Consequences of accelerated ovum transport, including a re-evaluation of Estes' operation

C. E. Adams

*A.R.C. Institute of Animal Physiology, Animal Research Station, 307 Huntingdon Road,
Cambridge CB3 0JQ, U.K.*

Introduction

Within the past decade the mammalian oviduct and its function has attracted considerable attention, having been the subject of three international symposia (Hafez & Blandau, 1969; Johnson & Foley, 1974; Harper *et al.*, 1976) and several reviews (Blandau, 1969, 1973; Hafez, 1973; Dukelow & Riegler, 1974).

The present review is concerned with the consequences of accelerated ovum transport, a topic which has recently assumed greater significance due to its potential as a means of contraception (World Health Organization, 1976), because of experimental evidence that eggs fail to survive if transferred prematurely to the uterus. However, just as with ectopic, tubal pregnancy, which is practically unknown in non-human primates (Adams, 1977), the position may well be different in women, as indicated by reports of successful Estes' operations (Iklé, 1961). This operation will therefore be considered particularly from the point of view of fertility subsequent to it.

In the Eutheria, with a few notable exceptions, which include the cat, dog, mink and pig, the eggs normally enter the uterus 3 to 4 days after ovulation. Perusal of the literature suggests that the mechanisms responsible for ovum transport are normally remarkably 'fail-free' in so far as the majority, if not all, fertilized eggs do apparently reach the uterus at the right time. Moreover, this seems to apply equally to unfertilized eggs, the only exceptions so far reported being those of the horse (see van Niekerk, 1976) and the long-tongued bat (Rasweiler, 1972). Reports of accelerated or retarded transport almost invariably relate to experimental situations, such as treatment with oestrogens or progestagens or 'abnormal' conditions, for example superovulation (see Bennett, 1970) or following induced ovulation during pregnancy (Adams, 1968).

The pattern of egg movement through the oviduct is neither continuous nor uniform—passage through the ampulla tends to be rapid and through the isthmus slow; the latter is thought to be purposeful, as expressed by Corner (1942): "I believe that one of the most important functions of the oviduct is to hold back eggs until the uterus is ready for them . . . I have long thought we ought not to emphasize the oviduct solely as an organ for transporting the ova, but rather as a means of delaying their transportation. The uterus must have time to get ready for its exigent tenant".

It is generally agreed that the timing of the eggs' entry into the uterus is critical, as the following statements serve to illustrate: "it is critical that fertilized eggs reach the uterus at an appropriate progestational stage of the ovarian cycle" (Hafez, 1973); "the delay in tubal ovum passage appears critical to successful ovum implantation" (Pauerstein, 1974); "it is firmly established that the timing of the passage of the cleaving ovum through the oviduct is absolutely critical" and "delivery of the embryo to the uterus at specifically the right time is required" (Greep, Koblinsky & Jaffe, 1976). Such statements are no doubt strongly influenced by the results of experiments involving the transfer of eggs between donor and recipient animals. With few exceptions research into synchronization requirements has tended to focus on defining optimal 'donor-recipient' combinations and, as a consequence, there are relatively few studies

involving the ‘young egg–young uterus’ combination, the equivalent of accelerated tubal transport. The egg transfer technique permits the most precise timing to be achieved in simulating accelerated transport. Until now, observations on egg transfer to the uterus before, at or shortly after ovulation, were available for only 4 species: rabbit (Chang, 1950; Adams, 1970), mouse (Tarkowski, 1959; Doyle, Gates & Noyes, 1963), rat (Noyes & Dickmann, 1960), and ferret (Chang, 1969).

Rabbit

Chang (1950) transferred a total of 148 eggs to 3 Day-0, i.e. at the time of hCG injection, and 8 Day-1 recipients; no young were obtained. In a further experiment to determine the fate of the eggs, Chang found that when Day-1 eggs were transferred to the uterus before ovulation all degenerated. Specific results are not distinguishable because several different groups were presented together. When Day-2 eggs were transferred to the Day-1 uterus only 5 (13·5%) of the 37 eggs were recovered 2 days later. It was inferred that the missing eggs had been expelled from the uterus shortly after transfer. In the follicular phase, eggs are expelled from the uterus within a few hours (Adams, 1970). Chang (1955) presented further observations on the transfer of eggs to the oestrogen-dominated uterus. He transferred a total of 82 recently ovulated eggs to the uteri of 6 does, which had been mated 12 h earlier, but by 24 h after transfer he was able to recover only 11 eggs (13%), all of which were degenerate, including 9 which had lost the zona pellucida. It was concluded that the transferred eggs had “probably disintegrated” *in utero*. For Day-2 eggs transferred to a Day-2 uterus 22% (17/76) survived to term (Chang, 1950), and very similar results have been obtained by C. E. Adams (unpublished), using 2½ day eggs (see Table 1).

Table 1. The outcome of transferring 2- or 2½-day eggs to the Day-2 uterus in the rabbit

	Chang (1950)	C. E. Adams
No. of recipients	7	6
No. pregnant	5	6
No. of eggs transferred	76	63
No. of implantations	?	27 (43%)
No. of young born	17 (22%)	17 (27%)

Mouse

Tarkowski (1959) transferred 41 2-cell eggs to 6 recipient mice on the 2nd day after copulation and obtained “no positive results”. Examinations on the 5th and 7th days revealed complete absence of any trace of implantation, and on the 4th day of any transplanted eggs in the uterus. Tarkowski concluded that the eggs could not maintain themselves, being “discharged within a short time outside the genital system”. However, no proof of expulsion was provided. Following the transfer of 82 eggs to the uteri of 14 mice on Day 2, Doyle *et al.* (1963) obtained no young.

Rat

Noyes & Dickmann (1960) transferred a total of 94 2-, 3- and 4-day rat eggs to the uterus on the 2nd day of pseudopregnancy: none survived. The actual fate of the eggs was not established.

Ferret

When pronuclear ferret eggs (8 to 18 h after ovulation) were transferred to the Day-2 uterus 15% (3/20) implanted and 1 live embryo was found at autopsy between 2 and 3 weeks after transfer (Chang, 1969). It was, therefore, suggested that the ferret uterus is less hostile than that of the rabbit.

As a result of these experiments, two possibilities have been considered to account for the loss of eggs, namely expulsion from the uterus and destruction *in utero*. However, their relative importance is still not known for any single species.

Present experiments

In an attempt to resolve the issue I have investigated the effect of time elapsing from ovulation on retention of eggs within the uterus of the rabbit. For this purpose, a total of 450 eggs was transferred to the uteri of 43 recipients at fixed times between 24 and 84 h after giving an ovulating injection of 30 i.u. hCG (Gonadotrophin: Paines & Byrne Ltd, Greenford, Middlesex) and then, after 24 h, recovery of the eggs was attempted at autopsy. The vagina was ligated before egg transfer. Text-figure 1 shows that of eggs transferred at 24 or 36 h after hCG practically all (82/85) were subsequently recovered from the vagina and even at 48 h, the majority (25/36) were still to be found there. It was only from about 60 h onwards that 80% or more of the eggs were retained *in utero*. Thus, the normal time of entry of rabbit eggs into the uterus, given recently as 78–84 h *p.c.* (Hodgson & Pauerstein, 1976), and maximal uterine retention appear to be highly correlated.

Whilst these findings make it clear why transfers to the very early luteal-phase uterus, especially before 48 h *p.c.*, have proved so unsuccessful they do not tell us what would be the fate of eggs if they could be held within such uteri. In an attempt to answer this question a further series of experiments was therefore undertaken, initially using uterine relaxants, including ritodrine hydrochloride, isoxsuprine hydrochloride, fenoterol and ibuprofen, but none gave entirely satisfactory results in so far as eggs were still expelled. For that reason ligation was used. At laparotomy eggs were recovered by flushing the oviducts of does mated 24, 36 or 48 h previously, and then transferred to the uterine horns immediately after applying ligatures just distal to the cervixes. In the case of 12 h recipients, the eggs came from donors 24 h *p.c.* Groups of recipients were autopsied 1 or 3 days later in order to recover eggs, or examined by

Text-fig. 1. The recovery of eggs from the uterus and vagina of rabbits 24 h after transfer to the uterus. (A total of 450 eggs was transferred to 43 rabbits.)

Text-fig. 2. The survival of rabbit eggs relative to the time of transfer to the ligated uterus: □, 24 h after transfer (16 rabbits: 137 eggs); ○, 72 h after transfer (11 rabbits: 96 eggs); △, implantation sites (13 rabbits: 137 eggs).

laparotomy on Days 8 or 10 to count implantations. The results are illustrated in Text-fig. 2. Clearly, the picture changes dramatically during the 36-h period following ovulation. Of eggs transferred at 12 or 24 h, the majority (28/40 and 23/42 respectively) perished within 24 h, whilst those that were recovered often appeared abnormal. Out of 36 eggs transferred at 36 h 20 could be recovered 3 days later but the majority (34/40) in this group failed to implant, apparently because of problems at the blastocyst stage. Nevertheless, the fact that a few (6/40) succeeded in implanting is noteworthy. Significantly, the majority (17/22) of eggs transferred at 48 h were able to survive and undergo implantation, thereby proving that the relative lack of success hitherto associated with this stage is due to expulsion rather than endometrial incompatibility. These findings suggest that endometrial and myometrial 'receptivity' evolve at different rates in the rabbit. The endometrial (secretions) rate of change, as reflected in egg recovery, development and survival, seems to progress quite uniformly.

Transplantation of an ovary into the uterus

Perhaps the most dramatic means of achieving accelerated ovum transport is by transplanting an ovary into the uterine cavity. However, according to Estes & Heitmeyer (1934) "experimental implantations or transplantation of the ovary into the uterus have been lamentably few". Apart from their own observations, they cited only two reports, those of Uffreduzzi (1911) and Mairano & Placeo (1928).

Uffreduzzi (1911), using rabbits and guinea-pigs, concluded that with implantation of ovary, or piece of ovary, into the uterine cavity, projecting into the cavity or in the uterine cornu, pregnancy cannot occur. Mairano & Placeo (1928) also used guinea-pigs, some of which they observed for as long as 15 months following implantation of the pedicled ovary into the cut apex of the uterine horn and removal of the other ovary. Satisfactory function of the graft can be inferred as oestrous cycles continued but pregnancy did not occur.

Estes & Heitmeyer (1934) themselves performed ovarian transplantation into the uterus of 18 rabbits, in 16 of which one or both ovaries persisted in the cavity of the uterus. Functionally normal ovarian tissue was demonstrated microscopically in 12 of the animals at autopsy. Moreover, evidence of recent ovulations was provided both in the form of corpora lutea and recovery of ova. However, in spite of frequent matings, no pregnancy resulted in animals observed for up to 8 months. Estes & Heitmeyer suggested, amongst other things, that the failure may have been due to expulsion of ova from the uterus soon after ovulation.

Fertilization *in utero*

In the rabbit fertilization *in utero* is possible, as demonstrated by Chang (1955), Bedford (1969), Adams (1970) and Glass (1972). Chang transferred 26 freshly ovulated eggs to the uterus of a mated doe; at recovery 6 h later the eggs were found to be fertilized but some showed signs of degeneration—a finding which the other investigators have each confirmed. Chang's postulate of an injurious uterine factor ("une certaine substance dans l'uterus qui s'avère nocive pour les oeufs fecondés") therefore seems well justified.

If the eggs are recovered shortly after (<2–3 h) transfer, by which time sperm penetration has occurred, and then re-transferred to the oviduct of a synchronous, pseudopregnant recipient normal development can occur (Adams, 1970; Glass, 1972).

The only other species in which fertilization *in utero* has been attempted appear to be the golden hamster, rat and pig. In the hamster fertilization did not occur (0/392 eggs) and, as in the rabbit, adverse effects of uterine fluid on the eggs were noted (Hunter, 1968). Similar experiments with rats also gave negative results (Bedford, 1969). In the pig, fertilization can occur *in utero* but the success rate (5%) is low (Baker & Polge, 1973).

Estes' operation

Estes' operation was evolved by W. L. Estes Sr in 1904–1905 following the pioneer work of Morris (1895), Franck (1898) and Dudley (1900). A detailed description of the operation was provided by Estes Jr (1924), who reported that it had been used in the Estes' clinic in about 100 selected cases, 27 of which he reviewed. The operation consists of bilateral salpingectomy and unilateral oophorectomy followed by implantation of the cut surface of the remaining ovary (still attached to its pedicle) into the resected stump of one oviduct and portion of uterine horn.

Table 2. Incidence of pregnancy following Estes' operation (abstracted from Iklé (1961))

Author	Location	No. of cases	No. becoming pregnant
Morris (1895)	New York	1	1
Estes & Heitmeyer (1934)	New York	50	4
V. Mikulicz (1936)	Königsberg	9	0
Tritschkoff (1936)	Sofia	20	4
Moraes (1949)	Rio de Janeiro	7	1
Westman (1950)	Stockholm	23	4
Preston (1953)	Nairobi	10	3
Ten Berge (1956)	Groningen	16	0
Vara (1958)	Helsinki	128	8
Gaillard-Slavinska (1959)	Tarbes	5	3
Iklé (1961)	St Gallen	1	1
Total		270	29

Table 3. Incidence of pregnancy relative to menstrual history in patients undergoing Estes’ operation

Author	No. of cases	No. of pregnancies	Estimated no. of menstrual cycles	Notes
Petit (1922)	1	1	24	Normal child
Bainbridge (1923b)	1	1	9	1st menstruation 4 months after operation (39 years) conceived 9 months later, normal child
Estes Jr (1924)	1	1	49	Operation 12.8.1916 (30 years), conceived Oct. 1920; live birth
Douglass (1926)	1	1	3	Operation to conception = 3 months, aborted at 2½ months
Estes Jr & Heitmeyer (1934)	1	4	114	Operation 1905 (18 years), last conceived June 1918: 3 full-term and 1 still-born
Preston (1953)	1	4	23	Operation Sept. 1946; last pregnancy ended Feb. 1952: 3 full-term and 1 abortion at 3 months
Preston (1953)	1	1	43	Operation Jan. 1947: pregnancy ended June, 1951
Iklé (1961)	1	1	2	Operation Nov. 1951 (32 years), aborted 17th week
Total	8	14	267	

To judge from the literature, much of which is noteworthy for its inaccessibility, Estes’ operation was employed quite widely during the first half of the century, though mostly involving relatively few cases. The most comprehensive reviews are those of Bainbridge (1923a), Estes Jr (1924), Estes & Heitmeyer (1934) and Iklé (1961) who listed 270 cases from 11 centres (Table 2). Nine of 11 reports included patients who became pregnant, a few on 2 or more occasions, giving a total of 29 pregnancies, some of which were carried to term. Traditionally, the success of Estes’ operation has been expressed in terms of “pregnancies per group of patients”, e.g. “pregnancy after operation occurred in 4 cases (15 per cent of 27)” (Estes, Jr, 1924), or “the percentage of successful results in the total number of patients operated upon is 14.28 (3/21) whereas the percentage of success in only those which I have been able to follow up is as high as 30 (3/10)” (Preston, 1953). This, undoubtedly, exaggerates the success rate for it neglects to take into account the number of occasions on which conception might have occurred. It may be recalled that young women were normally chosen for the operation—the mean age of the Estes’ (1924) sample was 27.7 years—because in older women there is “less desire for pregnancy and less likelihood of pregnancy occurring”. Estes Jr (1924) reported that following the operation there was “a fairly normal menstruation”; it was regular in 19 out of 24 patients (79%), irregular in 4 and absent in only 1 in which only a small piece of ovary could be saved. Moreover, according to Estes & Heitmeyer (1934) only rarely does the transplanted ovary become cystic and require removal.

Unfortunately, many of the case histories are incomplete. However, by selecting the best documented examples, it appears that the 8 individuals (14 pregnancies) listed in Table 3 may have experienced as many as 267 cycles, the equivalent of 19 per conception, after making due allowances for interruption of menstrual cycles during pregnancy and *post partum*. On that basis the odds of becoming pregnant after Estes’ operation could be as low as 1 in 177 ($220/29 \times 267/14$) or 6.78 per 100 woman years. In making this calculation, it was assumed that intercourse was regular and unprotected, since such patients were most probably highly motivated to have children.

Estes’ operation represents an extreme situation in so far as ovulation may take place directly into the uterus, though perhaps not invariably, depending upon the topographical relationship

between the point of ovulation and the resected stump of tube, thereby eliminating the tubal phase in the life of the egg. Yet, in women, as the above results testify, pregnancy is still possible, though at a reduced level. If, in women having normal oviducts, the egg was to arrive in the uterus a few hours after ovulation, already fertilized, the chances of pregnancy becoming established might well increase significantly, as J. H. Marston (personal communication) has demonstrated in the rhesus monkey by means of egg transfer. In that case, any agent designed to act as a contraceptive by accelerating ovum transport would need to be extremely efficacious; if not, it will be necessary to continue the attack at the uterine level. In any event, there is a growing need to augment our knowledge of uterine secretions and control of myometrial function during the immediate post-ovulatory phase, even for reasons other than contraception. To echo our Chairman's remarks, even if the quest for new contraceptive approaches ends in failure, the effort need not have been in vain.

References

- Adams, C.E. (1968) Ovarian response to human chorionic gonadotrophin and egg transport in the pregnant and post-parturient rabbit. *J. Endocr.* **40**, 101–105.
- Adams, C.E. (1970) Egg–uterus interrelationships. *Adv. Biosci.* **4**, 149–162.
- Adams, C.E. (1977) Ectopic pregnancy. *Bibliophy Reprod.* **30**, 97–98.
- Baker, R.D. & Polge, C. (1973) Sperm penetration of pig eggs *in utero*. *J. Reprod. Fert.* **33**, 347–350.
- Bainbridge, W.S. (1923a) Transplantation of human ovaries: present status and future possibilities. *Am. J. Obstet. Gynec.* **5**, 493–498.
- Bainbridge, W.S. (1923b) Double salping-oöphorectomy with partial auto-ovarian transplantation, followed by 12 years of normal menstruation, normal pregnancy and uncomplicated menopause at 51 years of age. *Am. J. Obstet. Gynec.* **5**, 379.
- Bedford, J.M. (1969) Limitations of the uterus in the development of the fertilizing ability (capacitation) of spermatozoa. *J. Reprod. Fert., Suppl.* **8**, 19–26.
- Bennett, J.P. (1970) The effect of drugs on egg transport. *Adv. Biosci.* **4**, 165–178.
- Blandau, R.J. (1969) Gamete transport. Comparative aspects. In *The Mammalian Oviduct, Comparative Biology and Methodology*, pp. 129–162. Eds E. S. E. Hafez & R. J. Blandau. University of Chicago Press.
- Blandau, R.J. (1973) Gamete transport in the female mammal. In *Handbook of Physiology*, Section 7, Endocrinology, Vol. 2, Pt. 2, pp. 153–163. Eds R. O. Greep & E. B. Astwood. American Physiological Society, Washington, D.C.
- Chang, M.C. (1950) Development and fate of transferred rabbit ova or blastocyst in relation to the ovulatory time of recipients. *J. exp. Zool.* **114**, 197–216.
- Chang, M.C. (1955) Développement de la capacité fertilisatrice des spermatozoïdes du lapin à l'intérieur du tractus genital femelle et fécondabilité des oeufs de lapine. In *La Fonction Tubaire et ses Troubles*, pp. 40–52. Masson, Paris.
- Chang, M.C. (1969) Development of transferred ferret eggs in relation to the age of corpora lutea. *J. exp. Zool.* **171**, 459–464.
- Corner, G.W. (1942) *The Hormones in Human Reproduction*. Princeton University Press.
- Douglass, M. (1926) Pregnancy after salpingectomy. *Surg. Gynec. Obstet.* **43**, 480–482.
- Doyle, L.L., Gates, A.M. & Noyes, R.W. (1963) Asynchronous transfer of mouse ova. *Fert. Steril.* **14**, 215–225.
- Dudley, A.P. (1900) Post-Graduate **15**, 546. (Cited by Estes & Heitmeyer, 1934.)
- Dukelow, W.R. & Riegler, G.D. (1974) Transport of gametes and survival of the ovum as functions of the oviduct. In *The Oviduct and its Functions*, pp. 193–220. Eds A. D. Johnson & C. W. Foley. Academic Press, New York.
- Estes, W.L., Jr (1924) Ovarian implantation. The preservation of ovarian function after operation for disease of the pelvic viscera. *Surg. Gynec. Obstet.* **38**, 394–398.
- Estes, W.L., Jr & Heitmeyer, P.L. (1934) Incidence of pregnancy following ovarian implantation. *Am. J. Surg.* **24**, 563–581.
- Franck, A. (1898) Ueber transplantation der Ovarien. *Zentbl. Gynäk.* **22**, 444–446. (Cited by Estes & Heitmeyer, 1934.)
- Glass, R.H. (1972) Fate of rabbit eggs fertilized in the uterus. *J. Reprod. Fert.* **31**, 139–141.
- Greep, R.O., Koblinsky, M.A. & Jaffe, F.S. (1976) *Reproduction and Human Welfare: A Challenge to Research*. The MIT Press, Cambridge, Massachusetts.
- Hafez, E.S.E. & Blandau, R.J. (1969) *The Mammalian Oviduct, Comparative Biology and Methodology*. University of Chicago Press.
- Hafez, E.S.E. (1973) Gamete transport. In *Human Reproduction, Conception and Contraception*, Ch. 5, pp. 85–118. Eds E. S. E. Hafez & T. N. Evans. Harper Row, New York.
- Harper, M.J.K., Pauerstein, C.J., Adams, C.E., Coutinho, E.M., Croxatto, M.B. & Paton, D.M. (Eds) (1976) *Ovum Transport and Fertility Regulation*. Scriptor, Copenhagen.
- Hodgson, B.J. & Pauerstein, C.J. (1976) Comparison of oviductal transport of fertilized and unfertilized ova after HCG or coitus induced ovulation in rabbits. *Biol. Reprod.* **14**, 377–380.
- Hunter, R.H.F. (1968) Attempted fertilization of

- hamster eggs following transplantation into the uterus. *J. exp. Zool.* **168**, 511–516.
- Iklé, F.A.** (1961) Schwangerschaft nach Implantation des ovars in den Uterus. *Gynaecologia* **151**, 95–99.
- Johnson, A.D. & Foley, C.W. (Eds)** (1974) *The Oviduct and its Functions*. Academic Press, New York.
- Noyes, R.W. & Dickmann, Z.** (1960) Relationship of ovular age to endometrial development. *J. Reprod. Fert.* **1**, 186–196.
- Mairano, M. & Placeo, F.** (1928) Sul comportamento degli autotrapianti ovarici peduncolati nella cavità dell'utero. *G. Accad. Med. Torino* **91**, 206–215. (Cited by Estes & Heitmeyer, 1934.)
- Morris, R.I.** (1895) The ovarian graft. *New York Med. J.* **42**, 436–437.
- Pauerstein, C.J.** (1974) *The Fallopian Tube: a Reappraisal*. Lea & Febiger, Philadelphia.
- Petit, R.** (1922) Conservation et transposition dans l'utérus d'un fragment d'ovaire après salpingectomie pour suppuration. Grossesse consecutive dans un cas. (Rapport de M. Tuffier.) *Bull. Mém. Soc. Chir. Paris* **48**, 1051–1053.
- Preston, P.G.** (1953) Transplantation of the ovary into the uterine cavity for the treatment of sterility in women. *J. Obstet. Gynaec. Br. Emp.* **60**, 862–964.
- Rasweiler, J.J.** (1972) Reproduction in the long-tongued bat, *Glossophaga soricina*. 1. Preimplantation development and histology of the oviduct. *J. Reprod. Fert.* **31**, 249–262.
- Tarkowski, A.K.** (1959) Experiments on the transplantation of ova in mice. *Acta theriol.* **2**, 251–267.
- Uffreduzzi, O.** (1911) Studio sperimentale su alcuni interventi conservativi nei genitali interni femminili. (Trapianto dell'ova; nell'utero; uterostomia salpingostomia.) *Annali Obstet., Milano* **ii**, 57–100. (Cited by Estes & Heitmeyer, 1934.)
- Van Niekerk, C.H.** (1976) Retention of unfertilized ova in the oviduct of mares. In *Ovum Transport and Fertility Regulation*, pp. 375–380. Eds M. J. K. Harper, C. J. Pauerstein, C. E. Adams, E. M. Coutinho, M. B. Croxatto & D. M. Paton. Scriptor, Copenhagen.
- World Health Organization** (1976) Expanded programme of research development and research training in human reproduction. *5th Ann. Rep.*, pp. 52–53. Geneva, Switzerland.