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Adherence and fusion between the extremities of adjacent embryonic sacs in the pig

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While collecting foetuses from sows slaughtered after known durations of pregnancy, we noticed that with advancing gestation the extremities of adjacent embryonic sacs became increasingly difficult to separate. This observation is not a new one (see historical introduction by Lesbre, 1910) but considerable diversity of opinion still exists concerning the anatomy of the extremities and the relationship between adjacent embryonic sacs (see for example, the accounts of Bonnet (1891), Bourdelle (1921), Patten (1948), Bruni & Zimmerl (1951), Amoroso (1952), and Zietzschmann & Krölling (1955)). We examined sacs from the uteri of 37 sows killed at 15–109 d of pregnancy and subsequently sacs from four sows killed during the second half of pregnancy and from one sow during parturition. The material was examined in the fresh state, and examination was completed within eight h after removal from the sow.

RESULTS

(1) *The anatomy of the embryonic sac*

Towards the end of the first half of pregnancy (39–55 d) adjacent extremities of the embryonic sacs are often adherent but are easily separated. The surface of each sac is divisible into five zones (Fig. 1). The large *placental zone* is central, and has a velvety surface with transverse foldings and numerous areolae. This zone is bordered by two *paraplacental zones* characterized by smooth, glistening surfaces, distinctive longitudinal blood vessels, and almost complete absence of areolae. The extremities of the sac (the so-called 'necrotic tips') are sharply demarcated from the paraplacental zones by constrictions. At these constrictions, the blood vessels of the paraplacental zones lose their fresh red colour and become suddenly brown or colourless. These extremities are the *ischaemic zones*, which are often dry, shrivelled, and caked with dark brown, sticky material. With a little care, in such cases, these shrivelled, empty extremities can be expanded by 'milking' allantoic fluid into them.

At later stages of pregnancy (70 d onwards) separation of adjacent sacs becomes more difficult. The separated sacs resemble those earlier in pregnancy but there are important differences (see Fig. 2). The ischaemic zone often encroaches on the paraplacental zone, sometimes almost replacing it by a sharply demarcated ischaemic area in which remnants of blood vessels are plainly visible. Islands of ischaemic tissue may lie within the paraplacental zone. The 'necrotic tip', terminal in position in early pregnancy, often becomes subterminal or lateral in later pregnancy and the terminal part of the sac is then not necessarily ischaemic but may be part of the paraplacental zone. The 'necrotic tips' remain throughout pregnancy; they are

separable by careful dissection and their cavities can be refilled with allantoic fluid. The isolated extremities lying in the ovarian ends of the uterine horns show all the changes described above.

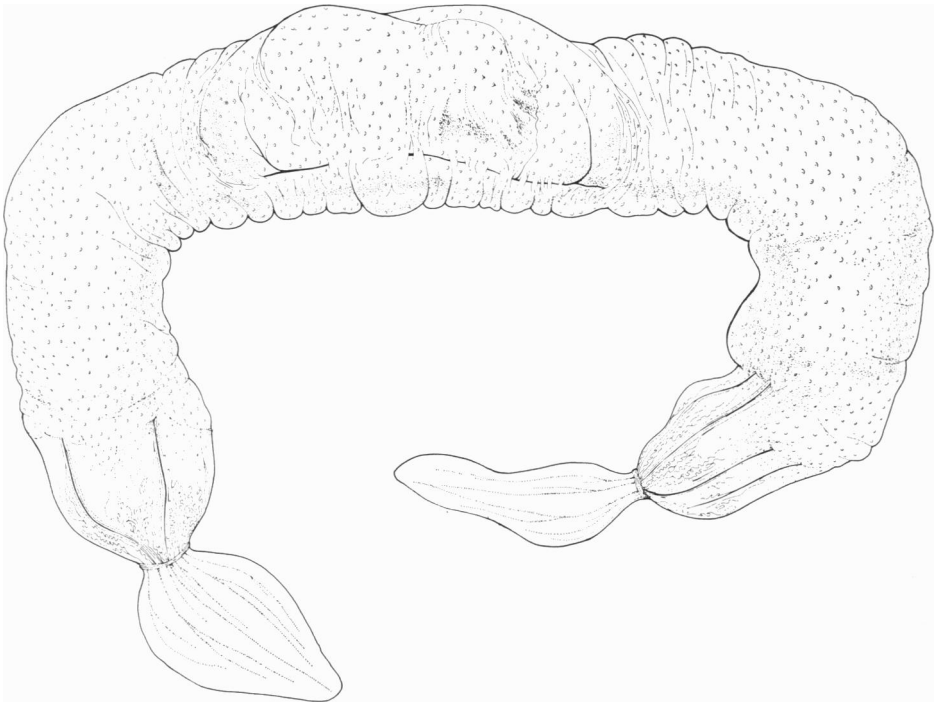

Fig. 1. Semi-diagrammatic representation of the embryonic sac of the pig at about 50 d pregnancy. Not drawn to scale. The central, *placental*, zone is stippled and areolae are indicated. The *paraplacental* zones are indicated by longitudinal blood vessels. The terminal *ischaemic* zones are delimited from the paraplacental zones by constrictions of the sac.

(2) *The relationships between extremities of adjacent embryonic sacs*

During the last two-thirds of pregnancy a progressive adherence and fusion seems to occur between many adjacent foetal sacs. It can be divided into three stages for descriptive purposes.

Stage 1. During this stage the ischaemic extremities are interlocked and adherent (Fig. 3*a*). The brown material lying around these interlocked extremities sticks them together. This relationship between sacs is characteristic of uteri examined at the end of the first third of pregnancy.

Stage 2. At the end of the second third of pregnancy, or even earlier, the paraplacental zones are apposed and adherent (Fig. 3*b*). Examination with a stereoscopic microscope during manual separation suggests that there is little histological fusion between adherent paraplacental zones. Arterial injections of coloured inks did not reveal any major vascular connexions between adjacent sacs. Occasionally, at the constriction that originally demarcated paraplacental from ischaemic zones, manual separation causes tissue and capillary damage, indicating a limited area of histological

fusion. We were repeatedly surprised that these thin, intimately apposed membranes could be separated so easily with no apparent tissue damage. The ischaemic extremities of adherent sacs were interlocked, invaginated one into the other, or lying free inside or outside the line of junction between sacs. With care, the tips could be filled with allantoic fluid; injection of coloured inks into the allantoic cavities did not establish any intercommunications between adjacent cavities as reported by Pomeroy (1960).

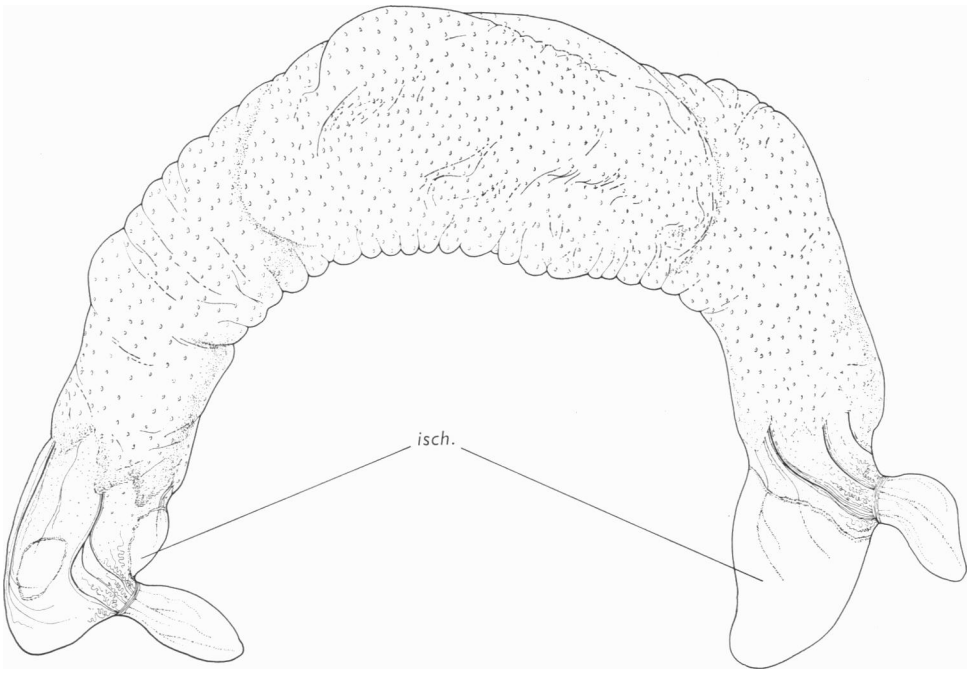

Fig. 2. Semi-diagrammatic representation of the embryonic sac of the pig at about 80 d pregnancy. Not drawn to scale. Note ischaemic areas (*isch.*) proximal to constrictions.

Stage. 3. This appears to be the final, most complete stage of union between adjacent sacs, and is characterized by partial or complete histological fusion of the adjacent edges of the placental zones (Fig. 3*c*). In late pregnancy, it may be difficult to detect the line of junction between two sacs but, with care, the joined placental zones can be torn apart; the underlying paraplacental zones are then exposed, and can be separated with little or no tearing of tissue. The ischaemic extremities and their allantoic cavities are disposed as in stage 2.

In the material we have examined, it is not until placental zones come together and fuse that a firm histological union is consistently established between two sacs. This occurs in some sacs in the last third of pregnancy, or even earlier. We found one complete example of fusion between placental zones in an estimated 60 d pregnancy, but at the end of pregnancy some sacs may not be adherent to adjacent sacs, or may show stage 1 or stage 2 adherences.

(3) *The process of adherence and fusion between adjacent sacs*

Our material shows considerable variation in the stages reached after a given duration of pregnancy. From 60 d onwards it is possible to find fully separate, adherent, or fused foetal sacs. Table 1 indicates the progressive nature of the process by listing the stages recovered from four pregnancies estimated as being 60, 70, 80 and 90 d duration. Fusion around the entire circumference of the placental zones is seldom seen before the last month of pregnancy. Even in late pregnancy, adjacent

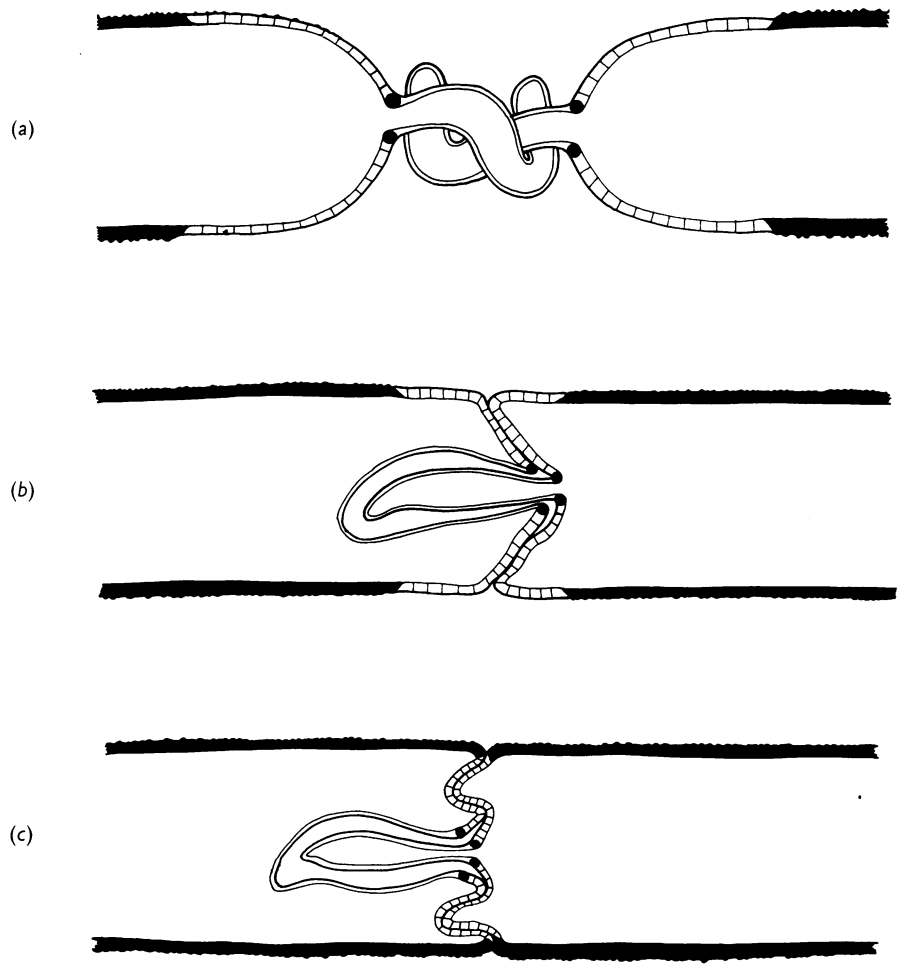

Fig. 3. Diagrammatic longitudinal sections through the extremities of two adjacent embryonic sacs, representing the three stages of adherence and fusion described in the text. Not to scale. (*a*) Stage 1, in which the terminal ischaemic zones are adherent. (*b*) Stage 2, in which paraplacental zones are adherent. (*c*) Stage 3, in which placental zones are fused. Placental zones are shown by irregular solid black lines; paraplacental zones by a hatched line; ischaemic zones by thin, wavy lines. The constriction between paraplacental and ischaemic zones is indicated in solid black, and for the sake of clarity no ischaemic areas are shown proximal to this constriction.

extremities may overlap without adherence, and at 109 d of pregnancy we found two sacs separated by a space of 5 cm.

Occasionally two sacs that were not adherent were separated by a dead embryo. Dead embryos and their sacs were occasionally (over 6 % of our series) immured in the adherent sacs of adjacent, living, embryos. At an estimated 80 d of pregnancy the membranes of two embryos (lengths 20·9 and 22·5 cm) surrounded a dead embryo (length 6·2 cm: estimated age at least 40 d) enclosed in its own necrotic membranes.

Table 1. *Classification of the relationships between adjacent foetal sacs in the uteri of four sows*

Sow number	M1	M2	M3	M4
*Estimated duration of pregnancy (in d)	60	70	80	90
Number of pairs showing no adherence or adherence restricted to ischaemic extremities	3	4	0	1
Number of pairs showing adherence between paraplacental zones but no fusion between placental zones	6	5	4	4
Number of pairs showing fusion between placental zones	2	3	7	1
Relationship not recorded	2	0	2	0
Total	13	12	13	6

* Estimated from mean embryonic length, using the data of Marrable & Ashdown (1967).

(4) *The fate of the membranes at parturition*

Eleven foetal sacs were recovered from one sow during parturition. In each sac the different zones could be clearly distinguished. Both ends of the sacs were torn; the tear was in the paraplacental zone, in all cases but one, in which the placental zone was torn. In two sacs one edge of the placental zone was torn into a narrow, ragged fringe, though the junction with its paraplacental zone was intact; this suggested that histologically fused placental zones had torn apart during birth. The placental zones of two sacs were still firmly fused; one of these placental zones was torn near the junction.

DISCUSSION

Our observations support the arguments of Lesbre (1910) concerning the anatomy of the 'necrotic tips'. They appear to be zones of the allanto-chorion that have been rendered ischaemic by vascular occlusion at the junction with the paraplacental zone. The anatomy of the ischaemic zone throughout pregnancy suggests that this change is progressive, involving more and more of the terminal part of the allanto-chorion as pregnancy proceeds. It does not seem to be associated with the process of adherence or fusion, since it starts before adjacent sacs begin to join, and occurs in the isolated extremities of foetal sacs at the ovarian ends of the uterine horns. (In one sac at the ovarian end of the uterine horn we found no true ischaemic zone on its ovarian extremity.) Hughes (1929) believed that the ischaemic extremities of embryo-

nic sacs in the pig prevent end-to-end fusion. However, although the ischaemic zones do not fuse, they do not prevent fusion between placental zones in late pregnancy. We have found no evidence to support the statements of Lesbre (1910) that the cavity of the 'necrotic tip' distal to the constriction is 'amputated' in late pregnancy.

The sequence of interlocking and adhesion between ischaemic zones, adherence between paraplacental zones and histological fusion between placental zones, progresses in the last two-thirds of pregnancy. The placental bed of each foetus extends until almost the whole of the available endometrial surface is utilized. This is not achieved by a gradual extension of the placental area over the whole of the foetal sac; the non-placental parts of the sac appear to become progressively affected by ischaemic degenerative changes and are not transformed into placental parts. Each placental zone seems to spread over the uterine surface until adjacent placental zones meet and fuse, forming placental tubes which sometimes join across the uterine body.

At birth the foetal sacs may be passed from the uterus still firmly fused at the edges of their placental zones. However, there is evidence to suggest that adherent and fused sacs may tear apart during the process of birth. The piglet seems to escape from the sac through a tear in the paraplacental zone; at the time of birth this zone is extensively affected by ischaemic degenerative changes.

SUMMARY

1. The superficial zonation of the allanto-chorion of the pig has been studied in embryonic sacs taken from 41 sows pregnant for periods within the range 15–109 d. (The gestation period is about 114 d.)

2. Towards the end of the first half of pregnancy (39–55 d) the surface of each sac bears five distinguishable regions; a large central *placental zone*, two bordering *paraplacental zones* and two terminal *ischaemic zones*. During this time the adjacent extremities of sacs are often adherent but are easily separated.

3. In the latter part of pregnancy, a progressive adherence and fusion seems to occur between many, but not all, neighbouring sacs. The final and most complete union is characterized by histological fusion of adjacent edges of placental zones.

4. Further observations on eleven sacs collected during parturition showed that at birth some sacs may be passed from the uterus still firmly fused at the edges of their placental zones. The piglet seems to escape through a tear in the paraplacental zone.

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