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OVA TRANSPORT AND FERTILITY AFTER RESECTION OF THE OVIDUCTAL ISTHMUS IN PIGS

By

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RODRIGUEZ-MARTINEZ, H., B. LARSSON and S. EINARSSON: *Ova transport and fertility after resection of the oviductal isthmus in pigs.* Acta vet. scand. 1985, *26*, 218—230. — The effect upon the reproduction of total resection of the oviductal isthmus, including the uterotubal and ampullary-isthmus junctions followed by end-to-end ampullo-cornual anastomosis was studied in pigs. Normal cycling gifts of proved fertility were submitted to bilateral isthmus deletion (Group I). Other gilts were submitted only to unilateral isthmic resection, while their contralateral medial isthmus was transversally cut and reanastomosed (Group II). A significantly lower nidation index was obtained after bilateral isthmic resection. The gilts in Group I which did not become pregnant and those in Group II were successfully mated during forthcoming standing oestruses, and were in association to that slaughtered on days 3, 4, or 5 of the cycle, and the characteristics and location of ova were determined. Spermatozoa fertilized the ova, and those cleaved normally in the isthmic-resected tube as well as in the sham-operated oviducts. The isthmic-resected oviduct, however, did not transport cleaved ova into the uterus at the time expected as normal. The findings suggest that the fertilized pig ova cannot reach the uterus at the normal expected time in case of total absence of the isthmic part of the oviduct, resulting in impaired fertility.

reproduction; gilts; embryos; uterus.

The surgical removal of particular segments of animal oviducts allow the assessment of the effects upon their function and the associated impact upon fertility (*Perez & Eddy* 1980, *McComb et al.* 1981).

In the pig, the newly ovulated ova are immediately picked up by the fimbriated infundibulum and then rapidly transported through most of the ampulla to the ampullary-isthmic junction

area (AIJ) where normally fertilization takes place (*Hunter* 1982). The sperm is sequentially transported into the pig oviducts, the number of spermatozoa reaching the fertilization site being regulated by the isthmic tubal segment (*Hunter* 1982). The fertilized ova remain at the AIJ area for 8—32 h after ovulation (*Alanko* 1974). Thereafter they are transported to the uterus, where they are only found after 120 h from onset of oestrus (*Oxenreider & Day* 1965, *Alanko* 1974).

The retention of the pig ova in the isthmus for such a long time has been related to a proper timing of the ova entry to the progestational uterus (*Murray et al.* 1971). The contractility of this segment is believed to be responsible for the arrest of ova in the oviducts of pigs (*Rodriguez-Martinez & Einarsson* 1982). Active peristaltic activity is namely present in the isthmic segment in pigs at the moment when ova are normally passing through to the uterus (*Rodriguez-Martinez et al.* 1982).

Extirpation of the isthmus in pigs, although it does not prevent fertilization and early cleavage of ova, was supposed to interfere with the proper timing of entry of the embryos to the uterine cavity and their subsequent implantation (*Hunter & Léglise* 1971). The conservation of either the uterotubal junction (UTJ) or the AIJ after removal of most of the isthmus has previously been proved to be associated with a reduction of embryo survival but, on the contrary, not the crude pregnancy rates in pig species (*Paterson et al.* 1981). Apparently, the presence of even a short part of the isthmus seems to be sufficient for normal reproduction in this species.

Since the junctions at either end of the isthmus might play an important role in its reproductive function, the real effect of isthmic deletion can only be evaluated after total removal, comprising UTJ and AIJ as well.

The aim of the present study was consequently to determine the fertility in gilts under controlled conditions before and after complete bilateral isthmic extirpation that included UTJ as well as AIJ. We also analyzed the sperm transport into the oviduct, the fertilization, cleavage and transport of the ova, and the morphological aspects of genital tracts after either bilateral or unilateral complete isthmic resection.

MATERIAL AND METHODS

Animals

The present series comprised 19 normally cycling (2nd or later oestrous cycles) crossbred gilts weighing 90—120 kg. The animals were housed indoors, 3—4 gilts per pen and they were fed a commercial pig feed. None of the gilts had been used previously in any experiment. Oestrus was registered by experienced personnel twice daily in the presence of a boar. The gilts were randomly allocated to the two groups of the experimental design:

Group I (n=8). The gilts were mated twice during a spontaneous standing oestrus with boars of proven fertility. Laparoscopy was performed 21—22 days after last mating in order to determine the number of ovulations, i.e., functional corpora lutea (CL) and possible pregnancy. The gilts were thereafter located in farrowing crates and abortion was induced by i.m. injection of ≥50 μg of Cloprostenol (Estrumate, ICI, England) at 30 days after mating. The number and characteristics of the aborted fetuses were noted. The gilts showed standing oestrus 3—10 days after abortion, and thereafter regular oestrous cycles with spontaneous standing oestrus periods. The gilts were then submitted to bilateral isthmic resection, including the UTJ and AIJ, followed by end-to-end ampullo-cornual anastomosis.

Group II (n=11). The gilts were submitted to total resection of one isthmus followed by end-to-end ampullo-cornual anastomosis. The contralateral medial isthmus, used as control, was transversally sectioned and reanastomosed (sham operation).

Surgical procedures (tubal resection)

Surgery was, in all cases, carried out between days 8—12 of the oestrous cycle (first day of standing oestrus is day 1). The gilts were anaesthetized with thiopental sodium (Penthotal sodium, Abbott) injected through a cannulated ear vein. The anaesthesia was maintained by intermittent injections as required. The reproductive tract was exposed through a midventral laparotomy. Hemostasis was secured by the use of bipolar diathermy. All exposed peritoneal surfaces were continuously moistened with saline, and any blood was immediately diluted with saline and aspirated. Because of the big size of the utero-tubal

region in this species, no operating microscope was found obligate to secure a surgical procedure, comparable to microsurgery in human beings. The ovaries were examined for the number of well-grown CL and growing follicles. Each appropriate vessel of the vascular arcade bordering the isthmic portion of the tube was ligated with 9-0 Dexon, (Davis and Geck, Pearly River, N. Y., USA). After that the oviduct close to the uterine horn was transected with microscissors. Mucosal fronds expelled invariably from the cut uterine lumen and were trimmed with microscissors. Next, the ampulla immediately adjacent to the AIJ was transected with microscissors. The ligamentum infundibulo-cornuale (LIC; *Schilling* 1968) was dissected from its insertion at the UTJ, along the isthmus to be removed, to the site of the transected ampulla. The isthmic segment (including the UTJ and AIJ) was then totally excised. The cut edges forming a V-shaped defect in the mesosalpinx were attached using two or three 6-0 Dexon sutures, roughly approximating the ampullary lumen to the uterine lumen. Hereby the LIC was in addition fixed to the uterine horn. One suture of 9-0 Dexon was placed at 6 o'clock (mesenteric) and one at 12 o'clock (antimesenteric) positions, approximating the ampulla and cornua. The sutures were placed in the outer layer of the muscular layers with care taken to avoid the endosalpinx. Four or five 9-0 additional sutures were placed at 8, 10, 2, and 4 o'clock positions. Any further adjustment of the luminal sizes of the anastomosed ends were not performed. The adnexum was then replaced to the peritoneal cavity. The same surgical procedure was repeated in the contralateral side in the appropriate animals (Group I). In the animals of Group II an unilateral and complete isthmic resection was performed on the right side. In order to provide an appropriate control side, the left medial isthmus was, in addition, exposed through the same abdominal incision and prepared with hemostatic ligatures. It was transected with microscissors and approximated with five or six 9-0 Dexon sutures placed through the serosa and the muscularis, but avoiding the mucosa (sham operation). The peritoneal cavity was then lavaged with saline and closed with interrupted sutures.

The removed isthmic segments were immediately fixed in Bouin's fluid for histological confirmation of complete isthmic resection.

Postoperative procedures

The gilts were left for convalescence for 3—4 weeks, isolated from boar's contact, penned in the proximity, but checked manually for standing oestrus. After that when the gilts showed spontaneous cyclicity, they were mated with the fertile boars as mentioned above. The gilts were carefully checked for standing oestrus or possible pregnancy for a 4-week period after mating. Those who did not get pregnant were mated again for up to 3 spontaneous oestrus periods. The animals that continued cycling (Group I) and those undergoing unilateral isthmus deletion (Group II) were after last mating slaughtered, either on days 3, 4, or 5 of the oestrous cycle.

Immediately after slaughter, the genital tract was excised en-bloc and examined. The presence of any adhesion was noted. The ovaries were examined and the total number of corpora lutea (CL) recorded. Each bloc, comprising the oviduct and uterine horn was divided into the following segments as registered from uterus to the fimbriated end of the tube:

Sham (control) side (Group II): distal 20 cm of uterine horn (*1*), UTJ and proximal isthmus (*2*), distal isthmus and proximal ampulla (*3*), mid and distal ampulla (*4*);

Isthmic deleted side (Groups I and II): distal 20 cm of uterine horn (*1*) and 1 cm of uterine horn adjacent to the anastomosis plus the remaining tube (ampulla) (*2*).

Each segment was flushed with 10—20 ml of tissue culture medium (Eagle Essential culture medium) and the separate flushings were examined under stereomicroscope for the presence of ova. The recovered ova were then fixed in 2.5 glutaraldehyde in 0.05 mol/l sodium cacodylate buffer (pH 7.2, 500 mOsm). After storage, at least overnight, the ova were postfixed in 2 % osmium tetroxide, dehydrated and embedded in EPON. These procedures were performed with the aid of a stereomicroscope. The ova were then serially sectioned (with 10—20 μm intervals) in semithin (about 1 μm) sections that were stained with buffered toluidine blue. The sections where then examined at 600—1000x on a Zeiss microscope, for their stage of development and the presence of spermatozoa. Particular attention was paid to the

nuclear structures. The number of spermatozoa on and in the zona pellucida (ZP) was recorded.

After flushing, the tubal segments comprising the anastomoses were cut into specimens 1 cm in length and fixed in Bouin's fluid for histological examination. The remaining parts of the genital tract were then opened and examined. The possible implantation sites were noted (noncycling gilt). The observed fetuses were macroscopically examined to determine their normality.

Statistics

A protected student's t-test was used for the statistical comparison of groups. A P value of less than 0.05 was considered significant.

RESULTS

Out of the 19 gilts operated on in this series, 4 were after slaughter excluded by the following reasons. Two gilts (Group I) developed peritubulae and periovarian adhesions impairing proper ova pick-up at ovulation. Two other gilts (Group II) developed bilaterally follicular cysts, without adhesions.

In the remaining 15 gilts, newly-formed CL on both ovaries were found when examined at slaughter. The macroscopical location of the anastomoses was identified solely by the presence of the sutures in the serosa. All anastomoses were patent, confirmed by presence and location of fertilized-cleaved ova, pregnancy, free passage of fluid, and histology. The histological examinations revealed that the epithelial folds of endosalpinx were continuous over the anastomosis (see Figs. 1 and 2). No sutures were found penetrating the endosalpinx. The tissues of anastomoses were completely healed, without signs of infection or pronounced foreign body reaction (see Fig. 2).

The total number of CL did not differ before and after surgery in any animal ($P > 0.05$). The mean fertility rate, i.e., the ratio of the total number of CL present in the ovaries and the number of fetuses of the gilts (Group I) before and after tubal resection of the isthmus is given in Table 1. Only 1 of the gilts operated on became pregnant. The uterus contained 2 fetuses, macroscopically normal, one in each horn. The number of functional CL in the ovaries of this animal was 10.

Table 1. Fertility of gilts before (control) and after bilateral surgical resection of the oviductal isthmus (treatment).

Group	Number of gilts		CL (total)	Fetuses (total)	Fertility rate (%)*
	Total	Pregnant			
Control	6	6	54	45	83
Treatment	6	1	53	2	4**

* Average nidation rate expressed as a percentage of the ovulation rate.
** $P < 0.001$.

In the non-pregnant animals (Group I), slaughtered on days 3, 4, or 5 of the cycle after being mated twice during a spontaneous standing oestrus, the location of ova after flushing (recovery rate = 69.1 % ± 3.51, $\bar{x}$ ± s.e.m.) revealed the presence of fertilized ova (81.7 % ± 3.82) in different stages of development (from 2 to 8 + cells) and in all animals restrained in the tubal (ampullar) segment. Not a single ova was found in the flushings from the uterine segment (Table 2).

Table 2. Distribution of ova in gilts undergoing bilateral isthmic-resection followed by ampullo-cornual anastomosis at different times after successful mating.

Day of cycle	n	Total CL	Ova recovered		Stage of ova development	Ova location	
			Total	Fertilized		Uterus (1)	Ampulla (2)
3	2	18	14	12	2—4 cells	0	14
4	1	9	6	4	4—8 cells	0	6
5	2	16	10	8	8+ cells	0	10

The distribution of ova at different times after successful mating of gilts having an unilateral isthmic-resection (Group II) is shown in Table 3. The ova recovery rate was significantly higher in the sham operated (control) tubes (90.5 % ± 3.45; $\bar{x}$ ± s.e.m.) than in the isthmic-resected ones (63.1 % ± 3.59) ($P < 0.01$). The percentage of ova recovered that underwent normal cleavage after fertilization did not differ significantly between the two sides (92.1 ± 3.76 for the control side and 80.4 % ± 3.34 for the isthmic resected side; $\bar{x}$ ± s.e.m.). The rate of ova cleavage did not show any significant difference between sides ($P > 0.05$) (Fig. 3). Ova descent to the uterus, however, did

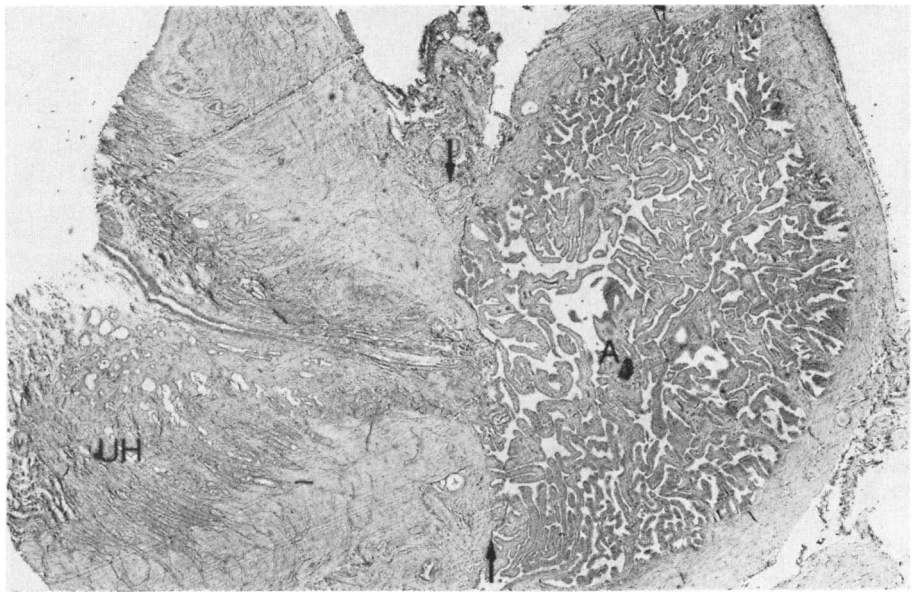

Figure 1. Longitudinal section of a patent ampullo-cornual anastomosis (arrows) (A: ampulla; UH: uterine horn). Note the absence of the fronds characteristic of the utero-tubal junction (He × 20).

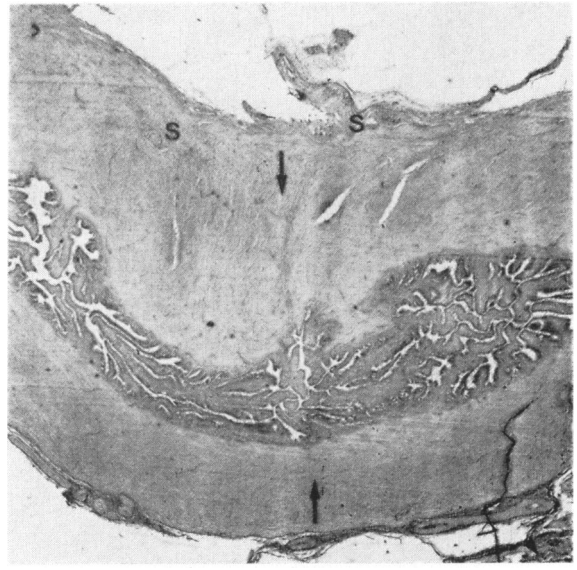

Figure 2. Longitudinal section of an isthmo-isthmic anastomosis (arrows) (S: suture material) (HE × 26).

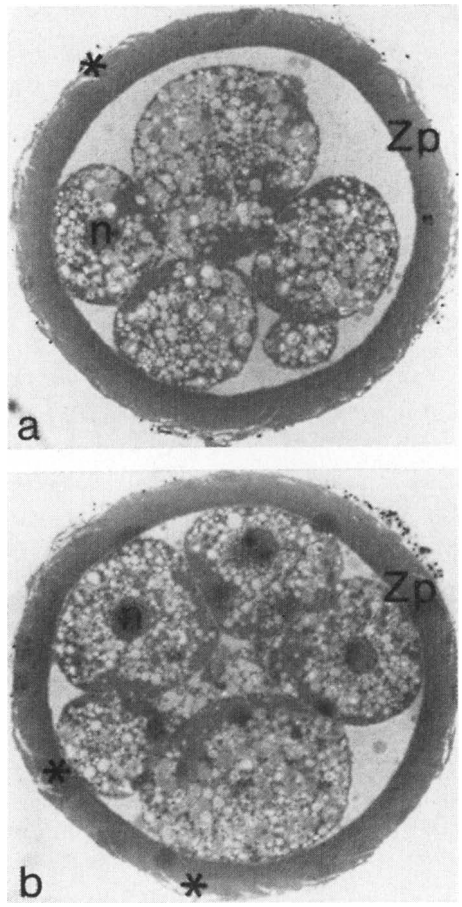

Figure 3. Early pig embryos recovered from (a) the isthmic resected side (ampullar segment) and (b) the uterine horn of the contralateral sham operated side, day 4 of the estrous cycle (Zp: Zona pellucida, *: sperm tracks, n: nuclei of blastosomeres) (toluidine blue × 700).

Table 3. Distribution of ova in gilts with an unilateral isthmic-resection at different times after successful mating on the second standing oestrus after surgery.

Day of cycle	n	Tubal side	Total CL	Ova recovered		Stage of ova development	Ova location			
				Total	Fertilized		Uterus (1)	Oviduct (2)	(3)	(4)
3	3	C	24	20	16	2—4 cells	0	17	2	1
		IR	23	14	12		0	14	—	—
4	3	C	13	11	11	4—8 cells	8	3	0	0
		IR	14	10	8		0	10	—	—
5	3	C	15	13	13	8+ cells	12	1	0	0
		IR	17	9	7		0	9	—	—

C control side (sham operation).
IR: isthmic-resected side.

not occur in the isthmic-resected oviducts either on day 4 or 5 of the oestrus cycle. The sham-operated side showed normal sequential ova distribution along the oviduct and presence of ova in the uterus at the expected time.

The number of spermatozoa attached to the zona pellucida (ZP) of recovered ova (Group II) was recorded from the serial sections. The mean number of spermatozoa on the ZP of ova recovered from the control side (n=30) was 234, with a range of 38—592 spermatozoa/ova. In ova recovered from the isthmic-resected tube, the mean number of spermatozoa was 389 (n=21) with a range of 24—725 spermatozoa/ova. In 6 gilts (Group II), a greater number of spermatozoa was present in the ZP of the ova recovered from the isthmic resected tube, compared to the control side. Within individual animals, however, the ova showed a great variation in the number of spermatozoa atached to the ZP. No consistent differences were observed in the mean number of spermatozoa in the ZP of ova collected at the different days of the oestrous cycle. No evidence of polyspermic fertilization was found in the examined ova. Failure of fertilization appeared in both control and isthmic-resected sides. Spermatozoa were present in the ZP, but no male pronuclei were seen. The number of spermatozoa were within the overall figures of the spermatozoa present in the ZP of the fertilized ova, but failure of fertilization could be due to improper timing of sperm or even ova transport.

DISCUSSION

The present study describes the effects of total resection of the isthmic segment of the oviduct (including the uterotubal and the ampullary-isthmic junctions) on the reproductive events in pigs.

The gilts used for the fertility trial (Group I) showed a normal fertility prior to surgery. In all but one animal, the bilateral isthmic resection prevented pregnancy. Fertilization in the pig usually accounts for more than 90 % of the ovulated ova (*Hunter* 1964) of which 30—35 % (estimated from the number of CL) fail to become viable embryos by 25—30 days of pregnancy (*Wrathall* 1971). The fact that only one of the gilts became pregnant, carrying only 2 fetuses, indicates that the absence of the isthmus significantly impairs fertility in pigs.

Establishment of pregnancy in the pig is not affected by removal of either the AIJ or the entire isthmus except for the 5 mm adjacent to the UTJ (*Paterson et al.* 1981). However, the reduction of oviductal length significantly diminished the nidation index in the operated animals (*Paterson et al.* 1981). The authors suggested that the presence of isthmic structures at the nonresected UTJ could have provided normal function for the tube, and achievement or normal crude pregnancy rates. The absence of any isthmic structure in the present experiments could account for the significantly diminished fertility of the gilts.

Many factors may singly or collectively explain the infertility achieved. Ovulation appeared unaltered by the excision of the AIJ, isthmus, and UTJ. However, the differences found in ova recovery rates between control and isthmic-resected sides indicate that the surgery produced deleterious effects, most probably on the ova pickup mechanism at ovulation. Besides the careful reimplantation of the ligamentum infundibulo-cornuale, an imperfect apposition of the bursa ovarica over the ovary could have caused the loss of some newly ovulated ova into the addominal cavity. In general, loss of ova in both sides could have occurred during the procedure of tubal and uterine flushing.

Other involved factors might include abnormal sperm or ova transport, abnormal fertilization, inappropriate retention of the ova at the anastomosis point, premature ova entry into the uterus or the impaired development of ths fertilized ova in an inappropriate tubal or uterine milieu.

An impeded sperm transport through the anastomoses might have been responsible for the infertility achieved. However, more than 80 % of the recovered ova from the studied gilts at the ampullar segment of the tubes were fertilized and showed normal cleavage rates. This confirms previous observations (*Hunter & Léglise* 1971, *Paterson et al.* 1981) that fertilization can take place in the pig species in the absence of the isthmus.

Polyspermic fertilization may led to lethal genetic combinations and thus contribute to cause infertility. The removal of the isthmus (but not UTJ) in the pig was regarded as increasing the incidence of polyspermic fertilization as a result of an unregulated spermatozoal ascent in great numbers to the fertilization site (*Hunter & Léglise* 1971). A higher incidence of polyspermic ova should have been the case in the present animal population, with the absence of the entire isthmus. Although a subsequent greater number of spermatozoa appeared attached to the zona pellucida in ova recovered from the isthmic-resected oviducts, no evidence of polyspermy in the blastomeres or the perivitelline space was seen in the ova examined in the present study. However, the small number of ova studied, sectioned at 10—20 μm intervals, prevents us from making further considerations about this subject.

The rate of ova cleavage did not show any difference between the left and right side in Group II. No implication of abnormal development of the early embryos (two- to eight-cell stages) was found. The fact that the ova cleaved in the isthmic-resected tube supports previous findings (*Hunter & Léglise* 1971, *Pope & Day* 1972) concluding that pig embryos are fully capable of undergoing normal development when restricted to the ampullar region of the oviduct. However, our data do not include the occurrence of blastulation (*Murray et al.* 1971).

In both groups of animals (Groups I and II), the ova recovered from the isthmic-resected side were located at the ampullar segment, above the anastomosis, and in no case was there any evidence of ova entering the uterine lumen. In contrast, the control side showed normal tubal and uterine location of ova at the expected times.

Our initial hypothesis was that the ova, in the absence of a tubal locking mechanism provided by the isthmic musculature would enter the uterus prematurely, leading to the failure of the fertilized ova to implant into a non-properly prepared endometrium (*Day & Polge* 1968). Although it might be presumed

that some ova had effectively passed the ampullo-cornual anastomosis prematurely, they should have been transported faster in the contralateral side to avoid the recovery after flushing of the ad-tubal segment of the uterine horns. Although this assumption may still be possible, we believe that the differences in ova recovery between sides found are a product of a subtle defective ova pick-up, rather than a faster ova transport, in the isthmic-resected side.

A prevented ova entrance into the uterus is the immediate suggestion that arises from the results obtained in the operated animals. Actually it suggests the presence of a mechanic barrier to the ova transport, absent in the control oviducts. However, the provided evidence of an existing vast anatomic lumen, connected to the uterine cavity does not support the existence of a barrier. The tubal anastomosis performed in the control tubes did not impede normal sperm or ova transport. There was no evidence from the histological sections that even partial obstruction had been produced at any of the anastomosis. Since no gilt was mated before 3 weeks after surgery, there is no reason to presume that healing following tubal anastomosis was incomplete and thus impeded the ova entrance into the uterus (*Eddy & Bajpai* 1982). The gilts examined were slaughtered and their reproductive organs flushed after having passed more than one oestrous cycle after surgery. In no case, ova or ova rests from the previous ovulation were recovered in the anastomosed tuba. This was also taken as an indication of patency of the tube to ova passage, independently of the timing of ova entry in the uterus.

Another possible explanation is that the isthmic-resected oviducts lack a propulsive mechanism that is normally needed to move the ova into the uterus. In the porcine species, a low ampullar spontaneous motility is recorded in the postovulatory period, when ova transport through this segment of the tubes has been verified (*Rodriguez-Martinez et al.* 1982). At the same time no peristaltic activity is seen in the isthmus during the period when ova are retained in the tubal compartment (Day 3 of the cycle) (*Rodriguez-Martinez et al.* 1982). Peristalsis is present in the isthmus (including AIJ and UTJ) when the ova are normally passing down to the uterus (days 4 and 5 of the cycle) (*Rodriguez-Martinez et al.* 1982). The spontaneous motility of the ampulla remains faible during this period. The lack of isthmus and therefore, the absence of this propulsive peristaltic force in the

operated gilts might explain the retention of the ova in the tubal compartment.

In any case, the pig ova seems not to enter the uterine cavity at the normal expected time when no isthmus is present. However, in some cases few ova may enter the uterus on time and succeed in their implantation, as in the case reported here. Lack of the isthmus (including the junctions) may either render the ova transportless and incapable of entering the uterus at the expected time or allow them to enter the cavity prematurely. Either the premature entry or the delayed transport of pig embryos into the uterus is detrimental to embryo survival (*Day & Polge* 1968, *Murray et al.* 1971). More extensive studies need to be carried out in order to determine the fate of the tube-delayed ova in this species, under these experimental conditions.

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SAMMANFATTNING

Äggtransport och fertilitet efter resektion av istmusdelen av äggledaren hos svin.

Ändamålet med denna undersökning var att studera inverkan av totalresektion av istmus på fertiliteten hos svin. Normalt cyklande gyltor med tidigare dokumenterad fertilitet användes för dubbelsidig istmusresektion (Grupp I). På några andra gyltor gjordes enbart ensidig istmusresektion, medan den kontralaterala sidans istmus delades transversalt och reanastomoserades (Grupp II). Signifikant lägre befruktningsindex erhölls efter dubbelsidig istmusresektion. De gyltor i grupperna I och II som inte blev dräktiga betäcktes vid efterföljande brunst. Gyltorna slaktades dag 3, 4 eller 5 i cykeln och äggen lokaliserades och bedömdes. Spermierna befruktade äggen och äggen delade sig normalt i den shamopererade äggledaren såväl som i den äggledare där istmus bortopererats. Den äggledare som saknade istmusdelen transporterade emellertid inte ner äggen i livmodern vid förväntad tidpunkt.

Resultaten visar att äggens förflyttning till livmodern är fördröjd då istmusdelen är bortopererad. Detta resulterar i dålig fertilitet.

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