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Survival of Porcine Embryos after Asynchronous Transfer[1–3] (41495)

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Abstract. Forty gilts and sows were used to transfer Day 5 embryos into one uterine horn, while Day 7 embryos were transferred into the other horn, of Day 6 nonpregnant recipients (Day 0 = first day of estrus). The survival of the transferred embryos was determined on Day 11 (Expt 1) and Days 60 to 70 (Expt 2). The percentage of Day 5 and 7 embryos surviving the transfer procedures on Day 11 was not different, 42 ± 10 and 43 ± 12, respectively. However, by midgestation (Day 60) more ($P < 0.001$) fetuses that developed from Day 7 embryos survived than fetuses that developed from Day 5 embryos, 63 ± 8 and 8 ± 7%, respectively. These experiments indicated that the presence of embryos more advanced in development caused the demise of younger embryos sometime between Days 11 and 60 of gestation.

Considerable variation exists in embryonic development within several polytocous species. This is not surprising because in pigs ovulation extends for a 6-hr period (1, 2). Accordingly, the first cleavage division of the fertilized ova occurs between 60 and 108 hr after the onset of estrus in sows (3). Anderson (4) noted marked variation in the morphology of porcine embryos between Days 11 and 13 of gestation. Within a uterine horn these embryos ranged in development from spherical and tubular to filamentous. Embryo survival was not altered when embryos were transferred 1 day from synchrony with the recipient (5). However, the biological significance of this normally occurring variation in embryonic development remains unclear.

Pigs naturally lose 40% of their embryos during gestation. Several physiological events, important for survival of the porcine embryo, are closely associated with morphological changes of the embryo. These events include estrogen synthesis (6), luteal maintenance (7), and transuterine migration of embryos (8). The possibility exists that embryos advanced in their development relative to their litter mates have a survival advantage.

The present experiment was conducted to determine if embryos more advanced in development migrated further within the uterine horns and had a greater chance for survival during pregnancy.

Materials and Methods. *Expt 1.* Sixteen cross-bred gilts and sows, checked daily for estrous behavior, were utilized in this experiment. Recipients, 6 days after the onset of estrus, received four to six embryos each from Day 5 and 7 donors (Day 0 = first day of estrus). Such a procedure allowed establishment of pregnancy with embryos 2 days apart in age but only 1 day from synchrony with the recipient. The uterine horn to which the embryos, within an age, were introduced was randomized. Gilts assigned on the appropriate days as donors were mated 4 and 24 hr after the onset of estrus. To maximize utilization of females, two recipients were used for each Day 5 and 7 donor. On Day 11, 5 days post-transfer, the recipients were slaughtered and the em-

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bryos recovered by flushing segments (10 to 20 cm) of the excised uterus with physiological saline. Age (Day 10 or 12) and location of the embryos and the length of uterine segments were recorded. The age of the recovered embryos was determined by morphology (spherical vs tubular or oblong) and size. Recovered embryos classified as originating from Day 5 donors, for example, were all spherically shaped, measuring 1 to 2 mm in diameter. Those identified as originating from Day 7 donors were spherical to tubular in shape, ranging from 4 to 15 mm in length. Only those paired recipients with recoverable embryos from each donor, as determined on Day 11 of gestation, were included in the statistical analysis.

The distance the embryos migrated (cm) was determined as follows: distance = {(sum of the length of uterine segments traversed by each embryo) − (the total number of Day 5 or 7 embryos transferred) × [the length of the anterior uterine segment (10 to 20 cm) containing the respective embryos]}. It was necessary to subtract the length of the anterior segments because the embryos were located in these segments before their migration. Distance the Day 5 or 7 embryos migrated was compared by using a least-squares analysis of variance.

Expt. 2. Twenty-four [eight purebred colored (Duroc, Spotted or Hampshire), eight purebred white (Yorkshire, Landrace or Large White) and eight crossbred] gilts and sows were utilized in this experiment. Day 6 recipients received embryos in accordance with procedures described in Expt 1 except donors were mated to colored or white boars. One recipient was used for each Day 5 and 7 donor. Attempts were made to balance the number of Day 5 and 7 embryos transferred into each recipient (10.9 ± 0.9 Day 5 and 10.4 ± 1.0 Day 7 embryos, $\bar{x}$ ± SE). Breed of donor (colored vs white) was randomized such that four Day 5 and four Day 7 colored donors were utilized. Fetuses were recovered between Days 60 and 70 of gestation, identified by skin pigmentation, weighed, and the distance between adjacent fetuses was noted. One gilt aborted on Day 60, in which case, all fetuses were recovered immediately and skin pigmentation was noted. Fetal weight was subsequently correlated with the distance between adjacent fetuses. The percentage of Day 5 and 7 embryos surviving to Day 11 (Expt 1) and 60 of gestation was compared by a nonparametric Mann–Whitney *U* test.

To confirm that Day 5 embryos can survive in Day 6 recipients in the absence of Day 7 embryos, 10 additional recipients received only Day 5 embryos (8.8 ± 0.7 embryos per uterine horn). Comparisons of the percentage of Day 5 and 7 embryos surviving to Day 60 (Expt 2) to the percentage of Day 5 embryos surviving to Day 60 alone were conducted by use of a nonparametric Mann–Whitney *U* test and unpaired *t* test, respectively.

Embryo manipulation. Embryos were collected surgically from donors by flushing the anterior half of each uterine horn toward a catheter (medical grade Teflon tubing, 1.50-mm i.d., 2.11-mm o.d.) located 1 to 2 cm posterior to the uterotubal junction. The flushing medium (Table I) was similar to that utilized by Davis and Day (9) except lactate and pyruvate were deleted; antibiotic–antimycotic was substituted for penicillin G and streptomycin, and sodium chloride increased to maintain physiological osmolarity. Recovered embryos were incubated (39°, 95% air–5% CO_2) for not more than 30 min before being transferred. This transfer procedure consisted of effluxing the embryos and medium (100 to 200 μl) into the uterine lumen (2 to 3 cm posterior to the uterotubal junction) through a catheter (medical grade Teflon tubing, 0.69-mm i.d., 0.99-mm o.d.) temporarily inserted through the posterior 3 to 4 cm of the oviduct. Only morphologically normal embryos were utilized. Transferable Day 5 embryos ranged from late compacted morula to blastocysts with a small to medium blastocoel and a recognizable inner cell mass. The Day 7 embryos had an expanded blastocoel and a comparably flattened inner cell mass and thinner zona pellucida.

Results and Discussion. Results of Expt 1 (Table II) indicated no difference in the ability of Day 5 and 7 embryos to survive

TABLE I. MODIFIED KREBS–RINGER BICARBONATE[a]

Ingredient	g/500 ml	m*M*
NaCl	3.500	119.78
KCl	0.178	4.78
$CaCl_2 \cdot 2H_2O$	0.125	1.71
KH_2PO_4	0.081	1.19
$MgSO_4 \cdot 7H_2O$	0.147	1.19
$NaHCO_3$	1.053	25.00
Glucose	0.500	5.56
Bovine serum albumin[b]	2.000	
Antibiotic–antimycotic[c]	50,000 units/5 ml	

[a] Davis and Day (1978).
[b] Pentex bovine albumin crystallized, Miles Laboratories.
[c] Antibiotic–antimycotic, lyophilized, Gibco Laboratories.

for 5 days after transfer. However, by midgestation (Day 60) more fetuses that developed from Day 7 embryos (Expt 2; Table II) survived than fetuses that developed from Day 5 embryos ($P < 0.001$). At Day 60 only two recipients had fetuses that developed from transferred Day 5 embryos. Only females that remained pregnant to Day 60 were included in the data of Expt 2. This increased the proportion of Day 7 embryos recovered at Day 60 as compared with Day 11 because the percentage of all transferred Day 5 vs Day 7 embryos surviving to Days 11 and 60 was 34 vs 44 and 4 vs 36, respectively.

More ($P < 0.01$) Day 5 embryos survived to Day 60 in the absence than in the presence (42 vs 8%) of Day 7 embryos (Table II). Webel *et al.* (5) observed an equivalent survival rate of embryos transferred one day from synchrony (49 to 53%). Alternatively, the percentage of Day 7 embryos surviving to Day 60 in the presence of Day 5 embryos was not different from the percentage of Day 5 embryos surviving to Day 60 alone (42 vs 63%, respectively). These observations confirm that each population of transferred embryos can survive alone but when forced to cohabit in the uterus, fewer younger embryos survived to Day 60.

Identification of embryos by size in Expt 1 was more subjective than skin pigmentation of the fetuses in Expt 2. Wright and Grammer (10) observed a 25-fold increase in protein content of the porcine embryos between Days 8 and 9 of gestation. This

TABLE II. PERCENTAGE SURVIVAL OF DAYS 5 AND 7 EMBRYOS TO DAYS 11 AND 60 OF GESTATION

			Day 5 embryos			Day 7 embryos		
	No. recipients utilized	No. recipients pregnant	No. embryos transferred	No. survived	Survival/ recipient (%)	No. embryos transferred	No. survived	Survival/ recipient (%)
Day 11	10	8[a]	45	19	42 ± 10[d]	42	18	43 ± 12[d]
Day 60	16	8[b]	87	6	8 ± 7[e]	83	53	63 ± 8[f]
Day 60	10	5[c]	88	35	42 ± 10			

[a] Two recipients were not included as 5 of the 10 transferred Day 7 embryos were recovered and none of the 11 Day 5 embryos.
[b] Eight recipients were not included after failing to maintain pregnancy following the introduction of a total of 78 Day 5 and 63 Day 7 embryos.
[c] Five recipients were not included after failing to maintain pregnancy following the introduction of a total of 89 Day 5 embryos.
[d,e,f] Means with different superscripts within rows are different ($P < 0.001$).

exponential growth of embryos continued between Days 9 and 16. Although considerable variation existed, spherical shaped Day 12 embryos were larger and contained fourfold more protein than spherical Day 10 embryos (4). Little difficulty was experienced in the present study in differentiating between the transferred Day 5 and 7 embryos on Day 11.

Transuterine migration of porcine embryos occurs between Days 7 and 12 (11). Recovered embryos 5 days post-transfer were mixed within both uterine horns as was previously observed with synchronous transfer (12). The older embryos (Day 12) failed to migrate further than the younger embryos (Day 10) when examined on Day 11, 160.1 ± 29.6 vs 113.5 ± 16.1 cm, respectively. However, the distance the embryos migrated may have been different if examined at an earlier time. Because only about 40% of the transferred embryos were viable on Day 11, differentiating the healthy from the dying embryos may have been difficult before this time.

Recovered fetuses ranged in weight from 60.1 to 274.5 g and crown–rump length from 7.5 to 19 cm. However, neither fetal weight nor length was associated with the age of the transferred embryo (172.7 ± 14.2 vs 157.0 ± 9.3 g and 14.5 ± 0.9 vs 13.9 ± 0.4 cm, Day 5 vs Day 7 embryos, respectively). After the variation in fetal weight between recipients was reduced by standardizing the weight of the heaviest fetus within each litter and then adjusting the weight of each remaining litter mates proportionately, the distance between adjacent fetuses was highly correlated with fetal weight ($r = 0.47$, $P < 0.01$). Rathnasabapathy *et al.* (13) observed a similar relationship with fetuses examined on Day 55 of gestation. Knight *et al.* (14) demonstrated a significant correlation of fetal weight to placental length suggesting a relationship between migration of the porcine embryo, outgrowth of the placenta, and the subsequent development of the fetus.

Although the majority of embryonic loss occurs by Day 30 (15–19) it is unknown when or why the young embryos (Day 5) died between Days 11 and 60 of gestation. Because the porcine embryo can elongate rapidly, 3 cm/hr (20), the possibility exists that older embryos (Day 7) elongated sooner and occupied more of the uterus than the younger (Day 5) embryos. Anderson (4) observed the inability of embryos to overlap each other regardless of the uterine space available. Knight *et al.* (14) observed an increase in mortality of crowded fetuses between Days 40 and 100 and suggested this was due to placental insufficiency. Perhaps in this experiment the younger embryos (Day 5) died because of placental insufficiency as a result of crowding by the older embryos (Day 7).

Another explanation for the loss of the younger embryos (transferred at Day 5) might include physiological advancement in the biochemical development of the recipient's uterus such that the younger embryos could no longer continue to develop. Beier *et al.* (21) and Adams (22) demonstrated the fragile relationship between synchronizing the pattern of uterine secretions and the age of successfully transferred rabbit embryos. Exogenous estrogen extends the length of the estrous cycle of nonpregnant pigs (23) possibly by altering secretion of uterine proteins (24), intrauterine sequestering of prostaglandin (25), and/or uterine blood flow (26). It is possible the older embryos (Day 7), by synthesizing estrogen earlier, advanced the secretory pattern(s) of the uterus resulting in the demise of the younger embryos (Day 5).

The precise mechanism by which some embryos survive and others die in polytocous species is unclear. These experiments indicated porcine embryos more embryologically advanced have a greater chance to survive and may have caused the demise of those less embryologically developed.

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