Br. J. Pharmac. (1970), **40**, 759–774.

Investigations into the mechanism of the antifertility action of minimal doses of megestrol acetate in the rabbit

K. E. KENDLE AND J. M. TELFORD

Biological Research Department, B.D.H. (Research) Limited, Borough Road, Godalming, Surrey

Summary

1. Experiments have been performed to investigate the mechanism by which low doses of megestrol acetate have an antifertility action.

2. It was found that the dose of megestrol acetate required to prevent pregnancy in nine of ten New Zealand White rabbits when given on 3 consecutive days, the last of which was the day of insemination, was 50 μg/kg daily.

3. This treatment reduced the numbers of sperm recoverable from the uterus at various times after insemination, but egg transfer experiments showed that neither egg viability nor fertilization was affected on day 1 of pregnancy.

4. Treatment with megestrol acetate for 3 days caused the fertilized eggs to enter the uterus approximately 48 h prematurely. Treatment with megestrol acetate for longer periods caused a similar but more marked increase in the rate of transport of eggs in the oviduct.

5. Megestrol acetate did not modify oviduct opening pressure, but the volume of oviduct fluid secretion was reduced earlier and transport of fluid through the oviduct was accelerated compared with controls.

6. Transfer of eggs on various days from the uteri of untreated rabbits to the uteri of treated rabbits indicated that the development of the endometrium which occurs in pregnancy may be advanced by megestrol administration. Histological examination of the endometrial proliferation induced by megestrol acetate confirmed that this was so.

7. It is concluded that the antifertility action of megestrol acetate in doses that do not necessarily inhibit ovulation is due to a combination of accelerated egg transport and advanced endometrial development.

Introduction

The idea of contraception without inhibition of ovulation was introduced in 1965 by Rudel, Martinez-Manautou & Maqueo-Topete. Their studies led to the development of a contraceptive regimen based on continuous administration of synthetic progestogens, in low dosage without added oestrogen (Martinez-Manautou, Cortez, Giner, Aznar, Casasola & Rudel, 1966; Martinez-Manautou, Giner-Velasquez, Cortez-Gallegos, Aznar, Rojas, Guittenez-Najar & Rudel, 1967a; Martinez-Manautou, Giner-Velasquez & Rudel, 1967b). Many successful trials of this regimen

using various progestogens including megestrol acetate have now been reported by other groups of workers (Mason, Cox, Mason & Grant, 1967 ; Macdonald, Lumley, Coulson & Stitch, 1968 ; Foss, Svendsen, Fotherby & Richards, 1968 ; Zanartu, Rodriguez, Pupkin, Salas & Guerrero, 1968b ; Howard, Elstein, Blair & Morris, 1969 ; Mears, Vessey, Andolsek & Oven, 1969 ; Cox, 1969).

The precise mechanism of this antifertility effect, however, has not been established although many suggestions have been made. Evidence of ovulation can be obtained in approximately 80% of patients (Martinez-Manautou *et al.*, 1966 ; Fotherby, Svendsen & Foss, 1968 ; Macdonald *et al.*, 1968) and so an effect at pituitary level is unlikely. While changes in cervical mucus resulting in inhibition of sperm penetration have been described (Martinez-Manautou *et al.*, 1966, 1967 ; Mason *et al.*, 1967 ; Roland, 1968 ; Zanartu, Pupkin, Rosenberg, Guerrero, Rodriguez-Bravo, Garcia-Huidobro & Puga, 1968a), the significance of these changes in relation to the antifertility effects of the regimen has been questioned (Mears *et al.*, 1969 ; Gibor, Cohen & Scommegna, 1969). It has been proposed that certain endometrial changes may occur (Martinez-Manautou *et al.*, 1967a ; Zanartu *et al.*, 1968a) or that the functioning of the corpus luteum may be modified (Foss *et al.*, 1968), but neither the precise nature of such changes nor their significance has been established.

Despite continuing speculation on the mechanism of the clinical antifertility effect of the low-dose progestogen regimen, only a few studies in laboratory animals have been reported. Bennett, Vallance & Vickery (1967) described inhibition of sperm migration and fertilization in the rabbit by megestrol acetate, melengestrol acetate and chlormadinone acetate. While there was a correlation between doses causing reduction in the number of spermatozoa recovered from the reproductive tract 20 h after insemination and those inhibiting fertilization as indicated by prevention of egg cleavage, the minimum effective antifertility doses were not established and so a cause and effect relationship could not be deduced. Chang (1966, 1967) has shown that progestogens increase the rate of transport of eggs in the fallopian tube in rabbits, and Vickery & Bennett (1969) have recently concluded that this property together with inhibition of blastocyst growth accounts for the antifertility effect of low doses of chlormadinone acetate.

The purpose of the present work was to establish the mechanism whereby low doses of megestrol acetate have an antifertility action in the rabbit. Low dose progestogen therapy in the human is not 100% effective and for this reason the studies were carried out using a dose of megestrol acetate which inhibited fertility in only 90% of the animals.

Methods

General

Primiparous New Zealand White does (3–5 kg) were obtained from a commercial breeder (Olac) then housed individually for a minimum of 1 week before being used in the experiments. Megestrol acetate (6-methyl-17α-acetoxy-pregna-4 : 6-diene-3 : 20-dione) was given by stomach tube as a suspension in an aqueous vehicle containing carboxy-methyl cellulose 1·2%, Tween 80 1·5% and distilled water 97·3%, the dose being 50 μg/kg body weight daily for 3 consecutive days, except where otherwise stated. The dose volume was 2 ml/kg and all control animals were given the aqueous vehicle only.

Insemination was carried out artificially on the day of the last dose, designated day 0 of pregnancy, using fresh ejaculates collected from New Zealand White bucks of proven fertility. Except where otherwise stated, each ejaculate was diluted with approximately 10 volumes of a phosphate buffer containing 1·4197 g Na_2HPO_4, 0·3728 g KCl, 0·1361 g KH_2PO_4, 0·2465 g $MgSO_4$ and 7·7154 g NaCl. Ovulation was induced by the intravenous administration of 25 IU human chorionic gonadotrophin (Pregnyl, Organon) dissolved in 1 ml of distilled water.

Laparotomies were carried out using pentobarbitone sodium supplemented by anaesthetic ether. Continuous silk sutures (Abrasilk grade 0) were used for closure of the peritoneum while divided cotton sutures were used for skin closure. A solution of 1% cetyltrimethylammonium bromide and 0·1% chlorhexidine gluconate in 70% ethanol was used for skin preparation and for storage of instruments during the operative procedure.

Animals were killed by cervical dislocation and where the experimental design involved autopsy of some animals during the dosing and insemination period, treatment appropriate to the day of autopsy was given at least 3 h before the animals were killed. Determination of oviduct opening pressures, oviduct fluid secretion, endometrial carbonic anhydrase activity and endometrial proliferation were carried out on the same animals, but are described separately for clarity of presentation.

Determination of the minimum effective antifertility dose

Various dose levels of megestrol acetate or the aqueous vehicle were given to groups of ten rabbits which were then inseminated and ovulated. The animals were killed on day 16 of pregnancy when the numbers of corpora lutea, live foetuses and resorptions were counted. Pregnancy was considered to be prevented if no live foetuses were found *in utero*.

Effect on the rate of sperm transport

Three groups of twenty rabbits were given 100 μg/kg megestrol acetate, 50 μg/kg megestrol acetate and the vehicle respectively. Insemination was carried out using a known volume of undiluted fresh ejaculates and ovulation induced. A 50 μl sample taken from each ejaculate was diluted to 10 ml with buffered formol solution and a sperm count made using a haemocytometer.

Five animals from each group were killed 3 h after insemination, five after 6 h, a further five after 12 h and the remaining five after 24 hours. At autopsy, each uterine horn was removed and flushed with 0·5 ml buffered formol solution. The washings from the two horns were pooled and a sperm count made using a haemocytometer. The number of sperm recovered from the uterus was expressed as a percentage of the number inseminated.

Influence of the number of sperm inseminated on the antifertility effect

Various dose levels of megestrol acetate or the aqueous vehicle were given to groups of ten rabbits. Five animals from each group were inseminated using diluted ejaculates, while for the remaining five, whole ejaculates were used. Ovulation was then induced.

The animals were killed on day 16 of pregnancy when the numbers of corpora lutea, live foetuses and resorptions were counted. Pregnancy was considered to be prevented if no live foetuses were found *in utero*.

Effect on the rate of egg transport

Two groups of twenty-five rabbits were given megestrol acetate and the vehicle respectively, then inseminated and ovulated. Five animals from each group were killed 24 h after insemination, five after 48 h, five after 72 h, a further five after 96 h and the remaining five after 120 hours.

At autopsy, the reproductive tracts were removed and each side divided into sections. The uterine horns were separated from the oviducts and the latter cut into five equal parts using a ruler and scalpel. Each section was flushed with 0·9% NaCl solution into a watch glass, and the number of contained eggs determined by microscopic examination of the washings (Nikon SMZ stereomicroscope $\times 25$ approximately). The numbers of ovulation sites or corpora lutea were counted and the eggs recovered expressed as a percentage of the eggs ovulated.

In a second experiment, two groups of fifteen rabbits were given megestrol acetate and the vehicle respectively as before. Administration was continued, however, up to and including the day of autopsy. The animals were inseminated and ovulated on the third day of dosing as before and five from each group killed 24 h after insemination, a further five after 48 h and the remaining five after 72 hours. The number and distribution of eggs within the reproductive tract was determined at autopsy as in the previous experiment.

Effect upon egg viability

Two groups of five donor rabbits were given megestrol acetate and the vehicle respectively, then ovulated and inseminated. Pseudopregnancy was induced in two groups of five vehicle dosed recipients by administration of the ovulating injection of human chorionic gonadotrophin.

The donors were killed on day 1 of pregnancy, that is approximately 24 h after insemination, when the contained eggs were recovered from the oviducts by flushing with sterile 0·9% NaCl solution into a sterile watch glass at laboratory temperature. The eggs were then washed in a further quantity of sterile saline and transferred to the oviducts of recipient animals which had also been ovulated approximately 24 h previously. The recipients were retained until day 16 of pregnancy when they were killed and the numbers of live and resorbing foetuses determined.

Effect on uterine receptivity

Two groups of five megestrol acetate treated rabbits and one group of five control animals were used as recipients. Pseudopregnancy was induced using human chorionic gonadotrophin (25 IU) given intravenously on the day of the last dose, designated day 0 of pseudopregnancy.

Donor rabbits, given the vehicle alone, then inseminated and ovulated, were killed on day 4 of pregnancy. Eggs were recovered from their uteri by flushing with sterile 0·9% NaCl solution at laboratory temperature. These were then transferred to the uteri of megestrol acetate treated recipients on day 4 of pseudopregnancy, control

animals on day 4 of pseudopregnancy and megestrol acetate treated animals on day 2 of pseudopregnancy. The recipients were killed on day 16 of pregnancy when the numbers of live and resorbing foetuses were determined.

Oviduct opening pressure

Five megestrol acetate treated and five control animals were examined on day −1 of pregnancy, that is, on the day of the second dose. Further groups were examined on days 0, 1, 2, 3 and 4. A polythene cannula was tied in the ovarian end of each right oviduct exposed by laparotomy. From the oviduct, the cannula passed to a T junction, one limb of which was connected to a motorized syringe (Palmer) calibrated to deliver 0·9% NaCl solution at a rate of 0·4 ml/min. A low displacement pressure transducer (Grass) was connected to the remaining limb of the junction and the pressure in mmHg (1.333 mbar) required to initiate and maintain flow of saline through the oviduct was recorded through an amplifier and pen recorder. Infusion of 0·9% NaCl solution down the cannulated oviduct was continued for approximately 5 min and the magnitude of the oviduct opening pressure, that is the maximum pressure recorded before a steady flow was established, was determined from the recording.

Oviduct fluid secretion

Groups of five megestrol acetate treated and five control animals were used. As in the previous investigation, one treated and one control group were killed on day −1 while further groups were killed on days 0, 1, 2, 3 and 4 of pregnancy. Each animal was laparotomized on the day before autopsy and the left oviduct exposed. A fine cotton ligature was placed at the base of the fimbria and a second just below the isthmo ampullar junction. Care was taken to avoid interference with the major blood vessels.

The animals were killed 24 h after ligation and the portion of the oviduct between the ligatures removed and weighed to the nearest 0·5 mg. An incision was then made in the wall of the oviduct and the intra-luminal fluid expressed. The amount of fluid was calculated by reweighing the tissue. The weight of a 20 μl sample of the fluid from each oviduct was then determined by weighing a disposable capillary pipette empty and then filled with the fluid. Thus, the volume was calculated.

Rate of passage of fluid through the oviduct

One group of five megestrol acetate treated animals and one group of five controls were used. A cotton ligature was placed at the base of the fimbria of the left oviduct exposed by laparotomy on day −1 of pregnancy. The ligated oviducts were then observed through small exploratory incisions made on each of days 0, 1 and 2. The animals were killed on day 3 of pregnancy when a final observation was made. On each occasion, the degree of distension observed was rated subjectively on a scale ranging from very marked distension given an arbitrary value of 7 to no distension given a value of 0. The mean score for treated and control animals on each day was then calculated. Scoring was performed blind.

Effect on uterine carbonic anhydrase activity and endometrial proliferation

Five megestrol acetate treated and five control animals were killed on day −1 of pregnancy while further groups were killed on days 0, 1, 2, 3 and 4. At autopsy,

approximately 0·5 g of tissue was removed from the uterus of each animal. The sample was weighed to the nearest 0·5 mg, placed in 10 volumes of ice cold distilled water and, after preliminary mincing with small scissors, homogenized using an Ultra-Turrax homogenizer. The supernatant liquid was removed after centrifugation and the carbonic anhydrase content determined by an automated (Technicon Auto-Analyzer) colourimetric method based on the decolourization of phenolphthalein by carbon dioxide liberated from bicarbonate by carbonic anhydrase. Briefly, the supernatant was added to an air-segmented stream of phosphate buffer and mixed in a single-length mixing coil set in an ice-bath. Bicarbonate solution (0·1M) was cooled to the same temperature as the sample/buffer mixture by passage through a second single-length mixing coil in the ice-bath. The two streams were joined and mixed in a third single-length coil, also in the ice-bath. After leaving the ice-bath, the liquid phase was pumped to waste and the gas phase, now containing carbon dioxide liberated from the bicarbonate solution, was used to segment a stream of colour reagent consisting of phenolphthalein in a carbonate—bicarbonate solution. The decrease in colour of the reagent was measured at 550 nm, using a 15 mm path-length continuous flow-cell. A standard curve was run using several dilutions of crystalline carbonic anhydrase dissolved in 0·05% peptone solution. By running serial dilutions of tissue homogenates, it was possible to determine enzyme concentration by reference to the standard curve.

A second sample of each uterus was fixed in buffered formol solution. After dehydration and wax embedding, 5 μm sections were cut and stained with haematoxylin and eosin. The degree of endometrial proliferation was then assessed according to the method of McPhail (1934).

Results

Determination of the minimum effective antifertility dose

The results given in Table 1 show that megestrol acetate had antifertility effects at dose levels ranging from 12·5 to 200 μg/kg daily. These effects were not associated with a reduction in the mean numbers of corpora lutea observed at autopsy. While dose levels of 12·5 and 25 μg/kg daily reduced the mean numbers of live foetuses to approximately half the control value, 50 μg/kg daily prevented pregnancy completely in nine of ten animals. Doses of 100 and 200 μg/kg daily were completely effective.

From these results, a dose of 50 μg/kg daily was selected for further study.

Effects on the rate of sperm transport

The results given in Fig. 1 indicate that doses of 50 and 100 μg/kg daily caused some reduction in the percentage of inseminated sperm recovered from the uteri 3, 6,

TABLE 1. *Antifertility effect of megestrol acetate, given by the oral route on days −2, −1 and 0 of pregnancy to groups of ten rabbits*

Dose μg/kg daily	Mean No. corpora lutea	Mean No. foetuses	No. not pregnant
—	10·4	7·9	2
12·5	8·7	4·0	4
25	11·3	4·0	3
50	8·9	0·5	9
100	10·7	0·0	10
200	10·7	0·0	10

12 and 24 h after insemination. This reduction was consistent in all treated groups and generally treatment with 50 μg/kg daily had as great an effect as treatment with 100 μg/kg daily. The mean numbers of sperm recovered were usually reduced by about 50%. However, the between animal variation was such that there was some overlap between individual control and treated animals at each of the various times.

The number of sperm recovered was maximal after 6 h both in the animals treated with 100 μg/kg and in the control groups, indicating no change in the rate of cervical transport. Maximal recovery in the animals given 50 μg/kg was 12 h after insemination, but the numbers recovered at this time were only marginally greater than the numbers recovered at 6 hours.

Influence of the number of sperm inseminated on the antifertility effect

The results in Table 2 show that 25, 50 and 100 μg/kg daily of megestrol acetate have antifertility effects in rabbits whether whole or diluted ejaculates are used for insemination. Pregnancy was prevented in all animals by a dose of 50 μg/kg in the whole ejaculate group and by 100 μg/kg in the diluted ejaculate group. Thus, there was no increase in the minimum effective dose when whole ejaculates were used.

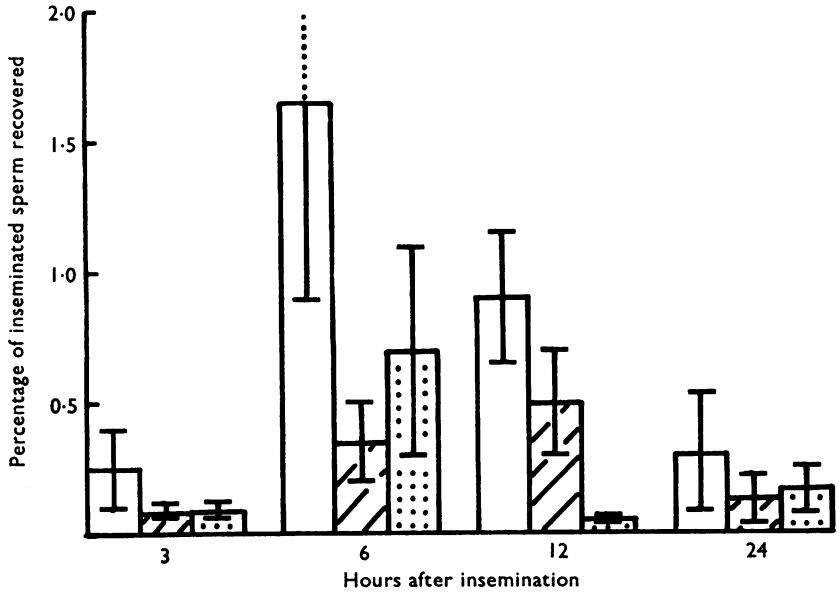

FIG. 1. Effect of megestrol acetate on the percentage of inseminated sperm recovered from the uteri of rabbits at various times. The doses of megestrol acetate were 50 μg/kg daily (hatched columns) or 100 μg/kg daily (dotted columns) given for 3 days before ovulation and insemination. Open columns are controls. Each column represents the mean percentage (±S.E.) of sperm recovered from groups of five rabbits.

TABLE 2. *Influence of sperm concentration on the antifertility effects of megestrol acetate in groups of five rabbits*

Dose μg/kg daily	No. not pregnant	
	Dilute ejaculate	Whole ejaculate
—	1	0
25	4	4
50	3	5
100	5	5

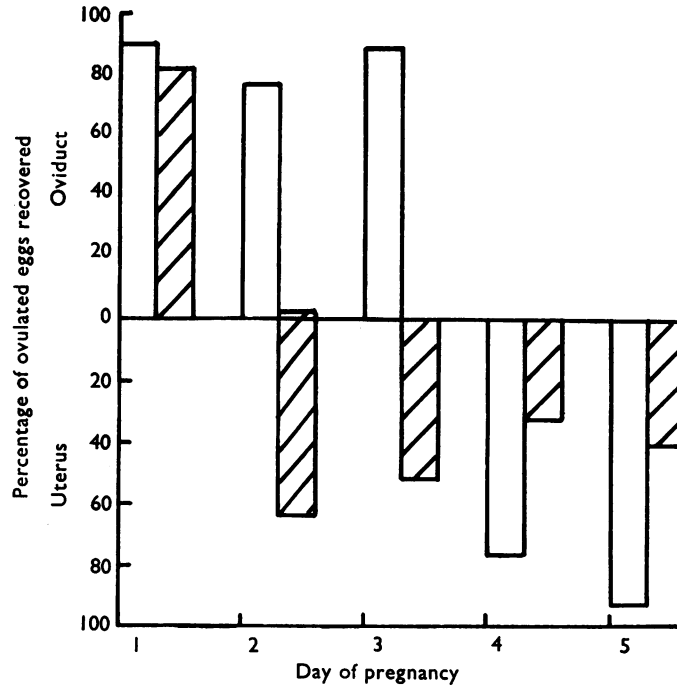

FIG. 2. Effect of megestrol acetate on the distribution of eggs within the reproductive tract in groups of five rabbits. The hatched columns refer to megestrol acetate (50 μg/kg) given for 3 days before ovulation, and the open columns are controls.

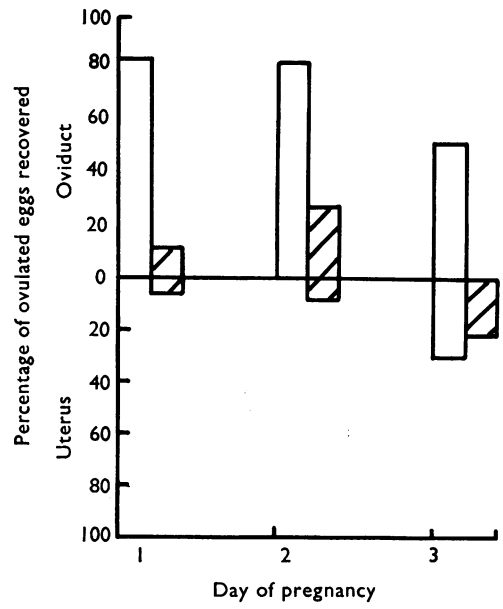

FIG. 3. Effect of continued administration of megestrol acetate on the distribution of eggs within the reproductive tract in groups of five rabbits. The hatched columns refer to megestrol acetate (50 μg/kg) given for the 3 days before ovulation and insemination, and continued thereafter to the day of autopsy. The open columns are controls.

Effects on the rate of egg transport

The results, summarized in Fig. 2, indicate that when megestrol acetate (50 μg/kg) was given for 3 days before ovulation, almost all the observed eggs were recovered from the uterus from day 2 of pregnancy onwards. In control animals, eggs were recovered from the oviducts on days 1, 2 and 3 and from the uterus on days 4 and 5. The rate of egg transport had been increased, therefore, by the megestrol acetate so that the eggs reached the uterus some 48 h earlier. The proportion of the ovulated eggs recovered in the treated animals declined from approximately control values of 82% on day 1 of pregnancy to 30–40% on days 4 and 5, whereas the recovery from control animals remained between 77 and 93%. Eggs recovered from the uteri of the treated animals showed anatomical signs of degeneration.

Fig. 3 indicates that when megestrol acetate administration is continued to include the days between ovulation and autopsy as well as the 3 days preceding ovulation, essentially similar results are obtained. There was, however, a greater loss of eggs from the reproductive tracts of treated animals, recoveries ranging from 15–35% of the total number ovulated, while recoveries from control animals remained between 83 and 88%. In this experiment, 35% of the recovered eggs were found in the uteri of control animals killed on day 3, indicating a slightly greater rate of transport than in the previous study. In the treated group, however, eggs were recovered from the uterus as early as day 1, thus the same acceleration of transport by approximately 48 h was apparent.

Effect on egg viability

The results in Table 3 indicate that eggs recovered from the oviducts of megestrol acetate treated rabbits on day 1 of pregnancy and transferred to pseudopregnant recipients gave rise to essentially the same proportion of viable foetuses as is obtained using eggs from control animals under the same conditions.

Effect on uterine receptivity

The results in Table 4 show that the proportion of implantations following the transfer of 4 day old eggs from untreated animals to the uteri of megestrol acetate treated rabbits on day 4 of pseudopregnancy was lower than that obtained when

TABLE 3. *Effect of megestrol acetate on egg viability in groups of five rabbits*

Dose μg/kg given to donors	No. of eggs transferred*	Percentage of transferred eggs giving:		
		Implantations	Resorptions	Viable foetuses
—	43	40	—	40
50	30	50	3·3	46·7

*Eggs from donors on day 1 of pregnancy transferred to the oviducts of day 1 pseudopregnant recipients.

TABLE 4. *Effect of megestrol acetate on uterine receptivity in groups of five rabbits*

Dose μg/kg given to recipients	Day of recipients' pseudo-pregnancy when eggs transferred*	No. of eggs transferred	Percentage of transferred eggs giving:		
			Implantations	Resorptions	Viable foetuses
—	4	41	66	—	66
50	4	49	49	18	31
50	2	35	60	3	57

* Eggs from donors on day 4 of pregnancy.

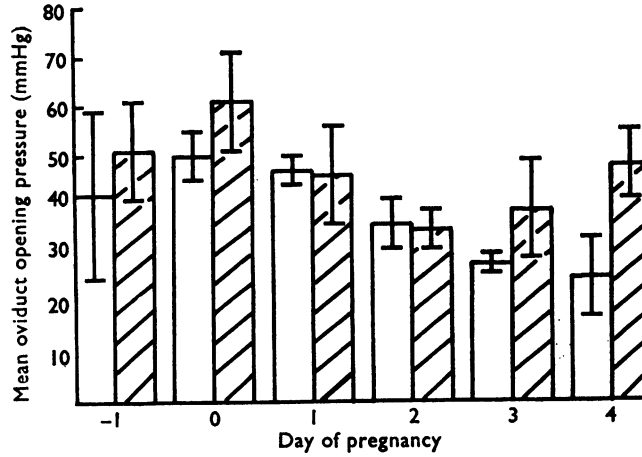

FIG. 4. Mean oviduct opening pressures (±S.E.) in groups of five rabbits on various days of pregnancy. The hatched columns are megestrol acetate treated rabbits (50 μg/kg daily for 3 days) and the open columns are control rabbits.

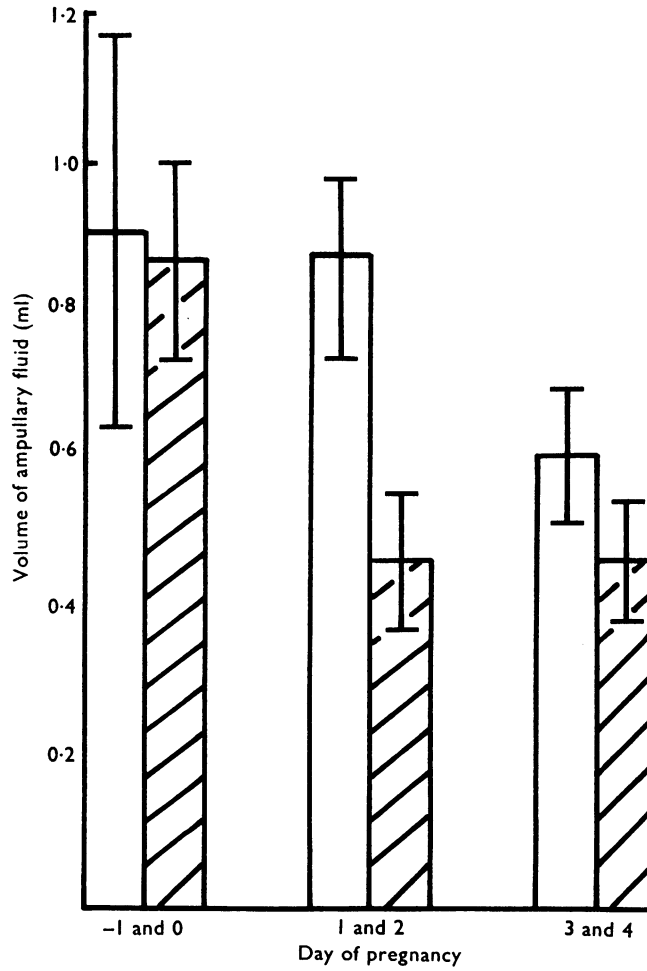

FIG. 5. Effect of megestrol acetate on the mean volume (±S.E.) of intraluminal fluid recovered from the ampullae of oviducts ligated at the fimbria and isthmo ampulla junction in groups of ten rabbits. Hatched columns, megestrol acetate 50 μg/kg given on days −2, −1 and 0 of pregnancy; open columns, controls.

control recipients were used, and over one-third of them subsequently resorbed. Thus the proportion of viable foetuses in the megestrol acetate treated animals was less than half the control value. However, if 4 day old eggs were transferred to megestrol acetate treated rabbits on day 2 of pseudopregnancy, the results were essentially similar to those obtained in synchronous day 4 control transfers.

Oviduct opening pressure

The mean pressures required to initiate the flow of saline through the oviducts of megestrol acetate treated and control rabbits are shown in Fig. 4. Treatment was apparently without effect upon this parameter, the between animal variation being greater than the between group variation. Similarly, examination of the recordings failed to reveal any consistent change in any other parameter of muscular activity such as pressure wave amplitude or frequency.

Oviduct fluid secretion

The mean volumes of fluid recovered from the oviducts of megestrol acetate treated and control animals are shown in Fig. 5. These values are considered over 2 day periods as within group variation tends to obscure the drug induced effect if daily values are used. The mean secretion rate in control animals on days −1 and 0 of pregnancy is essentially similar to the volume secreted on days 1 and 2, while on days 3 and 4 this is reduced by approximately 30%. In megestrol acetate treated animals, on the other hand, the volume on days −1 and 0 is the same as in the controls, but on days 1 and 2 and days 3 and 4, it is reduced to about 50% of the initial value.

These results indicate that the rate of secretion falls below the oestrus value on days 3–4 of pregnancy in control animals, but that this occurs on days 1–2 in megestrol acetate treated rabbits.

Rate of passage of fluid through the oviduct

The results given in Fig. 6 show that the degree of distension of oviducts ligated at the ovarian end falls during days 0–3 of pregnancy in control and megestrol

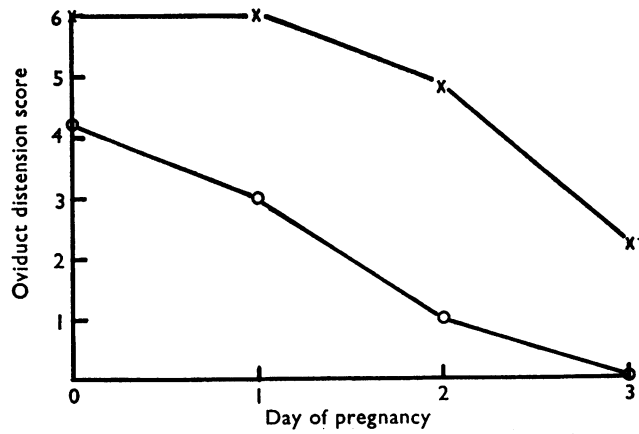

FIG. 6. Mean distension of oviducts ligated at the fimbria in groups of five control (×——×) and megestrol acetate (○——○) treated rabbits.

acetate treated rabbits. The decline is shown to occur approximately 48 h earlier in megestrol acetate treated animals than in controls.

Effect on uterine carbonic anhydrase and endometrial proliferation

The results given in Figs. 7 and 8 indicate that while no consistent change in uterine carbonic anhydrase activity with time or megestrol acetate treatment was

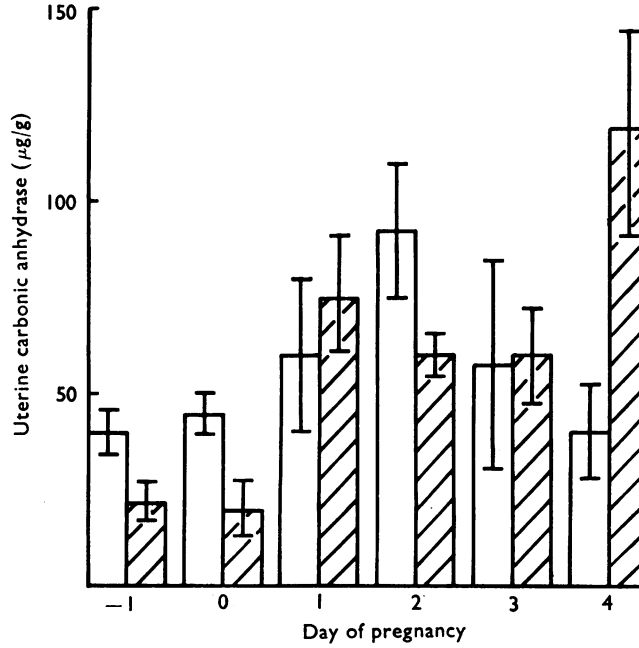

FIG. 7. Mean uterine carbonic anhydrase levels in groups of five megestrol acetate treated rabbits (hatched columns) and in control rabbits (open columns).

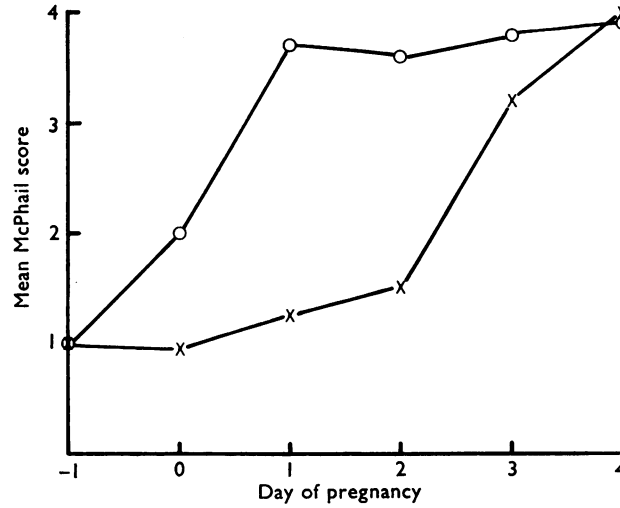

FIG. 8. Rate of endometrial proliferation during early pregnancy in groups of five rabbits. ×——×, Control rabbits; ○——○, megestrol acetate treated rabbits (50 μg/kg daily given on days −2, −1 and 0 of pregnancy).

obtained over the period studied, the McPhail index of endometrial proliferation did increase to maximal levels in both control and treated animals. The increase in the treated group however preceded that in the controls by approximately 55–60 hours.

Discussion

The results of the antifertility experiment indicate that the rabbit is a suitable species for investigation, in as much as antifertility effects are produced by low doses of megestrol acetate under conditions designed to exclude ovulation inhibition as a possible mechanism. This was achieved by inducing ovulation in all animals by exogenous gonadotrophin administration. Under these conditions, megestrol acetate was without effect on the numbers of corpora lutea observed at autopsy, indicating that the compound neither reduced ovarian sensitivity to gonadotrophins, nor induced macroscopically apparent regression of corpora lutea. A dose of 50 μg/kg was selected for further study. This was the dose that prevented pregnancy in nine of ten animals, and so corresponded better to the doses used clinically than would a fully effective, slightly supramaximal, dose. David, Edwards, Fellowes & Plummer (1963) showed that 62 μg/kg megestrol acetate blocked ovulation in ten out of twenty mated New Zealand White rabbits. Thus, like the clinical dose, it is probable that the selected dose would block natural ovulation in less than 50% of the animals.

Studies of the rate of sperm transport through the uterine cervix showed only small changes in the numbers of sperm recovered from the treated animals from 3 to 24 h after insemination. Despite a consistent reduction in the mean percentage of inseminated sperm recovered from the megestrol acetate treated animals, there was considerable overlap between individual values in the treated and control groups at each of the various times. It is unlikely, therefore, that changes in sperm transport through the cervix contribute to the antifertility effects of megestrol acetate under these conditions. This finding is in agreement with some of the more recent animal (Vickery & Bennett, 1969) and clinical (Mears *et al.*, 1969 ; Gibor *et al.*, 1969) experiments and contrasts with some of the earlier work in this field (Bennett *et al.*, 1967 ; Roland, 1968).

The antifertility experiments, like all other experiments in this investigation, except the sperm transport studies, were carried out using diluted ejaculates for insemination. In order to obtain high numbers of uterine sperm for accurate counting, however, the sperm transport studies used whole ejaculates. These experiments were therefore open to the criticism that a dose of 50 μg/kg megestrol acetate may not be an effective antifertility dose when whole ejaculates were used. A direct comparison of the antifertility effects of megestrol acetate in animals inseminated with diluted and whole ejaculates was therefore undertaken. This experiment showed that the compound was not less effective in animals inseminated with whole ejaculates.

Administration of megestrol acetate before ovulation was found to cause a subsequent increase in the rate of egg transport. Essentially similar observations with progesterone and medroxyprogesterone acetate have been reported previously by Chang (1966, 1967), who observed accelerated egg transport when progestogens were given before, but not after ovulation. In view of these results with such a low dose of megestrol acetate, it is possible that progesterone secretion has a role in the physiological control of egg transport through the oviduct. Such a hypothesis is not necessarily incompatible with the finding that ovariectomy after ovulation does not affect the rate of egg transport, as initiation of progesterone secretion has been shown

to precede ovulation by several hours (Hilliard, Archibald & Sawyer, 1963). Similar effects on egg transport were obtained when administration of the progestogen was continued after ovulation, indicating that these were effects of progestogen administration and not a result of progestogen withdrawal.

Although premature entry of fertilized eggs into the uterus has an adverse effect on fertility, Chang (1950) showed that 29% of 2 day ova recovered from the oviducts and transferred to the uteri subsequently developed into viable foetuses. Thus, while the observed acceleration in the rate of egg transport partly explains the antifertility effects of megestrol acetate, it is apparent that this is not the sole factor involved.

Transfer of eggs recovered from the oviducts of treated animals on day 1 of pregnancy to the oviducts of day 1 pseudopregnant recipients resulted in the same percentage viability as was obtained when control donors were used. This finding precludes any ovicidal activity of the drug on the growing follicle and any effect on sperm or fertilization. This finding, therefore, gives further support to the conclusion that changes in the rate of sperm transport are not involved in the antifertility effect of the drug.

Transfer of 4 day old eggs from untreated animals to megestrol acetate treated recipients on day 4 of pseudopregnancy resulted in a much lower percentage of viable foetuses than was obtained in the control animals. To determine whether this was due to an advancement of endometrial development in the treated animals, a series of asynchronous transfers were undertaken where 4 day old eggs were placed in the uteri of megestrol acetate treated recipients on day 2 of pseudopregnancy. The results of this experiment showed that the viability of these eggs was similar to that obtained in synchronous day 4 transfers in control animals, suggesting that administration of megestrol acetate advanced endometrial development by approximately 2 days. It would therefore appear that in megestrol acetate treated animals the eggs enter the uterus when only 2 days old as a result of accelerated egg transport, but at this stage, the development of the uterus corresponds to day 4 of pseudopregnancy. Chang (1950) has shown that the transfer of 2 day ova to the 4 day pseudopregnant uterus results in complete failure of the eggs to develop. It is therefore concluded that these two factors together, namely accelerated egg transport and advanced endometrial development, cause the total infertility observed with administration of minimal doses of megestrol acetate.

Further experiments were undertaken to confirm this hypothesis and to elucidate, if possible, how the progestogen affects the functioning of the oviduct and uterus to produce these changes. Investigation of indices of oviduct muscular activity such as oviduct opening pressure failed to reveal any significant effect of megestrol acetate treatment. However, no marked change in this parameter with time was apparent in control animals. If the effect of megestrol acetate is to advance the occurrence of the physiological changes of early pregnancy, effects of megestrol acetate can only be expected where these can be demonstrated in control animals.

Mastroianni & Wallach (1961) showed that the volume of fluid secreted by the rabbit ampulla decreased by day 3 of pregnancy to 50% of the oestrus value. Similar results were obtained in the present experiments. This fall in secretion rate occurred earlier in megestrol acetate treated animals, again suggesting advancement by approximately 2 days of the physiological changes characteristic of early preg-

nancy. It is hoped that further studies will elucidate whether the change in secretion rate has any role in the physiological control of egg transport.

In a further experiment, fluid was shown to pass through the oviducts of megestrol treated rabbits approximately 48 h earlier than in control animals. This indicates that patency of the oviduct is established earlier, possibly by premature relaxation of the uterotubal or isthmo ampullary junctions. This finding supports the observation of accelerated egg transport.

The postulated advance in endometrial development was confirmed histologically, but the significance of the changes in uterine carbonic anhydrase levels over the period studied is not clear. As no consistent pattern was seen however, it suggests that the hostility of the 4 day uterus to the 2 day egg is not related to uterine carbonic anhydrase levels.

The present studies will be extended in two ways. First, further work will be undertaken to elucidate how progestogen treatment affects the oviduct and why the advanced endometrium is hostile to the early egg. Second, the observations will be extended to other dosage regimens to make the experiments more comparable with the clinical situation where daily administration is continuous for long periods.

We are grateful to Dr. D. K. Vallance for his encouragement and interest in the progress of this work. Our thanks are also due to Mr. E. Woollett, of Glaxo Research Ltd., for the carbonic anhydrase determinations, and to Mrs. T. Bridle for skilled technical assistance.

REFERENCES

BENNETT, J. P., VALLANCE, D. K. & VICKERY, B. H. (1967). Inhibition of gamete migration and fertilisation in the rabbit. *Postgrad. med. J.*, Dec. suppl., 39–43.

CHANG, M. C. (1950). Development and fate of transferred rabbit ova or blastocysts in relation to ovulation time of recipients. *J. exp. Zool.*, **114**, 197–226.

CHANG, M. C. (1966). Effects of oral administration of medroxyprogesterone acetate and ethinylestradiol on the transportation and development of rabbit eggs. *Endocrinology*, **79**, 939–948.

CHANG, M. C. (1967). Effect of progesterone and related compounds on fertilisation, transportation and development of rabbit eggs. *Endocrinology*, **81**, 1251–1260.

COX, H. J. E. (1969). Progestogen only oral contraceptives. *Br. med. J.*, **3**, 471–472.

DAVID, A., EDWARDS, K., FELLOWS, K. P. & PLUMMER, J. M. (1963). Anti-ovulatory and other biological properties of megestrol acetate; 17 acetoxy 6 methyl pregna 4:6 diene 3:20 dione (BDH 1298). *J. Reprod. Fertil.*, **5**, 331–346.

FOSS, G. L., SVENDSEN, E. K., FOTHERBY, K. & RICHARDS, D. J. (1968). Contraceptive action of continuous low doses of Norgestrel. *Br. med. J.*, **4**, 489–491.

FOTHERBY, K., SVENDSEN, E. K. & FOSS, G. L. (1968). Ovarian function in women receiving low doses of a synthetic progestogen, Norgestrel. *J. Reprod. Fertil.*, suppl. 5, 155–166.

GIBOR, Y., COHEN, M. R. & SCOMMEGNA, A. (1969). Effect of continuous administration of small doses of chlormadinone acetate on the cervical mucus and post coital test. *Fertil. Steril.*, **20**, 572–580.

HILLIARD, J., ARCHIBALD, D. & SAWYER, C. H. (1963). Gonadotrophic activation of preovulatory synthesis and release of progestin in the rabbit. *Endocrinology*, **72**, 59–66.

HOWARD, G., ELSTEIN, M., BLAIR, M. & MORRIS, N. F. (1969). Low dose continuous chlormadinone acetate as an oral contraceptive. *Lancet*, **2**, 24–26.

MACDONALD, R. R., LUMLEY, I. B., COULSON, A. & STITCH, S. R. (1968). Chlormadinone acetate as an oral contraceptive: clinical results and the incidence of ovulation. *J. Obstet. Gynaec. Br. Commonw.*, **75**, 1123–1127.

MARTINEZ-MANAUTOU, J., CORTEZ, V., GINER, J., AZNAR, R., CASASOLA, J. & RUDEL, H. W. (1966). Low doses of progestogen as an approach to fertility control. *Fertil. Steril.*, **17**, 49–57.

MARTINEZ-MANAUTOU, J., GINER-VELASQUEZ, J., CORTES-GALLEGOS, V., AZNAR, R., ROJAS, B., GUITTEREZ-NAJAR, A. & RUDEL, H. W. (1967a). Daily progestogen for contraception: a clinical study. *Br. med. J.*, **11**, 730–732.

MARTINEZ-MANAUTOU, J., GINER-VELASQUEZ, J. & RUDEL, H. W. (1967b). Continouos progestogen contraception: a dose relationship study with chlormadinone acetate. *Fertil. Steril.*, **18**, 57–62.

MASON, B. A., COX, H. J. E., MASON, D. W. & GRANT, V. (1967). Clinical and experimental studies with low doses of megestrol acetate. *Postgrad. Med. J.*, Dec. suppl., 45–48.

MASTROIANNI, L. & WALLACH, R. C. (1961). Effect of ovulation and early gestation on oviduct secretions in the rabbit. *Am. J. Physiol.*, **200**, 815.

MCPHAIL, M. K. (1934). The assay of progestin. *J. Physiol., Lond.*, **83**, 145–156.

MEARS, E., VESSEY, M. P., ANDOLSEK, L. & OVEN, A. (1969). Preliminary evaluation of four oral contraceptives containing only progestogens. *Br. med. J.*, **2**, 730–734.

ROLAND, M. (1968). Norgestrel induced cervical barrier to sperm migration. *J. Reprod. Fertil.*, suppl. 5, 173–177.

RUDEL, H. W., MARTINEZ-MANAUTOU, J. & MAQUEO-TOPETE, M. (1965). The role of progestogens in the hormonal control of infertility. *Fertil. Steril.*, **16**, 158–169.

VICKERY, B. H. & BENNETT, J. P. (1969). Mechanisms of antifertility action of chlormadinone acetate in the rabbit. *Biol. Reprod.*, **1**, 372–377.

ZANARTU, J., PUPKIN, M., ROSENBURG, D., GUERRERO, R., RODRIGUEZ-BRAVO, GARCIA-HUIDOBRO, M., & PUGA, J. A. (1968a). Effect of oral continuous progestogen therapy in micro-dosage on human ovary and sperm transport. *Br. med. J.*, **2**, 266–269.

ZANARTU, J., RODRIGUEZ, G., PUPKIN, M., SALAS, O. & GUERRERO, R. (1968b). Antifertility effect of continuous low dose oral progestogen therapy. *Br. med. J.*, **2**, 263–266.

(*Received June* 11, 1970)