DEVELOPMENTAL FAILURE OF SWINE EMBRYOS RESTRICTED TO THE OVIDUCAL ENVIRONMENT*

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Restriction of embryos to the oviducal environment (tube-locked embryos) does not prevent embryonic development to the early blastocyst stage in mice (Kirby, 1962; Orsini & McLaren, 1967), rabbits (Pincus & Kirsch, 1956; Adams, 1958), rats (Alden, 1942) and sheep (Wintenberger-Torres, 1956), though further development of the tube-locked embryos has not been observed and they degenerate (Adams, 1958; Kirby, 1962; Orsini & McLaren, 1967). Kirby (1962) observed that mouse embryos recovered from the uterus continued to develop when transplanted beneath the kidney capsule; when tube-locked embryos were treated similarly only the trophoblast continued to develop. Kirby (1962) suggested that a 'uterine factor' was required for the development of mouse embryos beyond the blastocyst stage. Recently, Krishnan & Daniel (1967) isolated a specific uterine protein, blastokinin, from rabbit uteri and demonstrated that blastokinin stimulated blastulation and blastocyst expansion *in vitro*. A similar protein, designated uteroglobin, has also been demonstrated in rabbit uterine secretions by Beier (1968) and Kirchner (1969). Uteroglobin has been demonstrated in rabbit embryo blastocoelic fluid between Days 3 and 9 of pregnancy (Hamana & Hafez, 1970). This study was designed to determine the extent to which tube-locked swine embryos developed.

A total of nineteen crossbred gilts were checked for oestrus twice daily at 12-hr intervals with intact boars. The gilts were mated at 12 and at 24 hr, and surgery was performed on each animal between 48 and 72 hr, after the onset of oestrus. One oviduct was ligated at the uterotubal junction. The contralateral oviduct and uterine horn served as the control except that the uterus was ligated approximately 10 cm posterior to the uterotubal junction in ten of the gilts. The females were killed 4 to 8 days *post coitum*. Embryos were recovered from the uteri and oviducts by flushing with a physiological saline solution and were examined under a stereomicroscope to assess the stage of development. The diameter of embryos recovered from seven animals killed either 7 or 8 days *post coitum* was determined with an eyepiece micrometer. Gilts with no fertilized ova were eliminated from the experiment. Data were analysed by chi-square and the Student's *t* test (Steel & Torrie, 1960).

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TABLE 1
COMPARISON OF DEVELOPMENT OF TUBE-LOCKED VERSUS UTERINE EMBRYOS IN GILTS

Days post coitum	Site of recovery	No. of oviducts or uteri	No. of corpora lutea	No. of embryos recovered	Embryo recovery (%)	Stage of embryonic development	Average embryo diameter (mm)
4*	Uterus	2	13	9	69	4- to 32-cell	
	Oviduct	2	19	13	68	4- to 32-cell	
5*	Uterus	1	11	11	100	Blastocysts	
	Oviduct	1	7	4	71	Fragmented	
7*	Uterus	2	11	7	64	Blastocysts	
	Oviduct	2	14	3	21	Fragmented	
7†	Uterus	4‡	26	13	50	Blastocysts	0.22
	Oviduct	2	14	6	43	Fragmented, 4-cell to morula	0.18
8†	Uterus	5‡	31	24	77	Blastocysts	0.60
	Oviduct	3	19	10	63	Fragmented, 4-cell to morula	0.17

* The uterine horn contralateral to the ligated oviduct was not ligated and no measurements relative to diameter of the embryos were made for these animals.

† The uterine horn contralateral to the ligated oviduct was ligated about 10 cm posterior to the uterotubal junction and the diameter of each recovered embryo was determined.

‡ Includes one animal in which the oviduct ligation did not prevent passage of embryos into the uterus and all of the embryos developed to the blastocyst stage.

PLATE 1

FIG. 1. A tube-locked embryo recovered from Gilt 324 at 7 days *post coitum* $\times 150$.

FIG. 2. A uterine blastocyst recovered from Gilt 324 at 7 days *post coitum* $\times 150$.

FIG. 3. A tube-locked embryo recovered from Gilt 331 at 7 days *post coitum* $\times 150$.

The data are summarized in Table 1. Embryos were recovered from twelve of the nineteen gilts with an overall recovery rate of 61%. Recovery of tube-locked embryos (50.7%) tended to be less than recovery of uterine embryos (69.9%) but this difference was not statistically significant. The tendency suggests that many of the tube-locked embryos may have already disintegrated by the time of recovery, particularly by 5 days *post coitum*.

Tube-locked and uterine embryos were in a similar stage of development (four- to thirty-two-cells) at 4 days *post coitum*, implying that the oviducal environment is adequate for development of the pig embryo to the morula stage. However, pig embryos normally enter the uterus about 72 hr after the onset of oestrus (Pomeroy, 1955) in the three- to four-cell stage of cleavage with only a small percentage beyond the eight-cell stage (Oxenreider & Day, 1965). The blastocyst stage of development is reached between Days 5 and 6 *post coitum* (Perry & Rowlands, 1962). In this experiment, the uterine embryos had undergone blastulation by 5 days *post coitum* while the tube-locked embryos had begun to fragment. By 7 and 8 days *post coitum*, the difference between uterine and tube-locked embryos was striking. The tube-locked embryos were mostly fragmented and all remained within the zona pellucida while 83.8% of the recovered uterine blastocysts had emerged from the zona pellucida, formed a distinct inner cell mass and had expanded to about three times the diameter of the zona pellucida (Plate 1). This difference in size was highly significant ($P < 0.001$).

Since no blastocysts were recovered from ligated oviducts, these data suggest that pig embryos must reach the uterine environment in order for blastulation to occur. Experiments are now in progress to determine whether the uterine endometrium of swine secretes a blastokinin-like protein(s) which is required for continued development of the pig embryo.

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