

## Embryo Transfer Technology in Pigs : Experiences in Thailand <sup>1</sup>

เทคโนโลยีการย้ายฝากตัวอ่อนในสุกร : ประสบการณ์ในประเทศไทย

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การย้ายฝากตัวอ่อนในสุกร ให้ประโยชน์ในแง่การศึกษาสรีรวิทยาของระบบสืบพันธุ์และการตั้งครรภ์ในระยะแรก โดยที่สุกรมีอัตราการตกไข่สูง จึงเป็นตัวอย่างที่ดีในการผลิตไข่และตัวอ่อน เพื่อการศึกษาในหลอดทดลอง จุดประสงค์ที่สำคัญที่สุดในการย้ายฝากตัวอ่อนในสุกรเพื่อการค้า คือ การนำสายพันธุ์ใหม่เข้ามายังแหล่งเลี้ยงสุกรปิด เพื่อการควบคุมโรค

รายงานนี้กล่าวถึงหลักการและผลการศึกษา ตลอดจนเสนอแนะเรื่องที่สำคัญที่ควรจะได้รับ ความสนใจที่จะวิจัย และพัฒนาให้เกิดประโยชน์ต่อไป

**Abstract :** Peerasak Chantaraprateep. 1989. Embryo transfer technology in pigs : Experiences in Thailand. Thai J Hlth Resch 3 (2) : 127-131

Swine embryo transfer has been studied as an experimental tool for reproductive physiology and early pregnancy. Due to the high ovulation rate, the pig is a good animal amongst the domestic species for the provision of eggs and embryos for investigation in vitro. The most important goal for performing commercial embryo transfer in this species is the introduction of new genetic material into herds which are closed for the purpose of disease control.

The paper underlined some of the basic techniques, described the results achieved and drew attention to certain areas where more research and development are needed.

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## INTRODUCTION

Embryo transfer has been used successfully during the last 25 years both as an experimental tool in various studies related to reproductive physiology and early pregnancy in the pig. Due to the high ovulation rate the pig is also a good animal amongst the domestic species for the provision of eggs and embryos for investigation in vitro, the techniques used which have been developed (Hancock and Hovel, 1962 ; Polge, 1982) and for practical application in our condition are not complicated and effective (Kitrungrojcharoen *et al.*, 1980 ; Chantaraprateep *et al.*, 1985 ; Techakumphu *et al.*, 1987 ; Chantaraprateep *et al.*, 1987 a, b ; Techakumphu *et al.*, 1988). The most important goal for performing commercial embryo transfer in this species is the introduction of new genetic material into herds which are closed for the purpose of disease control and in this respect it can be regarded as an alternative to hysterectomy (Polge, 1982 ; Martin 1983, 1986 ; Einarsson, 1985). As the disease transmission potential of embryos is much less than those of either the live animal or semen.

It may be used for achieving an increasing rate of reproduction of superior genetic animals and there is also the possibility of export or import of embryos between countries particularly if long-term storage of swine embryos becomes possible. Furthermore, cryopreservation has been found to be effective in inactivating low levels of many viruses that can be adhered to embryos (Singh, 1987 ; 1988). Thus, theoretically, the disease transmission potential of embryos is limited. Table 1 shows the different reasons for introduction of swine embryos into recipient herds in the US in 1981.

**Table 1.** *Primary reasons for performing commercial embryo transfer in swine in the US in 1981 as well as the number of transfers and recipient herds involved (Martin, 1986).*

Group No.	Reason	No. of recipients (%)	No. of herds
1.	Establish new herds from herds with Pseudorabies	113 (43)	4
2.	Make additions to specific pathogen free (SPF) herds	67 (26)	13
3.	Obtain boars for closed commercial herd	61 (23)	11
4.	Obtain more offspring from superior gilts and sows	19 (7)	8
<b>Total</b>		<b>260</b>	<b>36</b>

The objective of the present paper is to underline some of the basic techniques we used, to describe the results achieved and to draw attention to certain areas where more research and development are needed.

### 1. The basic techniques

#### 1.1 Selection of donors and recipients

Depends on the needs of the owner. However, only animals that have the genetic potential to improve the breed should be selected as donors and they should be in good health. Sows are preferable as the source of embryos. Sound health and normal cycling animals without having a good genetic background can be served as recipients.

#### 1.2 Oestrus synchronization

Several methods are commonly employed for controlling estrus and ovulation for embryo transfer purposes. The first method involves weaning a group of sows on the same day, with estrus occurring 4 to 10 days later (Martin, 1986). Another method is using pregnant mare serum gonadotropin (PMSG)

and human chorionic gonadotropin (HCG) dose rate of 400/200 IU (PG 600<sup>®</sup>, Intervet) administered subcutaneously, 90.5% (19/21) of treated animals came in heat within 5 days after the treatment (Techakumphu *et al.*, 1987). The most effective treatment for estrus synchronization in mature gilts is by feeding allyl trenbolone (Regumate<sup>®</sup>, Roussel Uclaf) for 18 days. This progestational agent blocks follicular maturation during the time that it is administered and the animals then rebound into a follicular phase following withdrawal. Results of the experiments with Regumate<sup>®</sup> were 98.5% of 586 gilts came into estrus within 9 days after the end of treatment (Polge, 1982) and 80.7% of the heats were synchronized within a 2 day period on the 5th and 6th days (Chantaraprateep *et al.*, 1986 ; Chantaraprateep *et al.*, 1987 c). AI on the second day of estrus resulted in 90% of the animals with fertilized eggs (Polge, 1982).

### 1.3 Superovulation and breeding

When superovulation of donor is required, it is usually achieved by one injection of 1000-1500 IU PMSG at weaning or at the early follicular phase of the cycle, 15 or 16 days after the onset of estrus (Hunter, 1964) and the estrus occurs 3.5 - 4 days later. HCG at the dose rate of 500 IU can be used to enhance ovulatory responses and is given 3-4 days after the treatment of PMSG.

For optimum conception, donors should be bred every 12 h throughout estrus. The volume of the inseminate should be 50 to 100 ml and contain of least  $4-5 \times 10^9$  live spermatozoa.

### 1.4 Collection of embryos

Swine embryos are usually collected surgically 4 to 6 days after the onset of estrus. They are mostly at the 4 to 8 cell stage and expanded unhatched blastocyst stage respectively. At 4 days after the onset of estrus 4 to 8 cell embryos are easily identified and evaluated. The recovery rate of embryos were usually high 94-100% (n = 320 donor gilts) (Polge, 1982), 85-100% (Chantaraprateep *et al.*, 1987 a), 80-90% (Techakumphu *et al.*, 1987).

The techniques used is a mid-ventral laparotomy under general anaesthesia and the genital tract is exposed through a small incision. Ovulation in pigs occurs around 36-40 h after the onset of estrus and since the eggs remain in the oviducts for less than 48 h following ovulation and enter the uterus at the 4 cell stage, recovery from the uterine horn is practiced (Polge, 1982). Warm flushing medium (PBS) at 37° C is introduced from the fimbriated end of the oviduct or via oviduct with a blunt 12-14 gauge needle near the conjunction of each horn. The fluid about 20-50 ml is gently massaged along the horn and collected through a canula or a Foley catheter No. 12 inserted into the lumen via a small longitudinal incision. Embryos can be collected from the proximal one third of each horn. It is necessary to avoid bleeding during the operation in order to reduce adhesion and allows donor animals to be re-used again.

Donor can be re-used as shown by Chantaraprateep *et al.*, (1987b) 3 operations during 7 months period.

### 1.5 Handling embryos

The flushings are examined for embryos with a stereomicroscope at 10 × magnification and evaluation of embryos at 40 ×. As embryos are located they are transferred to culture plates. After several rinses, they are stored until transferred to the recipient. The embryos should not be chilled below 15° C.

Short-term storage of embryos can be made through flushing medium added with 15% bovine serum albumin (Whittingham, 1971) for several hours as shown by Cameron *et al.*, (1986) and Techakumphu *et al.* (1988) for conducting embryo transfer of pig between different farms.

### 1.6 Embryo transfer

Embryo is transferred surgically in a small volume of fluid to the anterior one fourth of one uterine horn only as they will migrate throughout the uterus (Dziuk *et al.*, 1964 ; Dziuk, 1985). A minimum of 4 embryos is required in order to avoid luteolysis around day 14-15 of the cycle (Polge *et al.*, 1966) gilt recipients seem to be better than those old age sows in term of farrowing rate 36.4% (n = 3) vs 11.8% (n = 4) as shown by Techakumphu *et al.* (1987).

## 2. Factors affecting results

The pregnancy rate is reduced when transfer are made to recipients which come in heat before the donors. There was no drop in pregnancy rate following transfers to recipients which come on heat 1-2 days after the donors (Polge, 1982). This phenomenon indicates that a greater degree of asynchrony between donor and recipients can be flexible in the pig than in cattle or sheep (Rowson *et al.*, 1969).

## 3. Certain areas to be more investigated

### *Preservation of embryos*

Simple media (PBS + 20% sheep serum) will support early cleavage at 37° C from single cell fertilized eggs to the 4 cell stage. Viability of embryos cultures for 1 day is good while 48 h culture results in poor survival.

Pig embryos are sensitive to cooling and few embryos survive following a reduction of temperature to below 15° C (Polge *et al.*, 1974).

Investigation on culture of pig embryos and in vitro fertilization transferring them to recipients as well as embryos manipulation and preservation are required in order to extend the possibilities of practical application of embryo transfer.

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