BRIEF COMMUNICATION

THE RECOVERY, TRANSFER AND SURVIVAL OF BLASTOCYSTS IN PIGS

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The technique of egg transplantation has been used in several species of laboratory animal to study factors involved in reproduction and growth; it has also been applied with considerable success to sheep. In the pig, most of the previous studies using egg transplantation have been principally concerned with the development of a satisfactory technique (Kvasnickii, 1951; Pomeroy, 1960; Hancock & Hovell, 1962; Vincent, Robison & Ulberg, 1964), but the method has been employed to study both migration of embryos (Dziuk, Polge & Rowson, 1964) and the effects of maternal environment on embryonic development (Smidt, 1965). In the above experiments, 2- to 8-celled eggs were transferred to recipients whose oestrous cycles were synchronized. The transplantation of embryos up to 12 days of age has been successful in sheep (Moor & Rowson, 1964, 1966), but transfer of pig eggs at stages later than 8-cells has, so far, not been reported. Since pig blastocysts also remain in the uterus for a relatively long period before implantation, the development of a technique for their transfer would permit further investigation of the physiology of early pregnancy.

Large White × Essex gilts, aged 6 to 8 months and weighing between 200 and 275 lb, were tested for oestrus once daily with a boar; those with cycles of 20 to 22 days were used in the experiment. Blastocyst donors were inseminated on the morning of the 2nd day of oestrus (Day 1) with 100 to 150 ml of fresh, undiluted semen collected from two fertile boars. Blastocysts were recovered in vivo on Days 7 to 9 by flushing the exposed tract with 40 ml of either untreated pig serum or Tyrode’s solution containing 1 mg of bovine plasma albumen/ml.

A mean of 10·2 blastocysts (range, 5 to 17) was transferred to each recipient using a Pasteur pipette. The pipette was introduced into the uterine lumen through a puncture wound made with a suturing needle about 10 cm from the utero-tubal junction, and the blastocysts were injected in 0·5 to 1·0 ml of fluid. During the interval between recovery and transfer, the fluid containing the blastocysts was maintained at 30 to 35°C in covered dishes. None of the transferred blastocysts (Pl. 1, Figs. 1 and 2) was stored in vitro for longer than 45 min. A number of recipients returned to oestrus; the remainder were slaughtered between Days 17 and 23.

Blastocysts were obtained from thirty-eight (90·4%) of the forty-two donors

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inseminated. Blastocysts were recovered on Days 7, 8 or 9, the mean percentage recovery for each group of donors being 75.3%, 77.1% and 81.3% respectively, assuming that each corpus luteum was represented by one blastocyst.

A total of 115 blastocysts recovered on Days 8 or 9 was transferred in serum to twelve recipients whose oestrous cycles were either synchronized or 1 day behind that of the donor. None of the recipients was pregnant at autopsy, nor were there any traces of embryos or membranous remains in the uteri of these animals. Corpora albicantia and large follicles (6 to 10 mm) were found on the ovaries, and the uterus in most instances was in an oedematous condition indicating the proximity of oestrus.

One hundred and seven blastocysts recovered on Day 7 were transferred in Tyrode’s solution to ten synchronous recipients; five of these returned to oestrus between Days 19 and 21, and the remaining five were slaughtered between Days 18 and 21. Three of these animals were pregnant, containing three, seven and eight embryos of normal appearance and size, which represented a survival of transferred blastocysts of 43%, 50% and 80% respectively. Following ten synchronous transfers of a total of 105 blastocysts on Day 8 in Tyrode’s solution, four recipients were found to be pregnant at autopsy on Days 21 and 23; the proportion of blastocysts surviving as normal embryos (Pl. 1, Fig. 3) varied from 20% to 33% in the individual animals. The corpora lutea in three of these pregnant animals were of 9 to 10 mm in diameter and of normal macroscopic appearance. However, in one animal which contained only one embryo in each uterine horn, there were two corpora albicantia of 5 mm diameter on the left ovary, whereas nine apparently normal corpora lutea were present on the right ovary. This situation therefore represented an instance of unilateral regression of the corpora lutea.

These results demonstrate that pregnancy can become established in unmated recipients to which blastocysts have been transferred on Days 7 or 8. However, the conditions under which blastocysts are recovered and transferred clearly require more investigation before the technique can be regarded as reliable and used as a basis for further studies of reproductive physiology in pigs.

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REFERENCES


Fig. 1. Pig blastocyst recovered from the uterus on Day 7. Note that the zona pellucida is still present and that it contains a large number of spermatozoa. × c. 260.

Fig. 2. Blastocysts recovered from the uterus on Day 8. The zona pellucida has been lost and the blastocyst has expanded considerably but has yet to undergo elongation. × c. 40.

Fig. 3. A normal 21-day embryo resulting from the synchronous transfer of blastocysts on Day 8 in Tyrode's solution. × c. 9.

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