decrease in the superovulatory response after repeated treatment with the same Gonadotrophin in donor cow.[27] In addition to commercial applications embryo transfer technology is being used at an ever - increasing rate in basic and applied research. The *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* culture (IVC) methods are developed in the present study to produce embryos in mass in order to increase exotic calves from native recipient cows in Thailand.

The aim of the experiment is to investigate the pregnancy rate and calves born from transferring the bovine embryo produced *in vitro*. An effort was made to produce a large number of embryos by *in vitro* maturation and fertilization of oocytes from slaughtered ovaries, that were capable of developing totally in culture with oviductal epithelial cells to the compact morula, early blastocysts and blastocyst stages which were transferred non-surgically to native cows.

MATERIALS AND METHODS

Oocyte collection and maturation *in vitro*

Bovine ovaries showing follicular development were obtained from a slaughterhouse. Ovaries were removed within 30 min after slaughtered and transported to the laboratory in a thermos of 0.9% NaCl with 100 IU/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml fungizone. The contents of follicles visible at the ovarian surface were aspirated from small vesicular follicles (1-5 mm in diameter) of ovaries into a 5-ml disposable syringe with 18 gauge needle containing 1 ml of HEPES buffer Tyrode's media (TALP-HEPES) and placed in petri dishes. Follicular contents were observed under a stereomicroscope and oocytes were removed with a Hamilton syringe.

Oocytes were recovered within 2-3 h after slaughtered depending on the number of ovaries collected a day. After recovery, oocytes were washed three times in TALP-HEPES supplemented with 10% heat-treated fetal calf serum (HTFCS) and 50 μg/ml gentamycin. Then they were classified according to their investments. The grades were defined and coded

as follows : compact and unexpanded cumulus as grade 1, fully expanded cumulus, partially denuded and completely denuded as grade 2, 3 and 4, respectively.

After washing, oocytes were cultured in CO_2 incubator at 39°C in 5% CO_2, 95% air with high humidity for 24 h in 50-µl droplets of a maturation medium under 10 ml of paraffin oil in 60x15 mm Falcon culture dishes. Each droplet contained 5-10 cumulus-oocyte complexes. Maturation medium was TCM-199 supplemented with 10% HTFCS and 15 µg/ml follicle-stimulating hormone (FSH), 1 µg/ml luteinizing hormone (LH) and 1 µg/ml estradiol diluted with ethanol. Although HEPES-buffer in culture medium maintained proper pH level for a longer culture period outside a 5% CO_2 incubator, TCM-199 was used to mature oocytes in the 5% CO_2 incubator. It was a better buffer to maintain pH in CO_2 environment. The pH of the maturation medium with 2.2 mg/ml $NaHCO_3$ was about 7.2-7.3 after equilibration in CO_2 incubator and slightly decreased at the end of maturation observed from the color of the medium with phenol red in the medium.

Semen collection

Ejaculated bovine sperm was obtained by artificial vaginal method from 5 bulls and extended 1:1 with an eggyolk/sodium citrate extender to protect them during transportation to the laboratory which took about 2 h. Progressively motile sperm were separated from the bovine semen by a swim-up method.

Sperm capacitation with heparin

Heparin (glucosamine-6-sulfate, glucuronic acid-2- sulfate and iduronic acid), a grade II sodium salt isolated from hog intestinal mucosa was obtained from Sigma Chemical Co., and dissolved in saline at a concentration of 0.2 µg/ml for stock solution. Vials (200 µl) of heparin stock were stored and frozen at-20 °C until used. Ten microliters of swim-up separated bovine sperm fraction was incubated in 50 µl culture drop of sperm-TALP-glucose free medium with 2 µl heparin (0.2 µg/ml) at 39°C for 4 h in a CO_2 incubator.

Isolation of oviductal tissue

Bovine and porcine oviducts of ovulatory phase were obtained from slaughterhouse and transported to the laboratory in 157 mM NaCl at 4°C containing 50 µg/ml gentamycin. Then

they were trimmed free of connective tissue and rinsed in 157 mM NaCl containing 100 IU/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B. After blotting on sterile gauze to remove excess fluid and blood, the oviducts were placed in 100 mm sterile petri dishes, grasped with a forceps at the isthmic end and scraped gently toward the infundibulum with a glass microslide. Mucosal tissue was then extruded from the ostium abdominale and transferred to a 12 ml conical centrifuge tube with 10 ml TALP-HEPES supplemented with 10% HTFCS. The tissue was then washed in 5-7 changes of TALP-HEPES containing 10% HTFCS, 50 μg/ml gentamycin and then resuspended in TALP medium to a ratio of 1:50. Five milliliters of the suspension was placed in a 60 mm Falcon dish and cultured at 39°C under an atmosphere of 5% CO_2, 95% air. The cells were used for co-culture when they were above 80% confluency (day 2-7).

***In vitro* fertilization and co-culture**

A 10 μl aliquot ($50x10^6$ sperm/ml) of swim-up separated sperm was placed in a culture dish with 50 μl of glucose free TALP containing 2 μl (0.2 μg/ml) heparin to enhance capacitation. Sperm were incubated for 4 h at 39°C in 5% CO_2 in air. Five to ten *in vitro* matured cumulus-oocyte complexed were added into the droplets of sperm suspension, after oocytes were washed three times with glucose free TALP medium containing 6 mg/ml BSA. To examine the fertilization rate at the pronuclear stage, sperm and cumulus-oocyte complexes were co-cultured for 18-22 h in a CO_2 incubator (5% CO_2 in air with high humidity at 39°C). After that the ova were removed from fertilization droplets and cumulus cells were stripped off by repeated pipetting through a small bore pipet. Then they were cultured in 10% HTFCS-TALP medium supplemented with oviductal tissue suspension (1:1) conducted in 50 μl droplets under paraffin oil (10-20 embryos/droplet). The medium was changed every 2 days.

Culture of embryos and assessment of development

Embryos were assessed every day for cleavage by using an inverted microscope in a 39°C culture chamber. All eggs, except the uncleaved ones, were cultured for 7-8 days, and at the end of the designated culture period all embryos at compact morular, early blastocyst and blastocyst stages were used for transfer to recipient.

Detection of estrous

Native cows in Thailand were used as recipients. The criteria of recipient were healthy, regular estrous cycle, not too thin or too fat and expand of sacro-lumbar-pelvis structure. All recipients were routinely treated for ecto-and endoparasites. All tests and treatments should be completed before starting the procedures for estrous detection. The many physiological and behavioral changed which occurred during estrous provide a variety of means of detecting its occurrence. Observation periods commened at 06.00, 11.00, 16.00 and 21.00 h and last for a minimum of 30 min and a maximum of 2 h. The definitive signs of estrous were when the recipient standed to be mounted by another animal or a recipient mounted the head of another animal. Besides, other activities such as attempted mounting, sniffing, butting and the presence of vaginal mucous were recorded to interpret the doubtful records and cases.

Recipients were selected for transfer in most circumstances, if they had been observed in standing estrous and had a good quality palpable corpus luteum or according to the chronological age (8 day after estrous). The stages of development of the embryo : compact morula, early blastocyst or blastocyst at day 7 were also used. The recipients should be handled in calm and orderly manner at all times and particularly at embryo transfer.

Non-surgical embryo transfer

The embryos were obtained from *in vitro* fertilization on day 7 after co-cultured with oviductal cells. Only morphologically normal compact morulae, early blastocysts and blastocysts were used in non-surgical embryo transfer experiments. At day 8 of recipient cows after estrous the embryos were washed three times in phosphate buffer saline (PBS) supplement with 20% HTFCS. Two embryos at the same stage were loaded into a 0.25 ml plastic artificial insemination straw in the following sequences. A column of PBS with 20% HTFCS about 5 mm long was aspirated into the straw followed by in air bubble of about the same length. This comprised the first chamber. Then the embryos which would be contained in a second column of PBS with 20% HTFCS about 5 mm long were aspirated into the straw followed by a second air bubble. This comprised the second chamber. Finally, a solution of PBS with 20% HTFCS was aspirated into the straw completing the filling process. This was the third chamber. Non-surgical transfer was done by using an AI breeding gun. The straw was loaded into the breeding gun and the

sterile plastic sheath was placed over it. The vulva area was cleaned by betadine, scrubbed and wiped dry. The recipient was examined for the presence of an appropriate corpus luteum and a normal uterus by rectal palpation. The AI breeding gun was inserted to the external cervical os, punched through the protective sheath and threaded through the cervix into the uterine horn ipsilateral to the corpus luteum. It was carefully advanced along the horn and the plunger firmly depressed to expel the embryos. The gun was carefully withdrawn. Pregnancies were determined between 60 and 120 days after transfer by rectal palpation or observe estrous cycle in the next cycle after transfer. The process of parturition marks the termination of pregnancy, at which time the fetus was capable of independent existence outside the uterus. The gestational length, sex of calves, birth weight of calves and twin calves born were recorded.

RESULTS

Embryos produced from IVM, IVF and IVC procedures were examined every 24 h from 1-7 days postinsemination (day of fertilization = day 0). All embryos were individually examined at 400x for development and quality. A high degree of variability was observed in morphological development and embryo quality. The overall diameter of the bovine embryo was 150-190 μm including a zona pellucida of approximately 12-15 μm thickness. The overall diameter of the embryo remained virtually changed from the zygote until blastocyst expansion. Early cleavage-stage embryos were commonly referred to by the number of cells present, such as the zygote, 2-cell, 4-cell, 8-cell, up to the 16-cell stage. Microscopic examination of living specimens revealed only an estimation of the number of cells present in embryos developed beyond the 16-cell stage. Consequently, other morphological criteria must be used. Compact morula was the stage that individual blastomeres had coalesced, forming a compact mass. The embryonic mass occupied most of the perivitelline space. (Figure 1A). Early blastocyst was an embryo that had formed a fluid-filled cavity or blastocoel and gave a general appearance of a signet ring. The embryo occupies 70-80% of the perivitelline space. Visual differentiation between trophoblast and the inner cell mass was possible at this stage of development (Figure 1D). The blastocyst pronounced the differentiation of the outer trophoblast layer and the compact

inner cell mass. The blastocoel was highly prominent with the embryo occupying most of the perivitelline space (Figure 1G). Quality of individual embryos was observed by the following criteria. The excellent was an ideal embryo, spherical, symmetrical with cells of uniform size, color and texture (Figures 1A, 1D, 1G). Good was trivial imperfections such as a few extruded blastomeres, irregular shape, few vesicles (Figures 1B, 1E, 1H). Fair was definite but not severe problems, presence of extruded blastomeres, vesiculation, few degenerated cells (Figures 1C, 1F , 1I).

The competence of bovine embryo in various stages was examined by non-surgical transfer to the recipient cows. Table 1 showed an overall view of the number of embryos, stages of embryos and percentage of pregnancy after transfer. Differences in pregnancy rate after unilateral transfer of two embryos at the same stage of compact morulae (55%, 6 of 11) or early blastocysts (75%, 9 of 12) and blastocysts (30%, 3 of 10) were observed. A total of 18 pregnancies were produced from 33 transfers (55%). Five of 18 pregnancies were twins and one of them (twin male fetus) aborted, at 150 days after transfer . All other calves appeared to be normal at birth. The length of gestation for single pregnancies ranged from 269-295 days (mean ± SD, 284 ± 8 days). The length of gestation for twin pregnancies ranged from 265-280 days (means ± SD, 275 ± 7 days). Seventy-one percent (15 of 21) of calves were male. The birth weight of twin calves (mean ± SD, 18 ± 4 kg) was lower than, but not statistically significant different from those of single calves (23 ± 4 kg). Single male (mean ± SD, 23 ± 4 kg) and single female (mean ± SD, 23 ± 3 kg) calves had similar birth weights. Dystocia or difficult of birth was not observed in recipients delivering twins, but calving assistance was necessary in many cases but all the calves were healthy.

DISCUSSION

Pregnancies and birth of live offspring in the present study has been established by the transfer of the embryos derived from *in vitro* culture system and the overall pregnancy rate was 55%. It is similar to the results reported earlier following transfer of embryos produced *in vitro*[28]. A total of 21 live calves were born from 17 recipients because 4 sets of twins were obtained. These results indicated that the number of calves produced by the experiment could

be increased in a herd. The twin pregnancy rate achieved after unilateral transfer of two embryos to the uterine horn ipsilateral to the corpus luteum was 28% and the abortion rate was 5.5%.

The twining rates in the present study is low (28% vs 45%, 44.4%, 61.5%) when compared to the results obtained with bilateral surgical transfer of embryos produced *in vivo*[1] as well as that reported after unilateral transfer of two embryos[5]. More meaningful results would have been generated by restricting twin-transfers of excellent or good embryos only.

A high (55%) pregnancy rate was achieved following embryo transfer when the relative synchronicity between recipient and embryo was-1 day (Table 2). Kajihara[16] obtained higher pregnancy rates (59.3%) after transfer of embryos produced *in vitro* to asynchronus recipients. Embryos produced *in vivo* by collecting from the uterus of superovulated donors should be returned to the uterus of recipients at approximately the same stage in their estrous cycle as the donors[12]. The present results for the transfer of embryos produced *in vitro* suggests that these embryos developed more slowly than those conceived *in vivo* and should therefore be transferred to - 1 day asynchronous recipients. Embryo evaluation to select the most viable for transfer was practised routinely in embryo tranfer programmers. A difference in pregnancy rates occurred when excellent or good, as opposed to fair or poor quality embryos recovered from superovulated donor cows were transferred[12]. The transfer of embryos produced *in vitro* evaluated as morphologically excellent in early blastocyst stage resulted in a higher pregnancy rate than the transfer of compact morula and blastocyst stages (Table 1). Pregnancy rate increased with the number of embryos transferred. The establishment of a "dialogue" between the embryo and uterus depended on the number and quality of the embryonic cells transferred. The trophoblastic cells gave the signal was maintaining the corpus luteum of the recipient cow, but the survival of the transferred embryo depended on the good viability of the cells of the embryonic disc[14].

The establishment of pregnancy in the cow involves a complex series of interrelationships between the embryo, its uterine environment and the corpus luteum. The embryo was known to produce proteins, steroids and prostaglandins with both luteotrophic and anti-luteolytic effects and also regulatory effects on uterine blood flow, nutrient transfer, embryo migration and other events associated with the establishment and maintainance of pregnancy[3]. The incidence of embryonic loss in the present study is 45%. Because the causes of embryonic

loss were complex, involving physiological, endocrinological, genetic, immunological and environmental components, it had not been possible to isolate the effects of specific factors on basal embryonic loss[13]. Embryo transfer procedures, however, did allow control of a number of factors that influence their subsequent survival. The embryonic and maternal or environmental-factors were important. Some of the factors that influence post transfer embryo survival were intrinsic to the embryo itself.

The recipient was one of the prime determinants of the success of the embryo transfer enterprise and improving recipient quality could produce significant savings in the cost of achieving a pregnancy[7]. Factors that affected the quality of the uterine environment affected embryo survival rate and the more important of these were discussed. Following fertilization, embryos grew within a continuously changing uterine environment. The changes that occurred within the uterus did so in response to a changing endocrine pattern. It had therefore, always been considered appropriate in embryo transfer programmes to ensure stage of embryo and recipient estrous cycles were synchronous. The estrous detection was one of the prime to determine the stage of embryo-recipient synchrony. Furthermore, it was obvious that effective estrous detection was the key to maximizing reproductive efficiency. Basically there were two estrous detection problems, 1) missed or unobserved estrous periods and 2) estrous detection errors[9]. There was clear evidence that pregnancy rate declined with the degree of asynchrony but as pointed out in a review by Seidel[23] there was a tolerance of 1.5 days in either direction. It was suggested that advanced cattle blastocysts might have less stringent synchrony requirements than morulae and early blastocysts[18,23] though experimental data on embryos produced *in vitro* (Table 1) would suggest the opposite direction.

In the cow as in the other domestic animals, progesterone secreted by the corpus luteum is one of the main steroids that determines the physiological state of the uterus and is essential for the establishment and maintainance of pregnancy. Progesterone supplementation therapy has been used in many species in an attempt to increase embryo survival rate after breeding. While supplementation of sub-fertile cows has been shown to increase embryo survival rate, there is no evidence of a beneficial effect in cows of normal fertility[10].

Studies on the effect of site of transfer within the uterus, whether to the tip or base

of the uterine horn had failed to show a clear advantage for either site[23]. However, site of transfer was determined by whether a surgical or non-surgical approach was used. One report[25] recorded a smaller difference in pregnancy rate (14%) but still in favour of ipsilateral transfer. For flank surgical transfers, it was easiest to transfer to the tip of the uterine horn while with non-surgical transfer the embryo can only be deposited between the base and mid-horn. Any attempt to non-surgically deposit the embryo further than mid-horn was likely to cause trauma and increase the probability of embryo loss. While pregnancy rates were generally lower and less consistent following non-surgical compared with flank surgical transfers in the cow. Nevertheless, because it is a fast and relatively inexpensive procedure it is now the method of choice in most commercial units. The biggest single factor in effecting a high non-surgical pregnancy rate is probably the skill and experience of the operator. Operator skill determines not only site of deposition of the embryo within the selected uterine horn but also the degree of trauma inflicted during the transfer.

Recipients in embryo transfer are usually in good body condition and on an adequate plane of nutrition to meet target growth rates. While many reports showed a positive relationship between level of nutrition and overall reproductive efficiency, few had established specific effects of nutrition level on embryo survival rates following either insemination or embryo transfer. The literature on inseminated animals suggested that extreme in body weight or level of nutrition of recipients should be avoided. For animals in poor body condition but enhanced feeding will increase pregnancy rates[19]. The level of enhanced feeding should be continued until implantation was complete as the developing foetus would seem to be most susceptible to nutritionally induced stress immediately prior to and during the implantation stage[20] which lasted from about day 21-day 45[15]. Conversely for animals in good body condition or that were overfat, pregnancy rates were depressed and enhanced feeding of such animals will even further depress pregnancy rates[11]. The birth weight of twin calves (mean, 18.3 kg) was lower than, but not statistically significant different from, those of cattle produced in Thailand from 1/2 indigenous crossbreed (mean, 19.8 kg) and single calves (mean, 23.1 kg). These results correspond to those of Key[17], who found that although twin calves weight significantly less at birth, at weaning and at 3 months of age, the weight difference at 1 year of age was insignificant. A knowledge of the mean birth weight of cattle was important not only in that it

gave an indication of the mature weight of cattle, but also in that it enabled an estimate to be made of the absolute daily weight gain that could be expected on a particular plane of nutrition and time that would be required to achieve a given stage of maturity. Moreover it had a value in interpreting the results of experiments on purchased calves of uncertain age.[22]

In conclusion, there are many other factors that influence pregnancy rate after transfer embryo produced *in vitro* [6,14,24] The results of the present study indicates that satisfactory pregnancy results can be obtained when excellent embryos at compact morula, early blastocyst and blastocyst stages, derived from *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* culture (IVC) methods, are transferred to-1 day asynchronous native cow recipients. In the present study, all calves appear to be normal at birth. The length of gestation for single pregnancies ranged from 269-295 days (mean, 284 days), for twin pregnancies ranged from 265-280 days (mean, 275 days) were similar to gestation in domestic cattle extends for approximately 280 days ranged from 270-292 days. Variability in length of gestation was influenced by fetal sex, number of fetuses, breed and genotype of the sire or dam or fetus, plane of nutrition and environmental temperature[26]. The birth weight of total calves (mean, 21.2 kg) was higher than the indigenous cattle in Thailand.

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Table 1. **The competence of bovine embryo in stages of compact morula, early blastocyst and blastocyst was examined by the percentage of pregnancy**

Stages	No. Embryos	No.Recipients	Preg/Trans	Pregnancy rate (%)
Compact morula	22	11	6/11	55
Early blastocyst	24	12	9/12	75
Blastocyst	20	10	3/10	30
Total	66	33	18/33	55

Table 2. **Summary of gestation period and birth weight of calves born from non-surgical embryo transfer**

Embryos breed	Age of Embryos (days)	Estrous cycle (days)	Gestational age (days)	Calves born
Dairy breed				
HF x NC	7	8	284	F 25 kg
HF x HF	7	8	270	F 27 kg
HF x HF	7	8	269	M 29 kg
HF x HF	7	8	265	F 19 kg, M 15 kg
SM x HF	7	8	278	F 24 kg
Beef breed				
LI x NC	7	8	282	M 27 kg
IB x NC	7	8	286	F 19 kg
IB x NC	7	8	282	F 21 kg
B x NC	7	8	289	M 26 kg
B x NC	7	8	295	M 20 kg
B x NC	7	8	292	M 18 kg
B x NC	7	8	295	M 17 kg
CH x NC	7	8	280	M 25 kg
Mix breed				
B x HF	7	8	285	M 22 kg
B x HF	7	8	278	M 16 kg, M 16 kg
B x HF	7	8	280	M 22 kg, M 25 kg
B x HF	7	8	abortion	M , M
CH x HF	7	8	278	M 16 kg, M 17 kg

B = Brahman CH = Charolais HF = Holstein IB = Indubrasin LI = Limousin NC = Native cow SM = Simmental

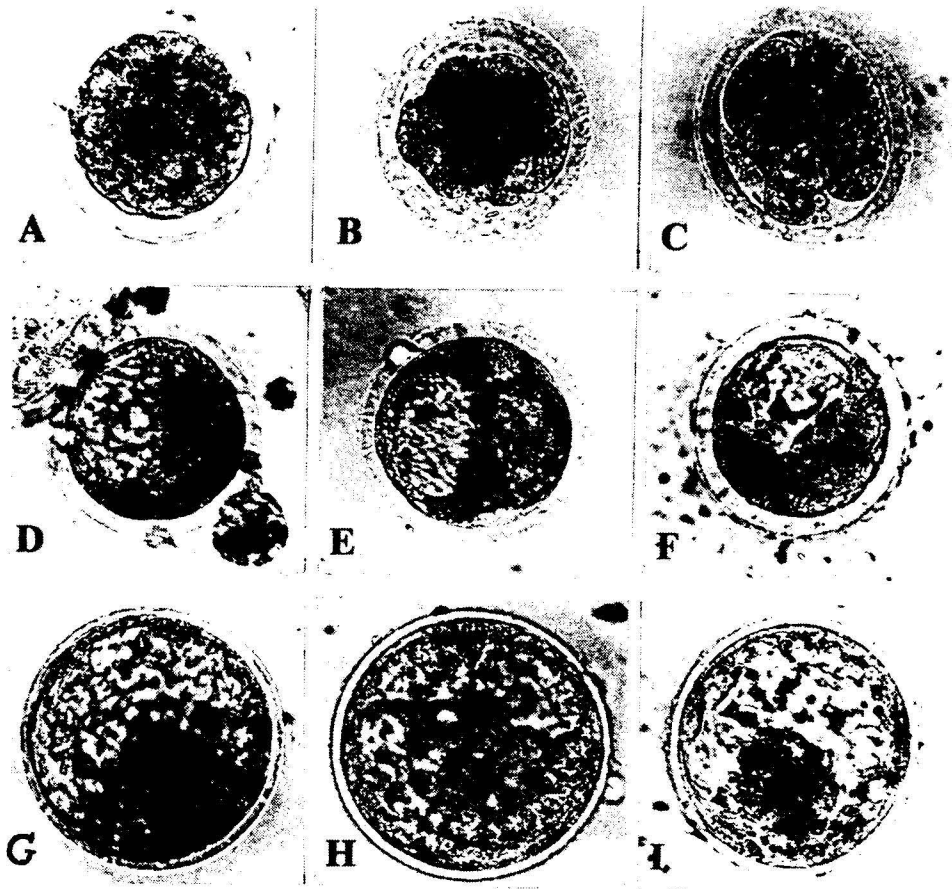

Fig. 1 Bovine embryos at the transferable stages in three grade of quality :

(A) Excellent compact morula

(B) Good compact morula

(C) Fair compact morula

(D) Excellent early blastocyst

(E) Good early blastocyst

(F) Fair early blastocyst

(G) Excellent blastocyst

(H) Good blastocyst

(I) Fair blastocyst