EMBRYONIC MORTALITY AND UTERINE INFECTION IN THE PIG

A. M. SCOFIELD,* F. G. CLEGG† AND G. E. LAMMING

*Department of Physiology and Environmental Studies,
University of Nottingham School of Agriculture, and*

*†Veterinary Investigation Centre, Ministry of Agriculture, Fisheries and Food,
Sutton Bonington, Loughborough, Leicestershire*

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Summary. Thirty-nine gilts were slaughtered 9 and 13 days *post coitum* (*p.c.*) to determine if the period of elongation and first attachment of the blastocyst to the uterus was critical in embryonic survival. The bacterial flora of the uteri of thirty-eight pigs were cultured to determine the relationship between bacterial infection and embryonic mortality at these times.

The embryonic loss in fifteen pregnant pigs killed 13 days *p.c.* was significantly greater than that in fifteen pregnant pigs killed 9 days *p.c.* Six pigs slaughtered 13 days *p.c.* and three 9 days *p.c.* were not pregnant. No abnormal embryos were found in the 9-day group but in nine pigs in the 13-day group, embryos or embryonic membranes tangled in mucus were recovered from the cervical end of the uterine horns. Most of the embryonic discs appeared normal but there was evidence that degeneration of embryos and their membranes occurred within the mucus. Leucocytic invasion of the mass appeared to be associated with advanced degeneration.

Uterine bacterial flora were compatible with pregnancy. There was, however, a significant difference in the embryonic loss between pigs with sterile and infected uteri at both 9 and 13 days *p.c.*, the difference being greater in the 13-day group. The findings suggest that infection of the uterus may be a major factor in the loss of embryos during early pregnancy and may result in a large proportion of infected pigs returning to service.

INTRODUCTION

At birth, prenatal mortality in the pig may approach 50% of the ova ovulated (Hanly, 1961). Most of this loss occurs by 25 days *post coitum* (*p.c.*), a stage commonly used by workers investigating prenatal mortality in the pig. Few attempts have been made to determine if any period during this first month is particularly critical for embryonic survival although such studies must be important if the factors associated with embryonic loss are to be understood

* Present address: Department of Biological Sciences, Wye College (University of London), nr Ashford, Kent.

and a rational approach to the problem implemented. Perry & Rowlands (1962) studied many stages of early pregnancy and their results suggested that most of the mortality occurred before 13 to 18 days *p.c.*, possibly between the 9th and 13th days, but conclusive evidence was lacking. Phillippo (1967) also concluded that most prenatal losses had occurred by the 13th day. In the present study, the period between 9 and 13 days *p.c.* (the time at which elongation and first attachment of the blastocyst to the uterine wall occurs) was studied. Routine bacteriological examinations were made of the uteri to determine the influence of bacterial flora on pregnancy.

MATERIALS AND METHODS

Animals

Virgin offspring of matings of Landrace boars to Large White $\times$ Landrace sows were mated with Landrace boars naturally on the first day of oestrus, or artificially on the day after oestrus was detected by daily testing with a vasectomized boar. Generally, a single mating was made at the third oestrus after puberty or the first oestrus after the pigs had reached 113 kg body weight if they had failed to cycle previously.

A standard complete sow diet was fed at the rate of 1.8 kg/pig/day. The animals were slaughtered 9 or 13 days *p.c.* The reproductive tract was removed about 20 min after death.

Bacteriological examination

Samples for bacteriological examination were taken from six uterine sites, i.e. from the centre and from the ovarian and cervical ends of each uterine horn. Bowel clamps were placed on the uterus to isolate a 5-cm length at the site. The surface between the clamps was seared with a hot spatula, a sterile Pasteur pipette filled with sterile peptone water was introduced through the wall and the contents were evacuated into the uterine lumen. The uterus was manipulated manually to spread the fluid and after 10 to 15 sec, some was removed with the pipette and transferred to the original bottle of peptone water. The procedure was repeated at the other sites. Aerobic cultures were made, using 5% sheep blood agar and McConkey plates incubated at 37°C.

The results of the bacteriological examinations were classified as follows: (1) pigs with 'sterile' uteri had negative cultures at five or six sites; (2) pigs with 'infected' uteri were those with at least four sites yielding positive cultures. The presence of one or two sterile sites in individual cases of infected pigs may be accounted for by the failure of the pipette to puncture the endometrium with the consequence that the sampling fluid was evacuated into the sub-epithelial stroma; (3) pigs with 'partially infected' uteri were those with three or four sites yielding negative cultures.

Examination of the reproductive tract

Except for minor modifications, the techniques used were those described by Perry & Rowlands (1962). No attempt was made to identify 'non-embryonated trophoblasts' in 13-day embryos (Perry, 1961; Phillippo, 1967) and the number

of embryonic discs recovered was recorded as the number of embryos present. The ovulation rate was determined by counting the number of corpora lutea on the ovaries (Cooper & Scofield, 1969). The embryos and samples of luteal tissue and uterus were examined histologically by standard techniques (Scofield, 1969).

RESULTS

Extent of embryonic mortality

The data on the reproductive performance of thirty-nine pigs slaughtered 9 and 13 days *p.c.* are summarized in Table 1. Five of the pigs pregnant 13 days *p.c.* had four embryos, or less. If, as suggested by Polge, Rowson & Chang (1966), five embryos are needed for the maintenance of early pregnancy, then these pigs would probably have returned to service. Without them, and assuming no further loss in the other pigs, the embryonic mortality would have been 38.7% at a later stage, had these pigs been allowed to continue pregnancy.

The difference in the number of ova lost between the groups of pregnant pigs killed 9 and 13 days *p.c.* was analysed by covariance analysis (Snedecor, 1956) to correct for any possible correlation with the ovulation rate. The analysis of the corrected means indicated that the loss of ova in the 13-day group was significantly greater than the loss in the 9-day group ($P < 0.01$). There was no significant correlation between the number of ova lost and the ovulation rate, and for subsequent calculations using these data the number of ova lost was not corrected for ovulation rate.

The average size of forty-seven gilt litters available from the breeding herd records was 9.21 ± 0.40 . The mean ovulation rate for the thirty-nine gilts slaughtered was 12.72 ± 0.35 . If the ovulation rate of the gilts in the herd was similar to the experimental animals, the embryonic loss by birth in those pigs which farrowed would have been 3.51 ova/pig (27.6%).

Examination of the reproductive tract and embryos

Microscopical examination showed no abnormalities in the corpora lutea or uteri of any of the pigs. All of the 165 embryos recovered 9 days *p.c.* appeared to be normal when examined under the dissecting microscope, despite variations in size within the same animal. At this stage, the blastocysts were either spherical with unfolded trophoblast or were just beginning to expand, so that they appeared oval. Forty-two embryos were sectioned and all appeared to be normally developed.

Apart from two pigs, from which large oval blastocysts were recovered, the embryos at 13 days *p.c.* were extremely elongated and possessed an embryonic disc in the form of a dome (Perry & Rowlands, 1962). In most cases, the amniotic folds were beginning to form around the dome and in a few cases, the neural groove was forming on the embryonic disc. Thirty-six macroscopically normal embryos were sectioned and all appeared to be normal when examined microscopically.

Abnormal embryos recovered 13 days *p.c.* included one embryo with a very small embryonic disc and one in which the trophoblast was tangled into a knot. From nine of the pregnant pigs, thirteen embryonic membranes (ten with

Table 1. Embryonic mortality in gilts at 9 and 13 days *post coitum*

	No. of pigs	Mean ovulation rate	Mean no. of normal embryos	Mean no. of abnormal embryos	Mean no. of embryos missing or abnormal	% loss of ova
9 days <i>p.c.</i>						
All pigs	18	13.61 ± 0.60	9.17 ± 1.23	4.44 ± 0.94	—	32.6
Pregnant pigs	15	14.00 ± 0.60	11.00 ± 0.88	3.00 ± 0.60	—	21.4
13 days <i>p.c.</i>						
All pigs	21	11.95 ± 0.33	4.19 ± 0.81	7.76 ± 0.71	0.57 ± 0.22	64.9
Pregnant pigs	15	12.33 ± 0.32	5.87 ± 0.79	6.46 ± 0.69	0.80 ± 0.30	52.4

Values are means ± S.E.M.

Table 2. Organisms isolated from cultures of the uterine contents of thirty-eight gilts at 9 and 13 days *post coitum*

Organism	No. of uteri infected at five or six sites†	No. of uteri with some sites infected	No. of sites† infected
Lancefield Group 'C' streptococci	—	1	1
<i>Corynebacterium pyogenes</i>	—	1	1
<i>Pasteurella septica</i>	1	—	6
<i>Escherichia coli</i> + α haemolytic* streptococci	2	1	2
<i>E. coli</i>	1	6	14
<i>Staphylococcus albus</i>	2	10	18
α Haemolytic streptococci	—	2	2
Anthracoïds, paracolonic organisms and Chromobacteria spp.	—	4	5

* Occurred together at each site. Not included under separate headings.

† Any other organisms found in these cases are included in the other columns.

‡ These figures do not include sites from uteri completely infected with a single organism or from two pigs with 'sterile' uteri in which one site was culturally positive.

Table 3. The influence of uterine bacterial flora on the embryonic mortality in gilts pregnant at 9 and 13 days post coitum

Condition of uterus	9 days p.c.				13 days p.c.			
	No. of pigs	Mean ovulation rate	Mean no. of missing ova	% ova lost	No. of pigs	Mean ovulation rate	Mean no. of missing and abnormal ova	% ova lost
Sterile	8	14.25 ± 0.88	1.75 ± 0.17	12.3	7	12.57 ± 0.60	4.71 ± 0.56	37.5
Infected	5	14.60 ± 0.68	3.40 ± 0.89	23.8	6	12.00 ± 0.45	8.33 ± 1.05	69.4

Values are means ± S.E.M.

discs) were recovered from the cervical end of either or both horns tangled in mucus. Macroscopically, the embryos were normal and at a stage of development similar to other embryos from the same pigs. Of five such embryonic discs sectioned, three appeared normal and the other two showed disintegration of the disc ectoderm although the trophoblast, mesoderm and endoderm appeared to be normal.

From each of four animals, a mass of embryonic membranes entangled in mucus was sectioned. In two of the animals, the membranes appeared normal, although there was slight infiltration of the mucus with leucocytes in one case. The other two showed evidence of degeneration of the trophoblast with massive infiltration of the mucous mass with neutrophils.

Bacteriology of the uterus

Thirty-eight pigs were examined for uterine bacterial flora. Twenty-two (57.9%) had no infection of the uterus, twelve (31.6%) had 'infected' uteri and four (10.5%) had 'partially infected' uteri. The organisms isolated and the extent of their occurrence are shown in Table 2.

Data are presented in Table 3 relating the presence or absence of bacterial flora to the embryonic mortality in pigs that were pregnant at slaughter. The differences in the mean number of ova lost or abnormal between the sterile and infected groups at 9 days *p.c.* (3.40 and 1.75 ova) and 13 days *p.c.* (8.33 and 4.71 ova) were both significant ($P < 0.05$). The differences between the number of ova lost or abnormal between 9 and 13 days *p.c.* within sterile (1.75 and 4.71 ova) and infected groups (3.40 and 8.33) were both significant ($P < 0.001$ and $P < 0.01$, respectively).

DISCUSSION

Embryonic mortality

The loss of embryos in pregnant pigs at 9 days *p.c.* was 3.00 ± 0.60 /pig, representing 21.4% of ova produced. It is possible that 5 to 10% of the loss may have been due to fertilization failure (Hancock, 1962; Perry & Rowlands, 1962). Although some of the remaining loss may have been due to failure to recover all the embryos, a problem which has been discussed by Perry & Rowlands (1962), it is felt that the losses recorded, which are higher than in some other experiments (Wrathall, 1971), are partly due to the action of various environmental factors. Nevertheless, as all the pigs were kept under identical conditions, the large increase in embryonic loss between the two stages suggests that the intervening period is critical for the survival of the embryo and confirms the suggestions of other workers that the bulk of embryonic mortality in the pig occurs around the time of implantation (Perry & Rowlands, 1962; Phillipppo, 1967). The estimated embryonic loss in gilts that farrowed in the University herd, from which the experimental animals originated, was 27.6% and this, compared with the mortality at 13 days *p.c.* in the experimental animals, would support the findings of other workers that post-implantation losses in the pig are relatively small.

Abnormalities of the embryos

Approximately a quarter of the 9-day embryos and half of the 13-day embryos were sectioned. In all cases, the microscopic diagnosis of normality or otherwise confirmed the opinion which had been obtained by examination of the fresh embryo under the dissecting microscope. It is probable, therefore, that the classification given to the other embryos was correct. The failure to find abnormal embryos at 9 days *p.c.* is similar to the experience of Perry & Rowlands (1962).

Of the 100 13-day embryos recovered, twelve were considered abnormal and of these, ten were recovered tangled in mucus. All were at a stage of development which was similar to normal embryos recovered from the same pigs. This suggests that the embryo had only recently become trapped in mucus. It is unlikely that they would have continued to develop as the mucus would probably have prevented normal trophoblast expansion. In most of these cases, the embryos and their membranes appeared cytologically normal. In the two cases where the embryos were cytologically abnormal, only the embryonic disc was disintegrating. In two cases, the trophoblast was cytologically abnormal and there was invasion of the mucous mass by leucocytes. From the limited data, it appears that the embryonic disc was the first embryonic tissue to degenerate. Later, degeneration of the trophoblast was associated with invasion of the debris by leucocytes. As both normal embryos and embryos engulfed in mucus were present in the same uterus, it is possible that all the latter embryos were dead and the mucus had accumulated once their normal metabolism had ceased. Embryonic death may have occurred at any time after elongation of the blastocysts. As no abnormal embryos were found among the 165 examined 9 days *p.c.*, however, other embryos must have died before or during expansion and were not detected by the method of dissecting the uterus.

Uterine infection and embryonic loss

Of the fifty pregnant pigs examined, twenty-three (46%) showed growth on culture. Bacteria (the commonest were *Escherichia coli* and *Staphylococcus albus*) have been isolated from pig uteri in other studies (Hájovský & Gamčík, 1966; Nikolić, 1967). The findings in three gilts suggest that infection of the conceptus with pathogenic organisms may occur. Lancefield Group 'C' streptococci (found at one site) are normally isolated from adult pigs affected with a wide variety of disease lesions. *Corynebacterium pyogenes* (found at one site) is associated with suppurative lesions such as pneumonia and abscess formation and probably becomes established in debilitated hosts or damaged tissues. *Pasteurella septica* (found at six sites in one pig) is usually isolated from pneumonic lesions and also tends to become established following other disease processes but it is interesting to note that only two of the thirteen ova ovulated were missing in this pig at 9 days *p.c.* The presence of these pathogens in the uterus suggests that they enter not from the vascular system but rather from the boar's preputial fluid, which has a high bacterial content (Reed, 1969), or from the semen itself, which in many cases may be contaminated with bacteria (Waltz, Foley, Hershler, Tiffany & Liska, 1968). Alternatively, they may be introduced by the

penis or insemination catheter contaminated with vaginal or faecal flora (Evans, 1967).

In cows and rabbits, the resistance to uterine infection is greatest at oestrus (Black, Simon, McNutt & Casida, 1953; Black, Ulberg, Kidder, Simon, McNutt & Casida, 1953; Rowson, Lamming & Fry, 1953) and although no comparable work has been conducted on the pig, it seems probable that some bacteria are able to survive this phase and multiply in the relatively poor antibacterial conditions of the uterus that may accompany the progestational phase.

There is much uncertainty in the literature as to the importance of infections of the reproductive tract in embryonic mortality. Hájovský & Gamčík (1966) found no relation between the presence of uterine infection and embryonic mortality in eighty sows. Evans (1967) suggested that the presence of haemolytic staphylococci in the vagina of gilts may be associated with embryonic death. He found a lower conception rate and mean litter size in infected gilts than in gilts without the organisms, but an attempt to increase litter size in sows by vaginal antiseptics just before mating failed (Scofield, 1972).

Although, in the present work, it was obvious that the presence of a uterine infection was compatible with a pregnancy, there was a significantly greater embryonic loss in the pigs with infected uteri than in those with sterile uteri at both stages of pregnancy examined. The difference in the number of ova missing between sterile and infected groups was much greater in the case of pigs killed 13 days *p.c.* where a uterine infection was associated with about 40% of the loss of ova. In these studies, it was not possible to determine the effect of individual species of bacteria or the relative density of infections on embryonic mortality and further investigations along these lines may be useful.

Assuming that at least five embryos are necessary for the maintenance of pregnancy (Polge *et al.*, 1966), four of the six pigs with infected uteri 13 days *p.c.* would have returned to service if allowed to continue pregnancy, whereas only one of the sterile group would have returned. It is possible, therefore, that uterine infection is a cause of returns to service, which applies to about 20% of all sows served in commercial herds (Scofield & Penny, 1969). Although uterine infection may be a factor concerned with the natural selection of embryos during early pregnancy, other factors must be of importance as there was a significant increase in the embryonic mortality between 9 and 13 days in pigs with sterile uteri. These are probably genetic in nature (Bishop, 1964) and possibly related to the adaptation the embryo may have to make in its metabolism during the period of its rapid expansion. These considerations have been discussed in more detail elsewhere (Scofield, 1972).

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