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PREGNANCY RESULTING FROM BOVINE OOCYTES MATURED, FERTILIZED AND CULTURED WITH OVIDUCTAL CELL *IN VITRO*[1]

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ABSTRACT

Bovine blastocysts derived from *in vitro* procedures (i.e. oocyte maturation, fertilization and embryonic development in oviductal cell culture) were non-surgically transferred to 4 recipient cows (3 embryos/recipient). Three recipients became pregnant from the first transfer and the fourth was pregnant after the second transfer. This is the first report of high pregnancy rate in cattle resulting from oocyte retrieved from a slaughterhouse, matured, fertilized, cultured with oviductal cell *in vitro*, and non-surgically transferred to the native recipients.

Key words : Bovine embryo, in-vitro production, pregnancy

INTRODUCTION

Bovine follicular oocytes have been cultured effectively till the matured stage in TCM 199 supplemented with 10% heated fetal calf serum (HTFCS). In vitro matured oocytes are fertilized *in vitro* (IVF) at high percentage by either fresh ejaculated or frozen sperm capacitated by a swim-up and heparin incubation methods (Pavasuthipaisit et al., 1989). Oocytes matured and fertilized *in vitro* have been shown to develop to the blastocyst stage after incubation in rabbit oviduct (Sirard et al., 1985).

Development of all early bovine embryos *in vitro* is generally arrested at the 8-cell to 16-cell stages (Wright & Bondioli, 1981). Successful *in vitro* culture to morula or blastocyst stage through the 8-cell block requires the presence of an active biological component such as Day-14 trophoblastic vesicles (Heyman et al., 1987), cumulus cell monolayer (Kajihara et al., 1987) or oviductal epithelium (Eyestone & First, 1989; Kitiyanant et al., 1989). Recently, it has been shown that the blastocysts obtained in cumulus cell co-culture (Goto et al., 1988; Fukuda et al., 1990) or from intermediate hosts (Xu et al., 1988) developed normally when they were transferred to recipient cows. However, the development of the embryos derived from IVF of *in vitro* matured *oocytes* and cultured with oviductal cells to normal calves through a normal pregnancy after transfer to recipients has not been reported. In the present study, we reported the results of pregnancy obtained from non-surgical transfer of embryos from oocytes matured, fertilized and cultured with oviductal epithelium *in vitro* up to the blastocyst stage.

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MATERIALS AND METHODS

Oocyte collection and maturation in vitro

Ovaries selected from either Holstein Friesian or mixed native cows were obtained from a local slaughterhouse and transported to the laboratory in a 157 mM NaCl solution with 50 ug/ml gentamycin. Oocytes were aspirated and matured as described previously (Pavasuthipaisit et al., 1990). Briefly, ten cumulus-oocyte complexes were cultured in 50 ul droplets of TCM 199 modified with Earle's salt medium for 24 h under 10 ml paraffin oil. The medium was supplemented with 0.25 mM pyruvate, 10% HTFCS, 15 ug/ml porcine follicle stimulating hormone, 1 ug/ml LH and 1 ug/ml oestradiol.

Sperm capacitation with heparin and in vitro fertilazation

Semen was collected with an artificial vagina from either Holstein Friesian or Simmentol bulls, diluted 1:1 with an egg yolk extender and stored at 4°C during transportation to the laboratory which took about 2 h. Capacitation of spermatozoa and *in vitro* fertilization were done according to the previous report (Pavasuthipaisit et al., 1990). Briefly, the motile sperm fraction was obtained by a swim-up separation procedure and then a 10 ul sperm aliquot was incubated with 50 ul of glucose free-TALP containing 0.2 ug/ml heparin to enhance capacitation for 4 h at 39°C in 5% CO_2 in air.

In vitro development and embryo transfer

The ability of inseminated oocytes to accomplish fertilization was judged by their development to the 2-cell stage. To evaluate the ability of *in vitro* fertilized embryos to develop beyond the 8-cell stage and up to the blastocyst stage, all oocytes inseminated with sperm capacitated in the presence of heparin were cultured in 50 ul droplets of TCM 199 containing 10% HTFCS under mineral oil in culture dishes. The oviductal cells were prepared and co-cultured with 2-cell embryos as described in the previous experiment (Kitiyanant et al., 1989). Embryos (37%) were developed to the compact morula or early blastocyst stage 7 days after the insemination.

The embryos were placed in PBS supplemented with 20% HTFCS for embryo transfer. Morphologically normal blastcysts were non-surgically transferred into the uterine horn ipsilateral to corpus luteum of the recipient (3 embryos/recipient). In two recipient native cows, the transfers were performed at Day 7 or 8 of the cycle (Day O = oestrus). In the other two recipients, the embryos were transferred at Day 11 after the second dose of 25 mg luteolyte (Lutalyser, Upjohn company, Belgium) injection. The first luteolyte administration was 12 days prior to the second dose. The pregnancy diagnosis was done by rectal palpation after 60 days.

RESULTS

The results of the embryo transfer are shown in Table 1. Normal fresh blastocysts derived from the *in vitro* procedures were non-surgically transferred to 4 recipents. Three recipients became pregnent after the first transfer. The fouth recipient received transfer for the second time 7 days after the third oestrus cycle, then it became pregnant.

DISCUSSION

This is the first report of the pregnancies in cows from non-surgical transfer of embryos resulting from oocytes matured, fertilized and cultured with oviductal cells *in vitro* up to the blastocyst stage. Most calves born after IVF have so far been originated from *in vivo* matured oocytes transferred surgically to the oviduct of synchronized recipient cattles (Brackett et al., 1982) or followed by using the rabbit oviduct as an incubator for 4-5 days (Sirard & Lambert, 1986). Xu et al. (1987) established pregnancy by transferring *in vivo* matured and *in vitro* fertilized oocytes to the oviducts of recipient heifers.

In vitro fertilization of *oocytes* matured *in vitro* has only resulted in the formation of early embryonic stages (Ball et al., 1983). By using sheep oviducts as the *in vivo* culture system to bring the embryo to the morula stage, one pregnancy was reported after *in vitro* fertilization of *in vitro* matured oocytes (Crister et al., 1986). In Japan, Hanada et al. (1986) reported the birth of three calves after the *in vitro* fertilization of *in vitro* matured oocytes but using rabbit oviducts as a temporary incubator. Recently, Fukuda et al. (1990) reported the birth of normal calves from oocytes matured, fertilized and cultured with cumulus cells *in vitro* Nevertheless, the present report differs from that of Fukuda et al. (1990) in (1) the types of co-culture, i.e. oviductal cell vs. cumulus cells, (2) the procedure of sperm capacitation, i.e. heparin vs. Ca^{2+} ionophore´A 23187, and (3) the percentage of blastocyst development, i.e. 37% vs. 9.0%.

Even though the birth of a live calf would be the ultimate proof of the efficiency of the *in vitro* culture and embryo transfer technology. Blastocyst formation and hatched blastocysts as we have previously reported (Kitiyanant et al., 1989; Pavasuthipaisit et al., 1990) may be considered as a valid and viable indication that normal oocyte maturation, fertilization and *in vitro* culture with oviductal cells have been accomplished. The positive pregnancy diagnosis reported herein further support this conclusion.

Table 1. Pregnancy of recipient cows after transfer of blastocysts derived from oocytes matured, fertilized and cultured with oviductal cells *in vitro*

Embryo breed	Age of embryos (Day)	Recipient no	Oestrus cycle (Day)	Synchronization (Day)	Date of ET	Pregnancy
HF × HF	7	1	-	11	21/3/90	+
HF × NC	7	2	8	-	17/4/90	+
HF × NC	7	3	-	11	22/5/90	+
HF × HF	7	4	7	-	4/4/90	–
SM × NC	7	4	7	-	26/6/90	+

Day of in vitro insemination = Day O
Day of oestrus = Day O
Day of second luteolyte injection = Day O
HF = Holstein Friesian
SM = Simmental
NC = Native cow
ET = Embryo transfer

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บทคัดย่อ

ได้ทำการฝากถ่ายตัวอ่อนโคในระยะบลาสโตซีสซึ่งได้มาจากขบวนการในห้องทดลองทั้งหมดได้แก่ การเพาะเลี้ยงเซลล์ไข่ให้สุกเต็มที่ การปฏิสนธิในหลอดทดลองและการพัฒนาตัวอ่อนด้วยเซลล์เยื่อบุท่อนำรังไข่ ให้กับแม่โคตัวรับ 4 แม่ โดยวิธีการไม่ผ่าตัด โดยฝากถ่ายตัวอ่อน 3 ตัว ให้กับแม่โคตัวรับ 1 แม่ ในมดลูกข้างที่มีคอร์พัสลูเตียม ผลปรากฏว่าแม่โค 3 แม่ ตั้งท้องจากการฝากถ่ายตัวอ่อนครั้งแรก และแม่โคแม่ที่ 4 ตั้งท้อง จากการฝากถ่ายตัวอ่อนครั้งที่ 2 การวิจัยนี้เป็นรายงานแรกที่ประสบความสำเร็จ ในการฝากถ่ายตัวอ่อนจากการเพาะเลี้ยงตัวอ่อนในเซลล์ท่อบุนำรังไข่ และขบวนการอื่น ๆ ในห้องปฏิบัติการโดยไม่ต้องผ่านสัตว์ทดลอง