Attempts to modify ovum transport in women

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Summary. The effect of various compounds upon ovum transport was investigated by comparing the location of the ovum in treated and untreated subjects between 24 and 120 h following the LH peak. The compounds were administered in the immediate post-ovulatory period. Methyl ergonovine and 15(S)15-methyl-prostaglandin F-2 α were given at doses which stimulate human tubal contractility *in vivo*. Ergonovine maleate, also at a dose which stimulates tubal contractility, was given in conjunction with a β -adrenergic agonist (Ritodrine) in order to prevent closure of the lumen in the narrow portions of the oviduct. Oestradiol-17 β was given i.m. as a single dose of 5 mg. Utilizing overall recovery of ova, their segmental distribution within the Fallopian tube or their distribution between Fallopian tubes and uterus as the criteria for comparison between control and treatment groups, no evidence was obtained that any of the treatments accelerated transport. Oestradiol and Ritodrine should be tested at higher doses and an experimental design to disclose delayed transport should be used before concluding that these treatments have no effect upon ovum transport in women. The present results permit the conclusion that compounds which stimulate tubal contractility do not necessarily accelerate ovum transport. The implications of these findings for our current concepts on the physiology and pharmacology of ovum transport are discussed.

Introduction

Since the demonstration by Burdick & Pincus in 1935 that oestrogen administration caused tube-locking of ova in mice, several reports have been published which show that ovum transport can be either accelerated or retarded in laboratory animals by provoking an excess or deficit of sex steroids or by administration of a variety of drugs. Most of the information on the subject has been reviewed at two recent symposia (Pauerstein, 1975; Harper *et al.*, 1976). Since then, several groups of workers have presented new data on ovum transport under the influence of hormonal changes and drug administration in rabbits (El-Banna, Sacher & Schilling, 1976; El-Banna & Sacher, 1976; Spilman, Beuving, Roseman & Larion, 1976; Hodgson, 1976; Aref & Hafez, 1976; Tsutsumi, Takeda, Yamamoto & Tanabe, 1976; Spilman & Beuving, 1977; Takeda, Tsutsumi, Tanabe & Yamamoto, 1977; Valenzuela, Ross, Hodgson, Harper & Pauerstein, 1977) and in guinea-pigs (Maia, Salinas, Fernández & Pauerstein, 1977).

One can infer from these observations that even if ovum transport is programmed by neuro-endocrine signals the programme is susceptible to modification by proper pharmacological manipulation. This being the case pharmacological modification of ovum transport becomes of interest to the physiologist as a tool to disclose the mechanisms involved in the regulation of the process. This experimental exercise is of interest also to the clinician, for it can eventually lead to the identification of compounds that can be used therapeutically to change the rate of ovum transport either for the improvement or derangement of fertility.

Methods for studying ovum transport in women have been developed recently and they have permitted us to establish the normal time course of the process (Croxatto *et al.*, 1978a). This in turn has been used as a base-line to assess the ability of several compounds to change the rate of transport.

This presentation is intended to review reported attempts to modify ovum transport and to describe additional unpublished observations.

Materials and Methods

Since all the procedures have been described in detail elsewhere (Croxatto *et al.*, 1978a), only a brief account will be given here. All the volunteers included in the study were normal and healthy women who underwent laparotomy for surgical sterilization. [The investigators from the Pontificia Universidad Católica did not participate in the selection or in the clinical and surgical management of patients undergoing surgical sterilization.] The use of hormonal contraceptives was avoided during 2 cycles preceding surgery and barrier methods were used instead. Beginning on Day 10 of the cycle a blood sample was taken each morning until the day of surgery for the radioimmunoassay of oestradiol, progesterone and LH. Oestradiol concentrations were assayed daily until the preovulatory oestrogen peak was detected, and this peak was used to time drug administration and ovum search. Treatment was usually started 48 h after the oestradiol peak by which time the ovum is already in the Fallopian tube. The tube was searched for ova 24 or 48 h after the beginning of treatment if the possibility of acceleration of transport was under investigation, whereas for identifying tube-locking effects the search was performed 120 or 144 h after the oestradiol peak because by this time the ovum should normally be in the uterus.

During laparotomy both ovaries were inspected to identify the ovulatory side. The ipsilateral oviduct was immediately divided by means of ligatures into four segments and removed. After the other oviduct was ligated and sectioned, the uterine cavity was flushed with saline (0.15 M-NaCl) by transfundal puncture. Recovery of injected fluid was not always successful and therefore the number of attempts for ovum recovery from the uterus is slightly less than for ovum searches in the oviducts. Each segment of the oviduct was flushed independently and the search for ova was carried out by examining the tubal and uterine flushings in culture dishes with the aid of a dissecting microscope ($\times 12$ –25 magnification).

When the hormone assays had been completed and the segment which contained the ovum had been identified histologically, the location of the ovum was correlated with the interval from LH peak to surgery and treatment if any. Intervals from LH peak are expressed in multiples of 24 h using the sample with the highest LH concentration to identify the day of the peak. Oestrogen and progesterone levels were used to assist in resolving doubtful cases.

The following compounds were tested for their ability to accelerate ovum transport. (1) Methyl ergonovine, 0.2 mg i.m. every 4 h, was tested in 32 subjects. The total dose administered varied from 0.6 to 4.8 mg (Guiloff *et al.*, 1978). (2) Ergonovine maleate (0.2 mg) plus 10 mg 4-hydroxy α -1-2-4-hydroxyphenyl ethylamino acetyl-benzyl alcohol hydrochloride (Ritodrine: Phillips-Duphar, Weesp, Netherlands) was given orally every 4 h for 4 doses in 12 subjects. (3) 15(S)-15-Methyl-prostaglandin F-2 α , infused i.v. at a rate of 1.5 μ g/kg/h was tested in 5 subjects. The total dose varied between 216 and 500 μ g (Croxatto *et al.*, 1978b). (4) Oestradiol-17 β , microcrystalline suspension in aqueous vehicle, given as a single dose of 5 mg i.m., was tested in 9 subjects.

Fifty-four (54) untreated subjects served as a control group (Croxatto *et al.*, 1978a).

Results

Details on the time relationships between the LH peak, treatment and surgery in subjects treated with oestradiol-17 β or ergonovine plus Ritodrine have not been published before and they are

presented in Table 1. For similar details in the groups treated with methyl ergonovine or the prostaglandin, the reader is referred to the original publication cited in the text.

Table 1. Distribution of women according to the interval between LH peak, treatment and surgery

Treatment*	No. of women	Interval between LH peak and treatment (h)	Interval between LH peak and surgery (h)
Oestradiol-17 β	1	24	72
	1	48	72
	6	48	96
	1	72	144
Ergonovine + Ritodrine	2†	0	48
	3	24	48
	4	24	72
	1	48	120
	2	72	120

* See text for details.

† One subject treated again 24 h after LH peak.

Table 2. Ovum recovery from the Fallopian tubes and uterus of women given various treatments

Treatment*	No. of women	
	Studied	From whom eggs recovered (%)
None (control)	54	24 (44.4)
Methyl ergonovine	32	13 (40.6)
Ergonovine + Ritodrine	12	10 (83.3)
15(S)15-Methyl-PGF-2 α	5	5 (100.0)
Oestradiol-17 β	9	6 (66.6)

* See text for details.

Overall ovum recovery in control and treated subjects is shown in Table 2. The lowest recovery rate 44%, was obtained in the control group and the highest one, 100%, was observed in the group treated with prostaglandin (PG) F-2 α . The highest recovery rates in all the groups were attained when the oviducts were searched for eggs 72 h after the LH peak.

The location of ova at various intervals following the LH peak in untreated subjects is shown in Tables 3 and 4. Up to 72 h after the LH peak the ova were recovered only from the oviduct, while at 96 h, 5 out of 6 ova were in the oviduct and one was in the uterus. After this time ova were found only in the uterus. Regardless of time of recovery, the most frequent location within the oviduct was the ampullary segment. Only 2 of the 19 ova found in the oviduct had passed beyond the ampullary-isthmic junction and these were at the latest time at which ova could be recovered from the tubes.

As shown in Tables 3 and 4 in none of the treated subjects were ova recovered from the uterus earlier than 96 h following the LH peak and within this interval the rate of recovery from the Fallopian tubes was comparable to or higher than that of the untreated control group. In one subject treated with methyl ergonovine (Table 4) and another treated with ergonovine plus Ritodrine (Table 4), the ovum was recovered from the oviduct at 120 h. In each of the treated groups most of the ova recovered from the Fallopian tubes were located in the ampulla (Table 3).

Table 3. Distribution of ova in the Fallopian tubes of untreated* and treated† women

Treatment	Interval between LH peak and surgery (h)	No. of ova recovered from:		
		Ampulla	Ampullary–isthmic junction	Isthmus
Control	24	1	1	—
Control	48	1	—	—
Control	72	7	4	—
Control	96	3	—	2
Ergonovine + Ritodrine	48–120	10	0	0
Methylegonovine‡	48–120	10	3	0
Oestradiol-17β	72–96	4	1	1
15(S)15-Methyl-PGF-2α§	48–96	3	1	1

* Modified from Croxatto *et al.* (1978a).

† See text for details.

‡ Taken from Guiloff *et al.* (1978).§ Taken from Croxatto *et al.* (1978b).

One ovum had passed beyond the ampullary–isthmic junction in the group treated with oestradiol and in the group treated with prostaglandin and in both instances the ovum was recovered at the 96 h time. One of the ova found in the segment containing the ampullary–isthmic junction was recovered at 48 h and all the others at later intervals.

Discussion

The distribution of ova in the control group can be used to establish the landmarks between normal, accelerated and retarded transport. According to this criterion transport should be considered to be definitely accelerated when ova are recovered from the uterus at 72 h or earlier following the LH peak. Recovery from the uterus at 96 h would confirm accelerated transport only if it involves a significantly greater proportion of ova. Accelerated transport through the Fallopian tubes could also be inferred from an increased proportion of ova recovered from the isthmic segment at 72 h or earlier. Finally, accelerated transport could be suspected if the overall rate of ovum recovery was significantly depressed. None of the results obtained in the treated groups fulfils any of the above criteria and therefore it can be concluded that the treatments utilized failed to accelerate ovum transport.

Retarded transport can be inferred when ova are found in the oviducts at 120 h or later, as was found with 2 subjects, both treated with ergot derivatives, but because of the small size of the sample we cannot conclude that the treatment caused delayed transport. Nevertheless, this possibility merits further investigation.

Concerning the biological activity of the compounds tested, previous studies have shown beyond doubt that methyl ergonovine (Guiloff *et al.*, 1978) and 15(S)15-methyl-PGF-2α (Croxatto *et al.*, 1978b) administered in the same manner as reported here do have a lasting stimulatory effect upon human oviductal motility *in vivo*. The observations on ovum transport reviewed here demonstrate that the increased motility of the tube produced by methyl ergonovine or PGF-2α does not lead to accelerated ovum transport.

One possible explanation of this result is that excessive tonic stimulation of the circular muscle could be associated with further narrowing or closure of the isthmic lumen, thus preventing passage of the ovum beyond the ampullary–isthmic junction. The same mechanism could be operating also at the level of the interstitial portion of the tube because both substances have the ability to stimulate myometrial contractions. Considering this argument it was proposed (Coutinho, 1976), that ovum transport could be accelerated if isthmic and/or myometrial muscle

Table 4. Ovum recovery from the Fallopian tubes (FT) and uterus (UT) of treated and untreated women at different times after the LH peak (no. of ova recovered/no. of cases)

Treatment	No. of women studied	Interval between LH peak and surgery											
		24 h		48 h		72 h		96 h		120 h		144 h	
		FT	UT	FT	UT	FT	UT	FT	UT	FT	UT	FT	UT
Control	54	2/4	0/3	1/6	0/5	11/19	0/17	5/13	1/12	0/6	2/3	0/6	2/5
Oestradiol-17 β	9					2/2	0/2	4/6	1/6			0/1	0/1
Ergonovine maleate + Ritodrine	12			5/5	0/4	4/4	0/3			1/3	0/1		
Methylexgonovine†	32	0/1	0/1	3/7	0/7	7/9	0/8	2/11	0/10	1/4	0/3		
15(S)15-Methyl-PGF-2 α §	5			2/2	0/2	2/2	0/2	1/1	0/1				

* Modified from Croxatto *et al.* (1978a).† Modified from Guiloff *et al.* (1978).‡ Modified from Croxatto *et al.* (1978b).

were relaxed concomitantly with the stimulation of tubal contractility. Since stimulation of β -adrenergic receptors has been shown to relax the circular muscle of the isthmus (Rosenblum & Stein, 1966), and also the myometrium (Coutinho, Maia & Filho, 1974), Ritodrine, a β -adrenergic agonist, was chosen for this purpose. Ergonovine maleate was selected to activate rhythmic contractions because it has been shown that, at the doses used in the present study, it does stimulate tubal contractility in women (Coutinho, 1971). Ritodrine, on the other hand, at the dose of 10 mg orally, relaxes the myometrium of the non-pregnant uterus but has little effect on the myosalpinx (Coutinho *et al.*, 1974). However, there are no data on the effect of these two compounds in combination, either upon the myometrium or upon the myosalpinx. This issue will have to be resolved before the present results with ergonovine and Ritodrine can be interpreted with certainty.

Post-ovulatory administration of oestrogens can disturb ovum transport in several species and each one responds in a particular fashion depending on the dose and time of administration (Croxatto & Ortiz, 1975). It would be of interest to find out not only the physiological role of oestrogens in the regulation of ovum transport in women but also the type of responses to pharmacological doses in this species. The experiments described here only show that a moderate dose of oestrogen with a short half-life does not accelerate ovum transport. Other possibilities remain to be disclosed: oestradiol may have no effect at all or it may tube-lock the ovum. Appropriate experiments can be designed to see if the ovum still remains in the tube at later intervals after the LH peak and also to test the effect of higher doses of potential blockers.

The initial attempts to modify ovum transport in women have been so far unsuccessful. We should not be discouraged, however, since only 4 compounds have been tested by us. Only 2 of these had been previously shown to be active in other species. PGF-2 α has been shown to accelerate transport in rabbits (Ellinger & Kirton, 1974; Spilman *et al.*, 1976; Hodgson, 1976; Takeda *et al.*, 1977) but not in hamsters or rats (Labhsetwar, 1972, 1973) or guinea-pigs (Maia *et al.*, 1977). Oestradiol has been shown to be active in all species tested, producing either accelerated or delayed transport, but only a single dose was tried in the women in this study and as with the other compounds the experiment was designed to give evidence only of accelerating effects. These considerations point to the fact that the task has just begun and there is much room for favourable developments.

One lesson we have learned from these results is that in choosing a compound one should bear in mind that its ability to stimulate oviductal contractility *in vivo* in women does not guarantee that it will accelerate ovum transport. Furthermore, this implies that we are not ready to use a simple mechanical index, such as the intraluminal pressure response to drug administration, to predict effects upon ovum transport and that the mechanics of ovum transport are still far from being in the domain of our understanding. The present limitations could be overcome perhaps by the combined effort of drug screening in laboratory animals and the experimental analysis of mechanical factors involved in normal, accelerated and delayed transport.

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