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Inhibition of Fertilization in Rabbits During Treatment with Progesterone

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Adult female rabbits (Dutch strain) were artificially inseminated with 0.5 ml of diluted semen (10×10^6 to 40×10^6 spermatozoa) and ovulated with 100 to 200 IU of HCG. Corn oil or progesterone in corn oil was injected daily subcutaneously for various periods, ranging from 0 to 6 days before insemination and continuing until sacrifice 48 hr after insemination. Fertilization of ova was inhibited when 1 mg progesterone was injected daily for 2 or 6 days before insemination but not when the treatment began on the day of insemination or 4 days before insemination. An increase in the transport rate of ova occurred only when treatment was initiated 2 days before insemination. The fertilization-inhibitory response to progesterone was dose dependent between doses of 0.05 and 4 mg/day and the ED_{50} was 0.4 mg. Although none of the levels employed was 100% effective, only 2% of the ova recovered from the group given 4 mg/day were fertilized. A sperm index with values ranging from 0 to 3 was employed to indicate the total number of sperm counted in the mucin layer, zona pellucida, or perivitelline space of ova. Values in all three areas were low in ova recovered from animals given 0.5 mg progesterone/day and were reduced to 0 or near zero with all higher doses. When semen was deposited into the uterus of animals given 1 mg progesterone/day for 6 days, nearly all ova were fertilized. In animals given 1 mg progesterone/day, an increase in the semen concentration from 10 to 100% failed to significantly increase the percentage of ova fertilized, but successive injections of 50 U.S.P. units of oxytocin 1 hr before insemination and 1 and 4 hr afterwards increased the percentage of ova fertilized from 0 to 45% and increased the sperm index values approximately 50% in each of the layers of the ovum. Spermatozoa were recovered from the cervix and serial segments of the uterus and oviducts at 4, 8, 16, and 24 hr following insemination; they were counted using a micropore filtration technique. The numbers of sperm in all segments of the uterus and oviducts of the progesterone-treated animals were consistently less than in corresponding segments of control organs; they were absent from the anterior 2/3 of the oviduct until 16 hr after insemination at which time only a few in the middle oviducal segment were found. Progesterone inhibited fertilization of ova, we conclude, primarily by interference with sperm transport mechanisms in the uterus and/or oviduct.

Fertilization of ova has been shown to be low in the cow (Casida, 1940, 1943), ewe (Murphree, Warwick, Casida, and McShan, 1944), sow (Tanabe, Warnick, Casida, and Grummer, 1949), rabbit (Murphree, Warwick, Casida, and McShan, 1947; Austin, 1949), and ferret (Chang, 1967a) when ovulation was artificially induced with gonadotropin during the luteal phase of the estrous cycle or during pseudopregnancy. In rabbits the infertile condition was shown to develop gradually during pseudopregnancy, and low fertilization rates were demonstrated in estrous rabbits treated with progesterone prior to fertilization (Boyarsky *et al.*, 1947). Improvement of fertility was obtained in pseudopregnant rabbits when sperm were deposited into the oviducts (Austin, 1949) or into the uterus (Murphree, Black, Otto, and Casida, 1951) before ovulation, suggesting that fertilization failure in rabbits during the luteal phase was caused by impairment of

sperm transport mechanisms. Chang (1967b) has demonstrated low fertility rates in the artificially inseminated, artificially ovulated rabbit treated with progesterone for 2 days before ovulation. He also demonstrated that under these conditions ova transport rate was increased. In humans, treatment with a progestin has been shown to reduce fertility (Martinez-Manautou *et al.*, 1966) without inhibiting ovulation. However, alterations in characteristics of the cervical mucus that were noted were judged to be unfavorable to sperm. Hence, these investigators speculated that the infertility was caused by a progestin-induced secretion of cervical mucus hostile to sperm.

Although the mode of action of small quantities of progestins on fertility remains equivocal, successful application of this contraceptive approach to world population control offers definite advantages over existing methods. For this reason we report a series of studies on the mechanism of antifertility action of progestins.

METHODS AND MATERIALS

Mature female rabbits (Dutch strain) weighing 1.7 to 2.8 kg were caged individually for 3 or more weeks before being treated. Animals were inseminated artificially with rabbit semen. Semen was collected from three or more mature bucks with the aid of an artificial vagina, pooled to reduce the chance occurrence of an infertile sample, and diluted with physiological saline. Unless otherwise stated in the results section, a semen dilution of 1:9 and an insemination volume of 0.5 ml, containing 10×10^6 to 40×10^6 spermatozoa, was employed as a standard practice. Immediately preceding artificial insemination, does were injected iv with 100 to 200 IU human chorionic gonadotrophin (HCG) to induce ovulation. The day of insemination was designated as day 0 of the experiment. Progesterone was dissolved or suspended in corn oil and injected daily by the subcutaneous route for periods ranging from 1 to 8 days. Animals were inseminated on various days of treatment and were routinely sacrificed 48 hr after insemination except as noted in the next section. Each experiment or replicate in an experiment contained a control group that was treated concurrently and injected with the corn oil vehicle at the indicated times.

At autopsy the reproductive tract was removed, and the oviducts and uterus were flushed with physiological saline. The washings were collected in a watch glass and viewed immediately beneath a dissecting microscope at a magnification of 80×. The stage and condition of development of ova were noted, and the external diameter of the mucin layer and zona pellucida were measured with an eyepiece reticle. Ova were then transferred to a hanging drop preparation in a depression slide to facilitate further examination at higher magnification using the light microscope and, in some cases, a phase contrast microscope. The numbers of spermatozoa in the mucin layer, zona pellucida, and perivitelline space were counted. Individual counts were transformed to sperm index values of 0, 1, 2, or 3 representing sperm frequency classes of 0, 1 to 3, 3 to 10, and > 10 spermatozoa, respectively. The ovaries were removed, and the number of corpora lutea was counted. Data from rabbits with corpora albicantia or with corpora lutea that were obviously larger than corpora lutea in control animals were excluded from the experimental results.

For ease of comparison the percentage of ova recovered (100 × number of ova recovered ÷ number of corpora lutea) and the percentage of ova fertilized (100 × number of cleaved ova ÷ number of ova recovered) were calculated from the totals in each group. The number of ova recovered and the number of ova fertilized were compared by means of a chi-square analysis using original rather than percentage values. The method of analysis of variance was used to determine the significance of differences between groups in the number of corpora lutea and the diameter of the mucin layer.

In the experiment concerned with sperm transport, the reproductive tract was removed and divided as follows: the cervix; three sections of each cornu designated as the posterior, middle, and anterior segments of the uterus; and three equal length segments of each oviduct designated as the posterior, middle, and anterior segments of the oviducts. Each of the thirteen sections was flushed separately with 2 ml physiological saline. The washings from sections on one side of the reproductive tract were pooled with the washings from the corresponding section on the contralateral side of the reproductive tract. Concentrations of sperm in each section were measured by a method previously described (Nutting, 1969). Briefly, washings were collected in a 5-ml hypodermic syringe attached to a filtering unit containing a 13-mm diameter, type HA micropore filter with a pore size of $0.45 \pm 0.02\mu$ (Millipore Filter Corp). The filters, containing sperm and accompanying detritus washed from sections of the reproductive tract, were stained with hematoxylin and eosin and

prepared for histological examination. The filters were then examined at 400×, and the sperm outlined in a calibrated eyepiece reticle grid were counted using a standardized sequence of seven microscopic fields with a total area of 0.4375 mm². The filters had an average effective filtration surface of 78.54 mm². The estimated total number of sperm on the surface of the filter was then calculated by simple proportion equations.

RESULTS

Fertilization rate during progesterone therapy. In the first experiment the minimum length of the preinsemination period of progesterone treatment to inhibit fertility was determined. Twenty-nine rabbits were assigned randomly to five treatment groups consisting of two replicates of three rabbits each. Progesterone or vehicle was injected daily for 2 to 8 days. The last injection was given approximately 24 hr before autopsy. Rabbits were killed 48 hr after insemination. Animals were inseminated on the 1st, 3rd, 5th, or 7th day of treatment.

In comparison with the control group, fertilization of ova was significantly lower when rabbits were inseminated on the 3rd or 7th day of treatment with progesterone (Table 1). Insemination on the 1st or 5th day of treatment had no effect on the fertilization rate of ova. The effectiveness of the longer period of treatment in preventing fertilization confirmed previous preliminary studies in which insemination on the 7th to 11th day of progesterone treatment consistently blocked fertilization of most ova recovered 48 hr later. These results show that treatment with progesterone for less than 6 days before insemination has a variable effect on fertilization and that treatment for longer periods inhibits fertilization more predictably.

The number of ova recovered was significantly less than controls only in the group inseminated on the 3rd day of progesterone treatment. In this group 2 of 16 ova were found in the uterus; in the control group and the other groups treated with progesterone all of the ova recovered were found in the oviducts.

In 57 groups of control rabbits with two to four rabbits per group, accumulated from related studies, the average percentage of recovered ova was 86% (range 51–100%), and the average percentage of fertilized ova was 93% (range 58–100%). Only four of the 169 rabbits comprising these groups were

TABLE 1

THE EFFECT OF THE LENGTH OF THE PREINSEMINATION PERIOD OF PROGESTERONE TREATMENT ON FERTILIZATION OF OVA IN ADULT RABBITS ARTIFICIALLY INSEMINATED WITH DILUTED FRESH SEMEN

Daily dose × days of treatment	Treatment day of insemination[a]	No. of rabbits with fertilized ova—no. with ova—no. treated	Total no. ova recovered[b]	% Ova fertilized	Diameter of mucin layer (mm)	Mean sperm index[c]		
						Mucin layer	Zona Pellucida	Perivitelline space
1 mg × 8	7	2–6–6	39 (78)	13	0.260	0	0.08	0.08
1 mg × 6	5	5–6–6	42 (95)	71	0.275	0.83	0.90	0.71
1 mg × 4	3	0–3–6	16 (24)[d,e]	0	0.245	0.18	0.06	0
1 mg × 2	1	6–6–6	39 (95)	97	0.333	0.74	1.5	1.5
None × 8	7	4–5–5	40 (82)	68	0.289	1.9	2.3	2.2

[a] Animals were autopsied 48 hr after insemination. All injections were subcutaneous.

[b] Number in parentheses is the percentage of ovulated ova that was recovered.

[c] Scores of 0, 1, 2, or 3 were given to each area of an ovum when the number of spermatozoa observed was 0, 1 to 3, 4 to 10, or >10, respectively.

[d] Two ova were found in the uterus. Spermatozoa were observed in the zona pellucida and in the perivitelline space of 3/16 uncleaved ova.

[e] Significantly different from control value, $p \leq 0.05$.

devoid of ova. Comparing the progesterone treatment data in Table 1 with this accumulated control data, ova recovery in animals inseminated on day 3 of treatment with progesterone and fertilization in animals inseminated on day 3 or day 7 of treatment were clearly less than the ranges of the corresponding control values. Thus, on the basis of comparison by statistics of proportion and the use of accumulated control data, separate periods of infertility, related to the length of the period of progesterone treatment before insemination, appear to occur.

The premature presence of ova in the uterus indicated that progesterone treatment, initiated 2 days before insemination, accelerated the movement of ova between the oviduct and uterus. The low recovery of ova suggested that ova either degenerated beyond recognition soon after arrival in the uterus or were expelled.

The minimum effective dose of progesterone to inhibit fertilization was determined in the second experiment. Progesterone was injected subcutaneously daily for 8 days. Rabbits were inseminated on the 7th day of treatment and autopsied on the 9th day. The data are summarized in Tables 2 and 3. Fertilization of ova was reduced by progesterone. The degree of inhibition was dose dependent in the dose range of 0.05 to 4 mg and the ED_{50} was 0.4 mg/day. A daily dose of as much as 4 mg did not completely block fertilization. The sperm index was reduced appreciably in the zona pellucida and perivitelline space of ova in groups treated with 0.5 mg progesterone/day. With larger doses (1 to 4 mg/day), the sperm index in the mucin layer was zero and approached zero in the other two areas. A significant difference from controls in the thickness of the mucin layer ($p < 0.05$) occurred only in the group treated with 0.1 mg progesterone/day. This difference may not be real since values as high as this have been seen occasionally in other studies in groups of similar control animals. The percentages of ova recovered in treatment and control groups were similar.

Effect of progesterone treatment on sperm transport. Since the preceding studies indicated that 1 mg of progesterone daily beginning 6 days before insemination consistently

TABLE 2

THE EFFECT OF VARIOUS DOSES OF PROGESTERONE ON FERTILIZATION OF OVA IN ADULT ESTROUS RABBITS TREATED DAILY FROM DAY −6 TO +1 OF EXPERIMENT IN WHICH THE DAY OF ARTIFICIAL INSEMINATION IS DESIGNATED AS DAY 0 AND AUTOPSY IS DONE ON DAY +2 OR 48 HR AFTER INSEMINATION

	Treated group				Control group			
Dose of progesterone (mg)	No. rabbits with fertilized ova—no. with ova—no. treated	Total no. of ova recovered[a]	No. of ova fertilized (%)	Diameter of mucin layer (mm)	No. rabbits with fertilized ova—no. with ova—no. treated	Total no. of ova recovered[a]	No. of ova fertilized (%)	Diameter of mucin layer (mm)
4	1– 6– 6	43 (84)	2[b]	.288	6– 6– 6	28 (85)	79	.294
2	1– 6– 6	36 (88)	19[b]	.275	6– 6– 6	34 (89)	88	.302
1	2–11–11	55 (65)[b]	10[b]	.322	10–11–12	79 (75)	74	.309
0.5	3– 6– 6	45 (80)	38[b]	.288	5– 5– 5	29 (87)	88	.285
0.2	3– 4– 4	24 (92)	75	.276	6– 6– 6	24 (89)	91	.303
0.1	5– 5– 6	38 (76)	100	.397[b]	5– 6– 6	31 (84)	71	.312
0.05	5– 5– 5	43 (84)	93	.292	5– 5– 5	24 (89)	92	.290

[a] Number in parentheses is the percentage of ovulated ova that were recovered.

[b] Significantly different from corresponding control value $p \leqslant 0.05$.

TABLE 3
THE EFFECT OF PROGESTERONE ON THE NUMBER OF SPERMATOZOA OBSERVED IN VARIOUS REGIONS OF FERTILIZED AND UNFERTILIZED OVA RECOVERED FROM ADULT RABBITS

Dose of progesterone (mg)	Sperm index in treatment group[a]			Sperm index in control group		
	Mucin layer	Zona pellucida	Perivitelline space	Mucin layer	Zona pellucida	Perivitelline space
4	0	0.025	0	1.02	0.89	1.54
2	0	0.06	0.06	0.76	1.18	1.49
1	0	0	0	1.12	2.23	2.04
0.5	0.70	0.80	0.85	0.50	1.00	1.80
0.2	0.29	1.09	1.07	0.76	1.18	1.52
0.1	0	1.52	1.52	0.23	1.42	1.78
0.05	0.67	1.39	1.29	0.50	1.00	1.80

[a] Index values of 0, 1, 2, or 3 were assigned to each of the designated areas of individual ova and represented sperm counts in these areas of 0, 1 to 3, 4 to 10, or > 10, spermatozoa, respectively.

lowered the percentages of fertilized ova, this quantity was used in the following series of experiments. In the first experiment animals were injected subcutaneously daily with progesterone or vehicle for 8 days. On the 7th day rabbits were anesthetized and 0.25 ml diluted semen was deposited into each horn of the uterus. The percentage of fertilized ova recovered on the 9th day from six control animals treated with corn oil and in five animals treated with 1 mg progesterone was 100 and 90%, respectively. This indicated that interference with sperm capacitation was not an important factor in the inhibition of fertilization produced with progesterone.

In a second experiment, the effect of semen dilution on fertilization was investigated to exclude the possibility that the inhibitory effect of progesterone on fertilization of ova was an experimental artifact produced by a combination of marginal numbers of spermatozoa in the diluted semen and the stimulatory effects of progestreone on the genitalia. It was thought that under this set of special conditions increasing the concentration of sperm inseminated into the anterior vagina might enable more sperm to enter the uterus and oviduct to accomplish a higher rate of fertilization. Consideration of this possibility was based on studies by Austin (1948) and Chang (1951) showing that the quantity of sperm recovered from the oviduct was directly related to the total number inseminated, but Braden (1953) demonstrated that the two values were independent when the number inseminated was above a certain level. Four groups of six rabbits were selected randomly. One group was injected daily with vehicle only, and three groups were injected daily with 1 mg progesterone for 8 days. Animals were inseminated on the 7th day and killed on the 9th day of the experiment. The control group was inseminated with a 1:9 dilution (10% concentration) of a sample of pooled semen. The three groups injected with progesterone were inseminated with concentrations of 10, 20, and 100%, respectively, of the same sample of semen (Table 4). An increase in the semen concentration did not increase the percentage of fertilized ova. These results, coupled with the above observation that the percentage of ova fertilized was normal when insemination was intrauterine, suggested that the low fertilization rate following artificial insemination into the anterior vagina of progesterone-treated rabbits was not related to the concentration of spermatozoa in the semen but was caused by

TABLE 4
THE INFLUENCE OF THE RATE OF DILUTION OF SEMEN ON THE ANTIFERTILITY EFFECT OF PROGESTERONE IN ARTIFICIALLY INSEMINATED RABBITS

Semen concentration (%)	Treatment	No. with ova/no. treated	Ave. no. ova	Fertilized ova (%)
10	Oil	6/6	5.2	80[a]
10	Progesterone, 1 mg	5/5	5.4	14[a,b,c]
30	Progesterone, 1 mg	6/6	7.6	12[a,b,c]
100	Progesterone, 1 mg	5/6	6.7	25[a,b,c]

[a] In unfertilized ova, no sperm were present in the mucin layer, zona pellucida, or perivitelline space.

[b] In fertilized ova, sperm were only occasionally seen in any of the above three areas and were always few in number.

[c] Significantly different from corn oil control value.

deficient sperm transport through the cervix from the anterior vagina to the posterior uterus.

A third experiment was undertaken to evaluate more directly the effect of progesterone on sperm transport through the reproductive tract. Animals were treated daily with 1 mg progesterone for 7 days, artificially inseminated into the anterior vagina on day 7, and autopsied 4, 8, 16, and 24 hr after insemination. The reproductive tract was removed, and sperm were recovered from the various segments of the reproductive tract (Table 5). In comparison with control rabbits the average number of spermatozoa recovered from animals treated with progesterone was consistently lower in practically all of the segments of the uterus and oviducts and at each of the four time periods studied. The reduction was apparent particularly in the uterus at the level of the middle and anterior segments while either no sperm, or only very few, were found in the middle and anterior segments of the oviduct during the 24-hr period following insemination. This suggested that progesterone impeded the migration of sperm from the cervix into the posterior region of the uterus and virtually blocked a further movement anteriorly beyond this position.

In a fourth experiment the stimulatory effect of oxytocin on uterine motility was applied in an attempt to overcome the adverse effect of progesterone on sperm transport. Three groups of 4–5 rabbits each were injected daily for 8 days with either vehicle, 1 mg progesterone or 1 mg progesterone plus three subcutaneous injections of 50 U.S.P.

TABLE 5
THE EFFECT OF PROGESTERONE ON THE NUMBER OF SPERMATOZA RECOVERED FROM VARIOUS SEGMENTS OF THE RABBIT GENITAL TRACT 4–24 HR AFTER ARTIFICIAL INSEMINATION WITH 10×10^6 TO 40×10^6 SPERMATOZA[a]

Segment of genital tract	Control, No. of hours after insemination				Progesterone No. of hours after insemination			
	4	8	16	24	4	8	16	24
Cervix	147,600	122,500	28,630	25,070	191,600	180,000	17,290	29,980
Uterus-posterior	54,040	34,590	12,390	34,590	41,830	24,470	2,993	8,796
Uterus-middle	105,200	5,724	5,445	19,330	6,462	838	2,693	7,060
Uterus-anterior	49,430	11,670	15,560	86,830	3,231	180	3,531	4,847
Oviduct-posterior	2,454	3,711	3,860	6,822	0	180	718	180
Oviduct-middle	300	4,847	2,154	957	0	0	120	120
Oviduct-anterior	0	3,531	2,244	838	0	0	0	0
Uterus-complete	208,670	51,984	33,395	140,750	51,523	25,488	9,217	20,703
Oviduct-complete	2,754	12,089	8,258	8,617	0	180	838	300

[a] Animals were treated daily with 1 mg progesterone in 0.2 cc corn oil or with corn oil only injected subcutaneously for 7 days and inseminated on the 7th day. Each value is the mean from three rabbits.

units oxytocin (Mann Research Laboratory, N.Y.) at 1 hr before insemination and 4 and 8 hr after insemination. Animals were inseminated on the 7th day and killed on the 9th day of treatment. The number of fertilized ova/total ova recovered from the three groups in the same order as above was 28/41, 0/32, and 7/17, respectively. Ova recovery rates for the groups were 100, 92, and 65%, respectively. Sperm were absent from the mucin layer, zona pellucida, and perivitelline space of ova from the group receiving progesterone only. The addition of oxytocin increased the sperm index values to approximately 50% of corresponding control values. Thus, the inhibitory effect of progesterone on sperm transport was partially reversed by oxytocin.

DISCUSSION

Rabbits under the influence of progesterone apparently pass through two distinct periods of lowered fertility. The first period occurs on the 3rd day of treatment and is short in duration. The second period of infertility is more prolonged. It begins about the 7th day of treatment and continues at least until the 11th day. In the interval of time between the two infertile periods, insemination results in normal fertility. With a continuous treatment period of 7 or more days, progesterone prevents fertilization primarily by inhibiting the transport of sperm. This conclusion is based on (1) the attainment of a normal fertilization rate after intrauterine insemination, (2) the drastic reduction of sperm recovered from the anterior ⅔ of the oviduct following vaginal insemination, (3) reversal of the inhibiting effect of progesterone on fertilization by the addition of oxytocin around the time of insemination, and (4) the observation that no sperm, or only a very few, were found in the zona pellucida and mucin layer of ova. Progesterone also appears to interfere with sperm transport when rabbits are inseminated on the 3rd day of treatment, evidenced by the scarcity of sperm found in the layers investing the ova, and, in addition, it accelerates the movement of ova through the oviducts and presumably through the uterus. The differences in the duration of the two infertile periods and in the movements of ova during those periods suggest that different mechanisms are responsible for the inhibition of fertilization following insemination on the 3rd and 7th day of progesterone treatment.

That progesterone prevents the fertilization of ova was first reported by Boyarsky *et al.* (1947) and more recently was confirmed by Nutting and Mares (1967) and Chang (1969), but conflicting results on fertilization and ova transport have been reported following insemination on day 3 of treatment (Chang, 1967, 1969). Chang (1969) has also compared the progesterone-treated rabbit to the pseudopregnant animal and has shown that the proportion of eggs fertilized in both groups remains the same for corresponding lengths of pseudopregnancy and treatment. On the basis of sperm found in mucosal smears, Austin (1949) obtained data from rabbits pseudopregnant for 4 to 12 days suggesting that sperm did not reach the oviducts. Our results in progesterone-treated animals were similar although small numbers of sperm were found in the oviducts 8 to 24 hr after insemination. Thus, progesterone treatment appears to mimic the effects of pseudopregnancy on fertilization of ova.

Migration of sperm through the cervix is probably accomplished by their own motility (Noyes, Adams, and Walton, 1958). Movement through the remainder of the reproductive tract is believed to be due primarily to an active transport of sperm as a result of the muscular activity of the uterus. Uterine motility is generally highest during estrus and decreases in pseudopregnancy (Reynolds and Friedman, 1930) and under the influence of progestational substances (Reynolds and Allen, 1932). Although the mechanism by which progesterone inhibits the motility of the uterus is still unclear, the altered con-

tractility patterns of the myometrium of the uterus and oviduct are probably responsible for the impairment of sperm transport through these organs in pseudopregnant and in progesterone-treated rabbits. Movement of sperm, however, was not completely prevented by progesterone treatment as a few sperm were recovered from the oviducts 8 to 24 hr after insemination and a few ova were fertilized. If it is assumed that the uterine musculature is quiescent as a result of treatment with progesterone, then the sperm found in the isthmic-ampullary region of the oviduct must have reached that position by their own motility. Traveling at a rate of 1 to 3 mm/min (Lloyd-Jones and Hays, 1918; Parker, 1931; Phillips, 1935), they could have traveled the distance from the cervix to the ampulla easily during the 8 to 16 hr that ensued before autopsy. Regardless of the mode of transport of these few sperm, the observation that at least some of the sperm were capable of fertilization when they reached the oviduct is particularly relevant to the possible involvement of progesterone in some aspect of sperm physiology within the female reproductive tract. It has been reported that capacitation of spermatozoa has been inhibited or prevented in pseudopregnant (Erikson, 1967; Hamner *et al.*, 1968) and in progesterone-treated rabbits (Chang, 1958), while Chang (1969) has postulated that interference in this process was at least partially responsible for the fertilization failure after progesterone treatment. The occurrence of a low incidence of fertilization even though the number of sperm in the oviduct was few, however, is evidence to the contrary. In addition, ova were fertilized when semen was deposited directly into the uterus of progesterone-treated animals. Similarly, fertilization occurred in pseudopregnant rabbits inseminated into the uterus (Murphree *et al.*, 1947) or via the oviduct (Austin, 1949). Furthermore, the virtual absence of sperm from the region of the oviduct where fertilization takes place indicates that if progesterone does interfere with capacitation of spermatozoa, it is of minor importance in comparison to the effect of the steroid on sperm transport.

The application of the micropore filter technique to the recovery of sperm from the genitalia of control animals resulted in a somewhat higher estimate of number of sperm in the oviducts than has been reported previously for other methods (Austin, 1949; Chang, 1951; Braden, 1953). The results obtained correspond sufficiently well, however, with those determined by other methods to demonstrate the potential usefulness of the micropore filtration technique in the study of sperm transport.

The primary cause of the inhibition of fertilization by progestins appears to be an interference with sperm transport mechanisms in the uterus and/or oviduct. Since continuous treatment of women with a progestin has been reported to be an effective means of contraception (Martinez-Manautou *et al.*, 1966) and alteration of sperm transport has been implicated as a mode of action, the rabbit, when treated according to the protocol reported herein, appears to offer some important advantages as an animal model for use in the testing and the selection of compounds with potential utility as contraceptive agents.

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