TRANSFER OF PREIMPLANTATION PIG EMBRYOS FOLLOWING IN VITRO CULTURE FOR 24 OR 48 HOURS

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SUMMARY
Two experiments were conducted to determine the viability of 258 pig ova cultured in vitro for 24 or 48 hr by transfer to 19 recipient gilts. One- to four-cell ova were recovered from donor gilts and cultured in droplets of Brinster’s medium under oil. A humidified gas mixture of 5% CO₂ and 95% air flowed through the incubator which was maintained at 37 C.

In Experiment I, a total of 69 ova was transferred to six unmated gilts after a 24 hr in vitro culture period. At recovery, 63 ova were at the two-cell stage, and at transfer 59 were at the four-cell stage. Five of six (83%) recipient gilts had an average of 7.6 (63%) normal embryos at slaughter on days 26 to 33 post-estrus.

In Experiment II, a total of 189 ova were transferred to 13 unmated gilts following a 48 hr in vitro culture period. Fifty-three percent of the ova were recovered at the two-cell stage and the remainder were recovered either as one- (21%) or four-cell (26%) ova. After 48 hr in culture 77% of the ova were transferred at the four- or six-cell stage of development. Two gilts (15%) were pregnant and slaughtered 28 and 34 days post-estrus. Fifteen of 29 (52%) ova transferred to the two pregnant recipients were present as normal embryos. The reduced pregnancy rate in Experiment II may have been due to factors other than the increased length of the culture period. During the course of Experiment I, 12 of 28 ova maintained in culture from the same donors as those which were transferred developed to the blastocyst stage after 96 to 120 hr in culture. None of the 97 ova cultivated for 72 additional hr after Experiment II ova were transferred reached the blastocyst stage.

(KEY WORDS: Swine, Reproduction, Embryos, In Vitro Culture.)

INTRODUCTION
A reliable method for the in vitro culture of preimplantation embryos of domestic animals is important not only for the knowledge to be gained about early embryonic development, but also for the potential economic value from practical application. Tervit et al. (1972) successfully cultured cattle ova to the blastocyst stage in a synthetic oviduct fluid (SOF) medium after recovery at the eight-cell stage. Development continued to day 35 of pregnancy following transfer of the cultured ova. More recently, Tervit and Rowson (1974) cultured sheep ova recovered at the early cleavage stages for 6 days in SOF medium and obtained the birth of live lambs following transfer.

Fewer reports are available on the in vitro culture and transfer of pig ova. Rundell and Vincent (1968) cultured pig ova in various media, but no pregnancies resulted following transfer to recipient gilts. Schneider et al. (1975) reported that four- to eight-cell pig ova, but not earlier cleavage stages, would develop to the blastocyst stage in SOF medium; however, the ova were not transferred after culture.

The purpose of the present study was to determine the viability of pig ova recovered at the one- to four-cell stage following an in vitro culture period of 24 or 48 hr by transfer to non-mated recipient gilts.

EXPERIMENTAL PROCEDURE
Ova Recovery and Culture. Twenty-four crossbred (Hampshire × Duroc × Yorkshire) gilts that ranged from 7 to 10 months of age were used as donors of ova for culture and transfer. Nine donor gilts were injected with 1,000 IU of pregnant mare serum gonadotropin (PMSG) on day 16 of the estrous cycle (day 0 = first day of estrus). All gilts were checked for...
estrus once daily using a mature boar, and donor gilts were bred, usually to a different boar, each day during estrus. Three boars were used in Experiment I and seven different boars were used in Experiment II. Ova were recovered from donor gilts at approximately 48 to 72 hr (day 2) after the onset of estrus. The procedure used for recovery and transfer of tubal ova has been described in an earlier report (Pope et al., 1972). Location and manipulation of recovered ova were conducted with a stereomicroscope enclosed in a plexiglass hood, the interior of which had been previously sterilized by ultraviolet light. An air curtain incubator maintained the temperature in the hood within the range of 30 to 37 C. A small constant temperature incubator was also located in the hood for holding ova during the interval between recovery and in vitro culture or transfer to recipient gilts. Ova recovered at the one-, two- or four-cell stage of development were deposited in .1 ml droplets of culture medium under paraffin or silicone oil (Dow Corning Dielectric Fluid 200) contained in a 15 x 60 mm sterile plastic culture dish (Falcon #1007). One to four droplets of medium were placed in each dish with one to 12 ova at a particular cell stage from one donor in each droplet. The paraffin or silicone oil had been sterilized, equilibrated with the culture medium and gassed with 5% CO₂ and 95% air prior to use. The culture medium described by Brinster (1963) was used with the addition of 1 mg of glucose/milliliter.

The medium was sterilized by filtration, gassed with 5% CO₂ in air and, if not used immediately, stored at 4 C in tightly stoppered bottles until needed 1 to 6 days later.

Ova were cultured in a water-jacketed CO₂ incubator (National, Model 3321) maintained at a temperature of 37 ± .5 C. A humidified gas mixture of 95% air and 5% CO₂ flowed at a rate of 4.0 and .2 liter per min, respectively, through a 250 mm I.D. glass dessicator which held the culture dishes. Ova were observed at 24-hr intervals during the culture period to estimate cleavage rate.

Experiment I. A total of 69 ova recovered from eight donor gilts was transferred after 24 hr in culture to the oviducts of six unmated, crossbred recipient gilts. Onset of estrus in four recipients was synchronous with the donor gilts while the other two recipients came into estrus 1 day later than the respective donor gilt. Ova were selected for transfer on the basis of their cleavage rate during the 48-hr culture period. Recipients that did not return to estrus following transfer were slaughtered at 28 to 34 days post-estrus and the number of embryos and/or corpora lutea were recorded.

RESULTS

Experiment I. The cell stages of cultured ova at the time of transfer are shown in table 1. Of the 69 transferred ova, 63 were recovered at the two-cell stage. Fifty-nine of the latter were at the four-cell stage and the remaining four ova recovered at the two-cell stage were at the six-
or eight-cell stage when transferred.

Table 2 summarizes the reproductive characteristics of recipient gilts at slaughter. Five of six (83%) recipient gilts were pregnant. The five pregnant gilts had received 11 to 13 cultured ova, while the remaining gilt had been the recipient of nine ova. The average number of normal embryos per pregnant recipient was 7.6 which represents an embryo survival rate of 63%. One of the two recipients which exhibited estrus 1 day after the respective donor gilt was pregnant and the other recipient, although non-pregnant, failed to show estrus prior to slaughter on day 27 after the last estrus. The ovaries of the non-pregnant gilt had several 8 mm follicles and the uterus appeared to be under an estrogenic influence.

The mean number of total embryos per pregnant recipient was 8.6. Thus, 72% of the ova transferred to gilts pregnant at slaughter or 62% of all embryos transferred were represented by either a normal or degenerate embryo.

**Experiment II.** The cell stages at recovery and at transfer for ova cultured in vitro for 48 hr are illustrated in Table 3. Approximately one-half of the ova were recovered at the two-cell stage. The remainder were recovered either as one- (21%) or four-cell (26%) ova. When transferred 48 hr after recovery, most of the cultured ova were at the four- (34%) or six-cell (42%) stage.

The majority (72%) of the one-cell ova had developed to the six- to eight-cell stage after 48-hr culture, while only one-half (48%) of two-cell ova at recovery had reached the same cleavage stage.

The reproductive characteristics of recipient gilts following transfer of ova cultured 48 hr are summarized in Table 4. Eleven of 13 recipients returned to estrus from 18 to 27 days ($X = 20.6$) after the previous estrus. Eight of the 11 gilts exhibited estrous cycles ranging from 18 to 21 days ($X = 19.0$) while the other three gilts returned to estrus in 24 to 27 days.

### Table 2. Reproductive Characteristics of Recipient Gilts at Slaughter

<table>
<thead>
<tr>
<th>Gilt no.</th>
<th>Onset of estrus(^a)</th>
<th>No. of ova transferred</th>
<th>No. of total embryos</th>
<th>No. of normal embryos</th>
<th>Embryo survival, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-1</td>
<td>0</td>
<td>12</td>
<td>9</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>8-3</td>
<td>0</td>
<td>13</td>
<td>7</td>
<td>7</td>
<td>54</td>
</tr>
<tr>
<td>54-1</td>
<td>0</td>
<td>11</td>
<td>10</td>
<td>10</td>
<td>91</td>
</tr>
<tr>
<td>29-9</td>
<td>0</td>
<td>11</td>
<td>8</td>
<td>8</td>
<td>73</td>
</tr>
<tr>
<td>34-2</td>
<td>+1</td>
<td>13</td>
<td>9</td>
<td>7</td>
<td>54</td>
</tr>
<tr>
<td>41-1</td>
<td>+1</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Average(^b)</td>
<td>11.5</td>
<td>8.6</td>
<td>7.6</td>
<td>66</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Onset of estrus occurred on the same day in both the donor and recipient. \(^b\) Onset of estrus occurred one day later in the recipient than in the donor.

**Includes pregnant recipients only.**

### Table 3. Cell Stages of Transferred Ova at Recovery and After 48 HR in Culture

<table>
<thead>
<tr>
<th>Cell stage at recovery</th>
<th>No. of ova</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>&gt;8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39</td>
<td>11</td>
<td>16</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>52</td>
<td>42</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>2</td>
<td>22</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>189</td>
<td>65</td>
<td>80</td>
<td>34</td>
<td>10</td>
</tr>
</tbody>
</table>
Viable embryos were present in two recipients at slaughter for a pregnancy rate of 15%. Of 29 cultured ova transferred to these two recipients, 15 (52%) were present as normal embryos with an additional two degenerate embryos in one pregnant recipient.

**Discussion**

The survival rate of cultured pig embryos in Experiment I is similar to that reported in other studies where the transfer was made immediately following recovery from the donor gilt (Webel et al., 1970; Pope et al., 1972). Also of significance is the fact that the ova in Experiment I were transferred to the oviducts of recipient gilts at a time when “native” ova would have already been transported through the oviducts into the uterus. The embryo survival rate following these transfers is in contrast to the low survival rate obtained when pig ova were recovered from the oviducts of the donor and transferred immediately to the uterus of the usually synchronous recipient gilt (Bazer et al., 1969).

In Experiment II, onset of estrus in the recipient gilt with seven normal embryos occurred on the same day as that of the donor gilt. Although the other pregnant recipient had shown estrus 2 days after the donor gilt, the donor was in estrus for 3 days so the time of ovulation was possibly not more than 1 day later in the recipient than in the donor. Due to limited results in Experiment II, it is difficult to evaluate the optimal relationship between stage of the estrous cycle of the recipients and developmental stage of cultured embryos. Webel et al. (1970) reported that transfer of pig embryos immediately after recovery to recipients that had received human chorionic gonadotropin (HCG) 1 or 2 days earlier than the donor were as successful as transfers between gilts which had been given HCG on the same day.

The results of Experiment I and II suggest that under the culture conditions of this study, most of the embryos maintained their viability and, hence, developmental potential during the first 24 hr in culture. However, during the period between 24 and 48 hr after recovery, in vitro conditions were not capable of maintaining the viability of the majority of the embryos since only 8% of the transferred ova survived.

When some of the recovered embryos were maintained in culture and others collected from the same donor were transferred, the ova from donors used in Experiment I showed a much greater rate of in vitro development after 96 to 120 hr in culture than did those from donors in Experiment II. The most obvious difference between these two groups of ova was the percentage which developed to the blastocyst stage during culture. Ova maintained in culture after
the other ova from the same donors were transferred at the 24-hr period showed a rate of blastocyst formation of 43% (12 of 28). Of 97 ova maintained in culture from gilts in which some ova were transferred after 48 hr in culture, none reached the blastocyst stage (C. E. Pope, unpublished data).

Since success had been obtained by transferring cultured ova at the four-cell stage to the oviducts of recipient gilts in Experiment I, ova at the four- to eight-cell stage were transferred to the oviducts of three recipient gilts that had shown estrus 1 day later than the donor gilt in Experiment II. All of the gilts in Experiment II in which tubal transfers were performed returned to estrus 19 days after the previous estrus. Recipient gilts in Experiment II in which ova were transferred to the oviduct after 48 hr in culture were at the same stage of the estrous cycle (day 3) as those gilts in which ova were transferred to the oviducts after culture for 24 hr (Experiment I). Thus, it seems that failure to maintain pregnancy in gilts in Experiment II when ova were placed in the oviducts was probably not due to failure of the ova to be transported to the uterus.

LITERATURE CITED