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Ectopic Pregnancy and Tubal Refractoriness to Implantation in Animals

Embarazo ectópico y refractariedad del oviducto a la implantación en los animales

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The widespread clinical incidence of tubal pregnancy appears to be the result of a complex and varying set of etiologic factors that may involve altered tubal physiology or aberrant trophoblastic function. The absence of spontaneous tubal pregnancy coupled with the inability to experimentally induce tubal gestation in infrahuman species makes it difficult to model the disorder and undertake systematic studies. Mounting evidence suggests the refractoriness of the infrahuman oviduct to implantation is due to the elaboration of a substance or substances by the endosalpinx that prevents implantation and suppresses embryo development. The nature and mechanism of action of such factors as well as their regulation is presently unknown.

INTRODUCTION

Ectopic pregnancy in women is a relatively frequent, poorly understood phenomenon. Approximately one in one hundred clinical pregnancies occurs outside the uterus (1, 2), most commonly in the ampullary segment of the fallopian tube (3). Only one third to one half of women who experience an ectopic pregnancy will subsequently give birth to a living child (4, 5) while up to twenty percent may experience a repeat ectopic (5, 6). This poor prognosis reflects a continuation of the underlying etiologic factors and the inability to clinically identify and correct them. If the mechanism, or mechanisms, that result in the human trophoblast developing and implanting in the tube were known, it would be possible perhaps to prevent or cure ectopic pregnancy.

ETIOLOGY

Etiologic factors contributing to tubal pregnancy in humans include those that involve the tube, manifested by altered embryo transport function and normal or increased receptivity to implantation, and those intrinsic to the conceptus itself. In nearly half of the patients with ectopic pregnancy some antecedent damage to the tubes is recognized on histopathologic examination (7-10). Because humans, unlike animals, contract infectious salpingitis which damages the tubal mucosa, the ability of the affected tube to efficiently transport the fertilized, cleaving egg into the uterus may be impaired or destroyed. In many cases of tubal pregnancy no preexisting tubal pathology can be documented highlighting the etiologic role of the conceptus itself and its ability to develop and implant in the tube (11).

In order for tubal pregnancy to occur, certain conditions must be met: 1) The fertilized egg must be located within the tube. This may reflect its continued presence within the tube due to its inability to reach the uterine lumen, its reflux back into the tube from the uterus, or its delayed entrance into the tube following intra-abdominal fertilization and delayed fimbrial pick up. 2) The tube must supply conditions conducive to support egg cleavage and differentiation. 3) Tubal receptivity leading to functional interaction between trophoblast and endosalpinx must exist.

ANIMAL MODELS

Attempts to create animal models in which tubal ectopic pregnancy can be replicated

and studied have proved difficult to achieve. Spontaneous ectopic pregnancies in animals do occur (12-25) but primarily involve non-tubal sites (Table 1). Spontaneous tubal pregnancy is rare in all infrahuman species particularly in nonhuman primates (26-28). Preimplantation cleavage stage eggs may implant in animals following transfer to a variety of non-tubal extrauterine sites (Table 2). The transfer of cleavage stage tubal eggs gives rise, with few exceptions, only to trophoblast and extraembryonic membranes whereas approximately one in four uterine blastocysts form morphologically normal embryos (29-47). In contrast cleavage stage eggs arrested in the oviduct never develop beyond the blastocyst stage and fail to implant in the guinea pig (48, 49), rabbit (50-52), rat (53), pig (54), or mouse (55-57). Uterine blastocysts returned to and confined to the oviduct similarly fail to implant (58, 59). The nonhuman mammalian oviduct appears either incapable of functionally interacting with endogenous trophoblastic tissue, for example via constitutional absence of cell surface receptors, inability to synthesize obligate embryo growth supporting factors, etc., or of actively preventing such interaction through synthesis and release of one or more substances that inhibit embryo growth and differentiation, tubal receptivity, trophoblastic invasiveness, etc. The virtual absence of spontaneous tubal pregnancy in animals in contrast to its common occurrence in humans, coupled with the inability to experimentally induce tubal pregnancy in animals strongly suggests fundamental differences in reproductive function between human and infrahuman species. The highly effective ability of the animal oviduct to avoid pregnancy appears not to have been conserved and passed on to the human. The mechanism that spares animals from tubal pregnancy seems either to be absent in the human, poorly developed, or subject to compromise in tubes damaged by salpingitis. While it may be possible that the human embryo is uniquely capable of advanced development and differentiation within the oviduct and that the tubal environment may be conducive to or may actively promote embryo development and trophoblastic invasiveness, the weight of evidence seems to support the hypothesis that the human tube, unlike that of animals, has an imperfectly developed ability to inhibit embryo growth, differentiation, and implantation.

TABLE 1

Spontaneous ectopic pregnancy in animals

Species	Site of ectopic	Reference
Cat	Abdominal cavity	12-15
Dog	Duodenal mesentery, abdominal wall	14, 16
Guinea pig	Stomach, abdominal wall	17
Hamster	Abdominal cavity	18, 19
Monkey	Mesentery	20
Rabbit	Omentum, small intestine	14, 21-24
Sheep	Abdominal wall, omentum	25

TABLE 2

Experimentally induced ectopic pregnancy in animals

Species	Site of ectopic	Reference
Mouse	Anterior eye chamber	29, 30
	Brain	31
	Kidney	32-38
	Lung	39
	Peritoneal cavity	29, 40
	Spleen	41
	Testis	42, 43
	Uterus, non-pregnant	44, 45
Rat	Peritoneal cavity	46
	Kidney	47

In animals the failure of tube locked embryos to develop to advanced stages and to implant suggests a tubal inhibitory mechanism. Mouse blastocysts retained in the oviductal lumen do not implant or differentiate into trophoblast. If transferred to the uterus 3.5 days after mating, about half develop into normal embryos. If kept in the tube for 4.5 days, none develop normal embryos or trophoblast after uterine transfer. The blastocysts do not degenerate until they are retained in the oviduct for eight days (56). Some factor in the mouse oviduct therefore appears to prevent implantation. Prior exposure of the embryo to the uterine environment does not overcome the ability of the oviduct to prevent implantation (56,

59, 59). Moreover, actively invasive trophoblast recovered from ectoplacental cones in the uterus when transferred to the tubal lumen does not interact with tubal epithelium but will with other tissue (59).

Arrest of the rabbit zygote in the oviduct results in similar failure of the embryo to develop beyond the early blastocyst state. If embryos retained in the ligated oviduct are transferred to the uterus 3.5 days after mating, almost all are capable of developing to term. With increased length of time in the oviduct this capacity progressively declines. After 4.5 days in the oviduct only 8% of blastocysts transferred to the uterus will develop to term. This capability is entirely lost by five days of retention in the tubal environment (51, 52).

In the rabbit, an estrogen-mediated factor found in serum and in oviductal fluid inhibits growth of the embryo beyond the morula stage (60). A small peptide that is produced under estrogen influence inhibits development of pronuclear stage embryos fertilized *in vivo* and then cultured *in vitro* (61, 62). Comparable results are obtained in the mouse (63). It is not clear why the oviductal environment, which is the site of fertilization and early embryo cleavage, should become hostile to advanced embryo development and survival. Such a function is, however, consistent with a mechanism to prevent tubal pregnancy.

In order to examine the mechanism by which the animal tube fails to support implantation, use was made of the technical capability to microsurgically autotransplant genital tissue in the rabbit (64). By transplanting tubal tissue into the mid portion of the rabbit uterine horn, the ability of a segment of transplanted endosalpinx to either support or inhibit implantation was examined in an organ normally conducive to implantation. Groups of animals underwent unilateral circumferential resection of endometrium from the mid uterine horn followed by its replacement with autologous endosalpinx or endometrium. Two months later animals were mated and examined on day 10 post coitum. All grafted tissue survived, underwent neovascularization, remained terminally differentiated, and exhibited normal histology by light microscopy (Figures 1, 2). All operated uterine horns were patent. The spatial distribution of implants throughout operated uterine horns relative to the position of the grafted endosalpinx or endometrium is shown in Figure 3. The presence of endosalpinx within the uterine horn was associated with a decrease in pregnancy rate, a lowering of the ratio of ova released to those that implanted, a decrease in the size of implantations and of embryonic growth, and a uniform failure of implantation to occur within the segment of uterine horn lined with endosalpinx (Table 3). In addition to these findings unattached blastocysts were seen within the endosalpinx lined segment of uterine horn in five of nine animals (Figure 4). No unattached blastocysts were seen

Fig. 1: Histologic appearance of autotransplanted endosalpinx 10 weeks after surgery. H & E x 300.

Fig. 2: Histologic appearance of autotransplanted endometrium 10 weeks after surgery. H & E x 240.

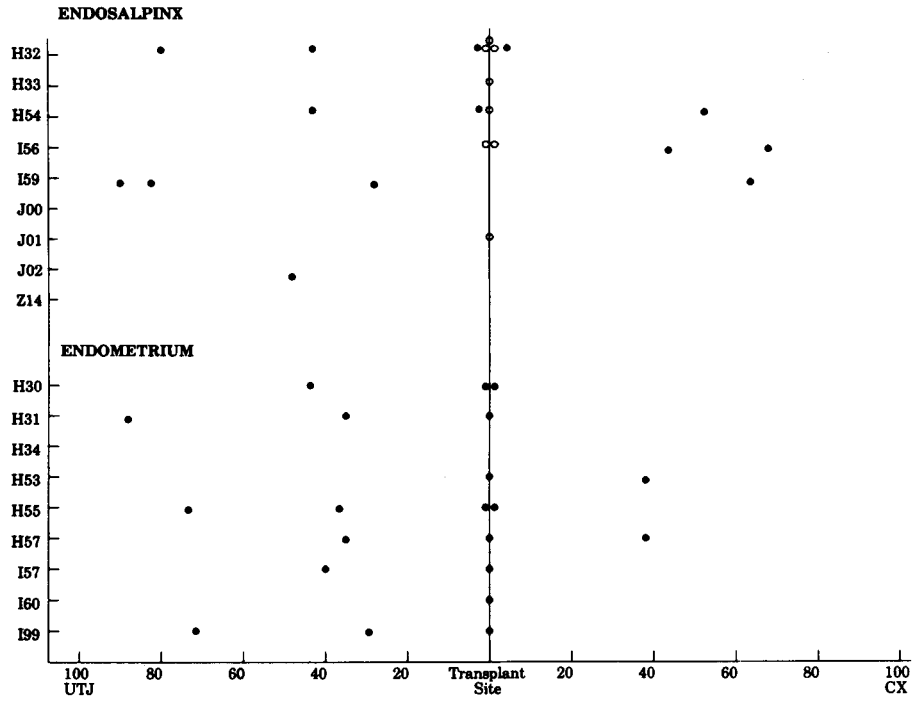

Fig. 3: Distribution of implantations along the length of uterine horns bearing a circumferential segment of autotransplanted endosalpinx (above) or endometrium (below). Distances have been normalized to the transplantation site. UTJ: utero tubal junction; CX: cervix; ●: implantation; o: unattached embryo.

TABLE 3

Effects of autotransplanted endosalpinx or endometrium on implantation and embryo development*

Tissue transplanted	N° animals pregnant	N° animals with implants at graft site	N° of implants with embryos
Endosalpinx	5/9	0/9	3/11
Endometrium	9/9	8/9	11/11

* Pauerstein *et al.*, (1964).

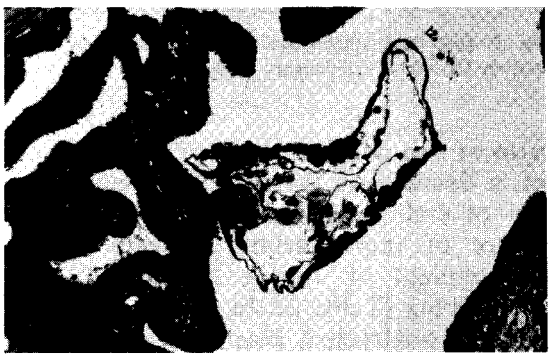

Fig. 4: Hatched, unattached blastocyst located within segment of uterine horn lined with autotransplanted endosalpinx, day 10 postcoitum. H & E x 180.

in control uterine horns grafted with endometrium.

These results document a marked inhibitory influence of grafted endosalpinx upon uterine-embryo interaction and suggest the rabbit endosalpinx produces a factor or factors that prevents implantation and suppresses embryo development. The nature and mechanisms of action of such factors as well as the regulation of their production is presently unknown. The uniform failure of implantation to occur within endosalpinx-lined segments of uterus, in contrast to its normal occurrence

in control uteri lined with grafted endometrium may reflect a cell-surface phenomenon, tissue-specific to the endosalpinx. In contrast, the decline in numbers of implantations and the inhibition in embryo development in uterine areas proximal and distal to grafted endosalpinx is consistent with the synthesis and release of inhibitory substances from the endosalpinx which affect the entire uterine horn. Follow-on studies are required to further examine the tube's ability to alter trophoblast-epithelial interaction and embryo development. Such studies will hopefully lead to a better fundamental understanding of tubal function and of the pathophysiology of tubal gestation.

REFERENCES

1. WESTROM, L.; BENGTSSON, L.P.H.; MARDH, P.A. (1981) Incidence, trends and risks of ectopic pregnancy in a population of women. *Br. Med. J. 282:* 15-18.
2. CENTERS FOR DISEASE CONTROL (1986) Ectopic pregnancy - United States, 1981-1983. *MMWR 35:* 289-293.
3. BREEN, J.L. (1970) A 21 years survey of 654 ectopic pregnancies. *Am. J. Obstet. Gynecol. 106:* 1004-1019.
4. PLOMAN, L.; WICKSELL, F. (1960) Fertility after conservative surgery in tubal pregnancy. *Acta Obstet. Gynecol. Scand. 39:* 143-146.
5. DeCHERNEY, A.H.; KASE, N. (1979) The conservative surgical management of unruptured ectopic pregnancy. *Obstet. Gynecol. 54:* 451-455.
6. SCHENKER, J.G.; EYAL, Z.; POLISHUK, W.Z. (1972) Fertility after tubal surgery. *Surg. Gynecol. Obstet. 135:* 74-76.
7. NILES, J.H.; CLARK, J.F.J. (1965) Pathogenesis of tubal pregnancy. *Am. J. Obstet. Gynecol. 105:* 1230-1234.
8. BRONSON, R.A. (1977) Tubal pregnancy and infertility. *Fertil. Steril 28:* 221-228.
9. HELVACIOGLU, A.; LONG, E.M.; YANG, S.L. (1979) Ectopic pregnancy, an eight year review. *J. Reprod. Med. 22:* 87-92.
10. VASQUEZ, G.; WINSTON, R.M.L.; BROSENS, I.A. (1983) Tubal mucosa and ectopic pregnancy. *Br. J. Obstet. Gynaecol. 90:* 468-474.
11. EMMRICH, P.; KOPPING, H. (1981) A study of placental villi in extrauterine gestation: a guide to the frequency of blighted ova. *Placenta 2:* 63-70.
12. FRY, P.D.; JONES, S.C. (1973) A case of ectopic pregnancy in a cat. *J. Small Anim Pract. 14:* 361-365.
13. CARRIG, C.B.; GOURLEY, I.M.; PHILBRICK, A.L. (1972) Primary abdominal pregnancy in a cat subsequent to ovariohysterectomy. *J. Am. Vet. Med. Assoc. 160:* 308-310.
14. BLAND-SUTTON, J. (1904) Abdominal pregnancy in women, cats, dogs and rabbits. *Lancet ii:* 1625-1627.
15. HUNTER, R.H. (1931) Abdominal pregnancy in a cat. *J. Anat. 66:* 261-263.
16. SCHLOTTHAUER, C.F.; WAKIM, K.G. (1955) Ectopic pregnancy in a dog. *J. Am. Vet. Med. Assoc. 127:* 213.
17. HONG, L.C.; ARMSTRONG, M.L. (1978) Ectopic pregnancy in 2 guinea pigs. *Lab. Anim. 12:* 243-244.
18. PETERS, L.J. (1982) Abdominal pregnancy in a golden hamster (*Mesocricetus auratus*). *Lab. Anim. Sci. 32:* 392-393
19. BUCKLEY, P.; CAINE, A. (1979) A high incidence of abdominal pregnancy in the Djungarian hamster (*Phodopus sungorus*). *J. Reprod. Fertil. 56:* 679-682.
20. McCLURE, H.M.; CHANG, J. (1975) Ectopic pregnancy in a squirrel monkey. *J. Am. Vet. Med. Assoc. 167:* 654-655.
21. JACOBSON, H.A.; KIBBE, D.P.; KIRPATRICK, R.L. (1975) Ectopic fetuses in 2 cottontail rabbits. *J. Wildlife Dis. 11:* 540-542.
22. FELLOW-NUTTING, M.D. (1942) A case of extrauterine pregnancy in the rabbit. *Anat. Rec. 84:* 215-219.
23. EALES, N.B. (1932) Abdominal pregnancy in animals, with an account of a case of multiple ectopic gestation in a rabbit. *J. Anat. 67:* 108-117.
24. BELL, W.B. (1910) Primary abdominal pregnancy in a rabbit. *Proc. Roy Soc. Med. 4:* 228-233.
25. DAVIES, P.T. (1982) Extrauterine pregnancy in a ewe. *Vet. Rec. 110:* 475.
26. WALDEYER, H.W. (1893) Ueber eine ectopische schwangerschaft bei einem mantel pavian. *Ztschrif Geburtschu Gynak Stuttg. 27:* 177-181.
27. LAPIN, B.A.; YAKOLEVA, L.A. (1963) *Comparative Pathology in Monkeys.* Springfield I.L.; Charles C. Thomas, pp. 215-216.
28. HODGEN, G. (1984) Letters to editor. *J. Am. Med. Asso. 251:* 2660.
29. FAWCETT, D.W.; WISLOCKI, G.B.; WALDO, C.M. (1947) The development of mouse ova in the anterior chamber of the eye and in the abdominal cavity. *Am. J. Anat. 81:* 413-443.
30. RUNNER, M.N. (1947) Development of mouse eggs in anterior chamber of the eye. *Anat. Rec. 98:* 1-13.
31. KIRBY, D.R.S. (1965) The invasiveness of the trophoblast. In Park, A. *The Early Conceptus, Normal and Abnormal.* St. Andrews Press, Edinburg, pp. 68-73.
32. FAWCETT, D.W. (1950) The development of mouse ova under the capsule of the kidney. *Anat Rec. 108:* 71-91.
33. JONES, C.L. (1951) Endocrine effects in the virgin female host of fertilized mouse ova developing in extrauterine sites. *J. Endocrinol. 7:* lxiii.
34. WHITTEN, W.K. (1958) Endocrine studies on delayed implantation in lactating mice. Role of the pituitary in implantation. *J. Endocrinol. 16.* 435-441.
35. KIRBY, D.R.S. (1960) Development of mouse eggs beneath the kidney capsule. *Nature* (Lond.) *187:* 707-708.
36. KIRBY, D.R.S. (1962) The effect of the uterine environment on the development of mouse eggs. *J. Embriol. Exp. Morph. 10:* 496-506.
37. KIRBY, D.R.S. (1962) Reciprocal transplantations of blastocysts between rats and mice. *Nature* (Lond.) *194:* 785-786.
38. KIRBY, D.R.S. (1967) Ectopic autografts of blastocysts in mice maintaned in delayed implantation. *J. Reprod. Fert. 14:* 515-517.

39. CARR, D.H. (1979) Trophoblast growth in the lungs of mice. *Obstet. Gynecol. 54:* 461-466.
40. McLAREN, A.; TARKOWSKI, A. (1963) Implantation of mouse eggs in the peritoneal cavity. *J. Reprod. Fert. 6.* 385-392.
41. KIRBY, D.R.S. (1963) Development of the mouse blastocyst transplanted to the spleen. *J. Reprod. Fert. 5:* 1-12.
42. KIRBY, D.R.S. (1963) The development of mouse blastocysts transplanted to the scrotal and cryptorchid testis. *J. Anat. 97:* 119-130.
43. STEVENS, L.C. (1967) The development of teratomas from intratesticular grafts of 2-cell mouse eggs. *Anat. Rec. 157:* 328.
44. KIRBY, D.R.S.; COWELL, T.P. (1968) Trophoblast-host interactions. In: Fleischmajor, Billingham. *Epithelia-Mesenchymal Interactions.* Williams and Wilkins, Baltimore, pp. 64-77.
45. COWELL, T.P. (1969) Implantation and development of mouse eggs transferred to the uteri of nonprogestational mice. *J. Reprod. Fert. 19:* 239-245.
46. JOLLIE, W.P. (1961) The incidence of experimentally produced abdominal implantations in the rat. *Anat. Rec. 141:* 159-167.
47. NICHOLAS, J.S. (1942) Experiments on developing rats. IV. The growth and differentiation of eggs and egg cyclinders when transplanted under the kidney capsule. *J. Exp. Zool. 90:* 41-64.
48. LOEB, L.; HUNTER, J.W. (1908) Experiments concerning extrauterine pregnancy and the influence of operation on the course of pregnancy. *Univ. Penn. Med. Bull. 21:* 294-298.
49. BLAND, K.P.; DONOVAN, B.T. (1965) Experimental ectopic implantation of eggs and early embryos in guinea pigs. *J. Reprod. Fert. 10:* 189-196.
50. PINCUS, G.; KIRSCH, R.E. (1936). The sterility in rabbits produced by injections of oestrone and related compounds. *Am. J. Physiol. 115:* 219-228.
51. ADAMS, C.E. (1956) A study of fertilization in the rabbit: the effect of postcoital ligation of the fallopian tube or uterine horn. *J. Endocrinol. 13:* 296-306.
52. ADAMS, C.E. (1958) Egg development in the rabbit: the influence of post-coital ligation of the uterine tube and of ovariectomy. *J. Endocrinol. 16:* 283-293.
53. ALDEN, R.H. (1942) Oviduct and egg transport in the albino rat. *Anat. Rec. 84:* 137-141.
54. MURRAY, F.G.; BAZER, F.W.; KUNDELL, J.W.; VINCENT, C.K.; WALLACE, M.D.; WARNICK, A.C. (1971) Developmental failure of swine embryos restricted to the oviductal environment. *J. Reprod. Fertil. 24:* 445-448.
55. BURDICK, H.O.; WHITNEY, R.; PINCUS, G. (1937) The fate of mouse ova tube-locked by injections of oestrogenic substances. *Anat. Rec. 67:* 513-519.
56. KIRBY, D.R.S. (1962) The influence of the uterine environment on the development of mouse eggs. *J. Embr. Exp. Morph. 10:* 496-506.
57. ORSINI, M.W.; McLAREN, A. (1967) Loss of the zona pellucida in mice and the effect of tubal ligation and ovariectomy. *J. Reprod. Fert. 13:* 485-499.
58. BRONSON, R.; CUNNANE, M. (1975) Transfer of uterine implantation blastocysts to the oviduct in mice. *Fertil. Steril. 26:* 455-459.
59. TUTTON, D.A.; CARR, D.H. (1984) The fate of trophoblast retained within the oviduct in the mouse. *Gynecol. Obstet. Invest. 17:* 18-24.
60. STONE, S.L.; HAMNER, C.E. (1977) Hormonal and regional influences of the oviduct on the development of rabbit embryos. *Biol. Reprod. 16:* 638-646.
61. STONE, S.L.; RICHARDSON, L.L.; HAMNER, C. E.; OLIPHANT, G. (1977) Partial characterization of hormone-mediated inhibition of embryo development in rabbit oviductal fluid. *Biol. Reprod. 16:* 647-653.
62. RICHARDSON, L.L.; HAMNER, C.E.; OLIPHANT, G. (1980) Some characteristics of an inhibitor of embryonic development. from rabbit oviductal fluid. *Biol. Reprod. 22:* 553-559.
63. CLINE, E.M.; RANDALL, P.A.; OLIPHANT G. (1977) Hormone-mediated oviductal influence on mouse embryo development. *Fertil. Steril. 28:* 766-771.
64. PAUERSTEIN, C.J.; EDDY, C.A.; KOONG, M.K.; MOORE, G.D. (1990) Rabbit endosalpinx suppresses ectopic implantation. *Fertil. Steril. 54:* 522-526.