Increased Numbers of Sperm in the Oviducts and Improved Fertilization Rates in Rabbits after Administration of Phenylephrine or Ergonovine Near the Time of Insemination

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INCREASED NUMBERS OF SPERM IN THE OVIDUCTS AND IMPROVED FERTILIZATION RATES IN RABBITS AFTER ADMINISTRATION OF PHENYLEPHRINE OR ERGONOVINE NEAR THE TIME OF INSEMINATION

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Summary

Phenylephrine, an alpha-adrenoceptor agonist, was administered im to does near the time of mating or insemination. The treatment increased sperm numbers in the oviducts by about 50-fold and in the uterus by about 10-fold at 2 or 2.5 h after insemination. Methoxamine, another alpha-adrenoceptor agonist that was given im, did not increase sperm numbers, although both phenylephrine and methoxamine significantly increased the number and amplitude of uterine contractions when contractions were measured by strain gauge force transducers attached to the uterus of conscious does. Ergonovine, an ergot derivative given im, increased sperm numbers more than 10-fold in the oviducts and five to 10-fold in the uterus at 2 or 2.5 h after insemination. Ergonovine increased the frequency and amplitude of uterine contractions when given iv but not when given im. In tests with a range of doses of phenylephrine and ergonovine, 5 mg of phenylephrine and .6 mg of ergonovine appeared to be near optimal for maximizing the number of sperm in the uterus and oviducts at 2.5 h after insemination. Phenoxybenzamine, an alpha-adrenoceptor blocking agent, prevented the phenylephrine-induced increases in both uterine contractions and sperm numbers in the oviducts and uterus. Phenoxybenzamine also prevented the effect of ergonovine on sperm numbers. In does inseminated with low numbers of sperm (92,000; an inseminate selected to result in a low fertilization rate in control does), the administration of phenylephrine or ergonovine significantly increased ovum fertilization rates (16% for control does, 52 and 63%, respectively, for phenylephrine- and ergonovine-treated does).

(Key Words: Rabbits, Sperm Transport, Ovum Fertilization, Phenylephrine, Ergonovine, Uterine Contractions.)

Introduction

Experimental treatments that improve sperm transport in laboratory animals have potential relevance to problems of fertilization failure in the larger domestic animals. Fertilization failure in cattle causes nearly as many infertile services as does embryonic mortality (Bellows et al., 1979; Hawk, 1979).

Three compounds, when added to semen used for insemination or injected into females near the time of insemination, have increased the number of sperm found in the oviducts. These compounds include prostaglandin E_1 or prostaglandin $F_{2\alpha}$ for rabbits (Mandl, 1972; Spilman et al., 1973; Hawk and Cooper, 1979), prostaglandin E_1 and $F_{2\alpha}$ combined for sheep (Edquist et al., 1975) and estradiol-17β for rabbits and sheep (Hawk and Cooper, 1975, 1978).

Alpha-adrenoceptor agonists increase the number and strength of uterine contractions in rabbits under estrogenic influence (Marshall, 1970; Nesheim, 1972). Such compounds given near the time of insemination might also improve sperm transport and ovum fertilization. This study was done to determine (1) whether phenylephrine or methoxamine, alpha-adrenoceptor agonists (Innes and Nickerson, 1975) would increase the number of sperm in the reproductive tract of the doe after insemination,

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(2) whether ergonovine, a lysergic acid-amide that stimulates uterine motor activity (Saameli, 1978), would increase sperm numbers in the tract, (3) an approximate optimum dose of phenylephrine and ergonovine to increase sperm numbers, (4) the effect of phenylephrine, methoxamine and ergonovine on uterine contractions, and (5) whether phenylephrine or ergonovine would improve the ovum fertilization rate.

Materials and Methods

Does were sexually-mature New Zealand Whites weighing about 4 kg each. Eight bucks of various breeds, selected for high sperm concentration and high sperm motility, were used in rotation for either mating or collecton of semen through the course of the experiments. No buck was used more than three times weekly. Natural mating was used in the first and fourth experiments (the initial experiments with phenylephrine or ergonovine). Natural mating and artificial insemination were compared in the second experiment to ascertain that the response to phenylephrine was not affected greatly by method of insemination. Artificial insemination was used in the other experiments to provide known numbers of sperm in the inseminate.

Exp. 1. Phenylephrine. Does were mated once to each of two bucks within 5 min. Does were mated by pairs; immediately after the does were mated, 1 ml of saline solution (.9% NaCl) was injected im into the control doe of each pair. One milliliter of saline solution was added to 5 mg of phenylephrine hydrochloride and the solution was injected immediately into the other doe. Injections were completed within 2 min after mating.

At 2 h after mating, the does were sacrificed by an overdose of pentobarbital injected into the marginal ear vein. The entire reproductive tract, including the vulva, was removed and the oviducts, uterine horns, cervices and vagina were flushed or washed with saline soluton. The procedures and efficiency of sperm recovery have been described (Hawk and Cooper, 1978). Briefly, each oviduct was flushed with 6 ml of saline solution and each uterine horn with 20 ml. The cervices and vagina were cut into strips and soaked overnight in saline solution. The flask was then shaken to remove sperm adhering to the tissues or glass. Aliquots of the flushings or washings of the tract were counted at 200× in a hemacytometer under a phase contrast microscope.

In this the seubsequent experiments, individual sperm counts were transformed to $\log_{10}$, and data were analyzed statistically by t-test or by analysis of variance. Sperm counts were converted to logarithms to normalize the distribution. Arithmetic means and standard errors are given in tables 2 to 5 and 7.

Exp. 2. Phenylephrine and Methoxamine. The experimental design was 2 × 3 factorial (natural mating or artificial insemination; treatment with saline solution, phenylephrine or methoxamine). Does assigned to be mated naturally were mated to two bucks as in Exp. 1. Does assigned to be inseminated artificially were first checked for estrus by vasectomized bucks, but mating was prevented to avoid the deposition of seminal fluids in the vagina. Does in estrus were inseminated with .2 ml of freshly collected undiluted semen; each inseminate contained about 100 million sperm.

Saline solution or phenylephrine was injected as in Exp. 1, except that injections were made 5 min before mating or artificial insemination. Methoxamine hydrochloride, β-hydroxy-β-(2,5-dimethyloxyphenyl) isopropylamine[4], was also injected im (5 mg in .25 ml of vehicle) 5 min before mating or insemination. Does were necropsied 2 h after insemination and sperm were recovered as described in Exp. 1. Data were analyzed by analysis of variance.

Exp. 3. Phenylephrine Dosage. Three doses of phenylephrine were compared for effects on numbers of sperm in the reproductive tract at 2.5 h after insemination. Four does/replicate were inseminated artificially as in Exp. 2. Individual does were treated immediately after insemination with an im injection of 1, 5 or 25 mg of phenylephrine in 1 ml of saline solution or with 1 ml of saline solution (control). Eight replicates were used and sperm were recovered as in Exp. 1. Data were analyzed by analysis of variance and regression of sperm numbers on dose of phenylephrine (Steel and Torrie, 1960).

Exp. 4. Ergonovine. Sixteen does in eight pairs were used. One doe of each pair was given an im injection of 3 ml of saline solution and the other was given 3 ml of vehicle containing .6 mg of ergonovine maleate[5].

[4] Vasoxyl; Burroughs Wellcome Co., Research Triangle Park, NC.

[5] Ergotrate maleate, Eli Lilly, Indianapolis, IN.

Ten min after treatment, each doe was mated once to each of two bucks. Does were necropsied 2 h after mating. Sperm were recovered as in Exp. 1 and data were analyzed by t-test.

Exp. 5. Ergonovine Dosage. Eight replicates of four does each were used to compare three doses of ergonovine for effects on sperm numbers. Does were checked for estrus by vasectomized bucks, but mating was prevented. Ergonovine maleate was injected im into three estrous does in each replicate at .1, .5 or 2.5 mg/doe. Control does were treated with 2.5 ml of saline solution. Ten minutes after treatment, does were inseminated with .2 ml of freshly collected undiluted semen containing about 100 million sperm.

At 2.5 h after insemination, does were necropsied and sperm were recovered and counted. Data were analyzed by analysis of variance and regression of sperm numbers on dose of ergonovine.

Exp. 6. Uterine Contractions. Frequency and amplitude or uterine contractions were measured by strain gauge force transducers attached to one uterine horn. The transducers were assembled and waterproofed by the method of Bass and Wiley (1972) with minor modifications and were similar to those of Bass and Wiley in size and radius of curvature. A small loop of 28-guage stainless steel wire was fixed to each end of the transducer with epoxy adhesive to facilitate attachment to the uterus and the transducer was encapsulated with RTV 102 or 112 adhesive[6].

Calibration of strain gauge transducers in vitro showed a linear relationship between increments of force applied to the gauge and additional deflection of the writing arm of the physiograph. Approximately 3 g of force in vitro was required to deflect the writing arm 10 mm, although the force required to cause a standard deflection varied somewhat among transducers.

Does in estrus were laparotomized under pentobarbital anesthesia. The loop on each end of a transducer was sutured to the uterine serosa with 4-0 thread. The transducer was placed about midway along the anterior one-half of one uterine horn (1.5 to 2 cm from the tubouterine juction). The wire leading from the transducer to the exterior was passed through silastic tubing. The tubing was sutured to the broad ligament in three places, with a loop of tubing left between sutures so that movements of the rabbit would not result in tension on the transducer. The tubing was pulled through the body wall and routed beneath the skin to the base of the neck, where it was exteriorized and sutured to the skin.

Uterine contractions were recorded 5 to 10 d after the transducer was installed. The does were in estrus at the time of recording. They were conscious and sitting in a small box, but were otherwise not restrained. The lead wire was attached to a strain gauge coupler (NARCO type 7172) in a NARCO Six-B physiograph. Amplification was set to obtain an average contraction amplitude during the control period of 25 to 40 mm on the recording paper. The recording paper moved at 3 cm/min.

Contractions were recorded for about 1 h before treatment and for 1.5 h after administration of the test compound. Test compounds were injected im in the following doses: phenylephrine, 5 mg; methoxamine, 5 mg; ergonovine, .6 mg. A higher dose of ergonovine (1.2 mg) was given to five does by im injection, and .6 mg of ergonovine was given to four does by iv injection. For statistical analysis, contraction characteristics were measured for three 20-min periods: (1) the last 20 min before injection of the test compound (control period); (2) between 10 and 30 min after injection of the compound; (3) between 60 and 80 min after injection of the compound.

Contraction characteristics were measured as described by Rexroad and Barb (1975): (1) number of primary contractions/20 min, reflecting either a single contraction peak that rose from and returned to the baseline or a period of increased uterine tone during which the recording pen remained above the baseline and secondary contractions were superimposed on the primary contraction; (2) number of secondary contractions superimposed on primary contractions during each 20-min period; (3) total number of contractions, both primary and secondary, within the 20 min; (4) the amplitude of primary contractions, measured as the distance (mm) from the baseline to the point of the highest peak in that contraction. Vertical movements of at least 3 mm (about 10% of the average amplitude of a primary contraction) were considered to represent a contraction, either primary or secondary. The

[6] General Electric Co., Waterford, NY.

amplitude of primary contractions was averaged for each doe within each time period; the standard errors listed in table 6 were calculated from mean values within each period for each doe.

Contraction characteristics were analyzed among the three periods by analysis of variance within treatment groups, with each doe serving as its own control. The control period was compared with the two test periods, then one test period was compared with the other.

Exp. 7. Alpha-Adrenergic Blockade and Sperm Numbers. An alpha-adrenoceptor blocking agent, phenoxybenzamine hydrochloride[7], was administered to determine whether it would prevent the effects of phenylephrine and ergonovine in increasing the number of sperm reaching the oviducts at 2 h after insemination. Phenoxybenzamine blocked the stimulatory effect of alpha-adrenergic agonists, such as phenylephrine, on uterine contractions (Marshall, 1970; Fuchs, 1972) and prevented the oxytocic effect of ergonovine on uterine contractions (Fregnan and Glasser, 1964); however, phenoxybenzamine did not prevent the stimulatory effect of prostaglandin $F_{2\alpha}$ on contractions of the rabbit oviduct (Spilman and Harper, 1974). Therefore, phenoxybenzamine was also administered to does given prostaglandin $F_{2\alpha}$ with the expectation that prostaglandin would induce high sperm numbers despite the alpha-adrenoceptor blockade.

All solutions were given im. Phenoxybenzamine was given 15 min before insemination to allow time for the alpha-adrenoceptor block to be established. The other solutions were given immediately after insemination. In the experiment, of 2 × 4 factorial design, estrous does were assigned to the following treatment groups: (1) saline solution; (2) phenoxybenzamine (20 mg in 1 ml of ethanol diluted to 1.5 ml with saline solution) 15 min before insemination; (3) prostaglandin $F_{2\alpha}$[8] (.75 mg); (4) phenoxybenzamine plus prostaglandin; (5) phenylephrine (5 mg); (6) phenoxybenzamine plus phenylephrine; (7) ergonovine (.6 mg) or (8) phenoxybenzamine plus ergonovine. Does were inseminated artificially with .2 ml of freshly-collected buck semen that contained about 100 million sperm and necropsied at 2 h after insemination. Sperm were recovered and counted as described previously. Data were analyzed by analysis of variance.

Exp. 8. Alpha-Adrenoceptor Blockade and Uterine Contractions. Uterine contractions were recorded, as described in Exp. 6, in rabbits with a strain gauge transducer attached to one uterine horn. Contractions were recorded for at least 30 min, then phenoxybenzamine (20 mg) was administered by im injection followed in 15 min by administration of saline solution, phenylephrine (5 mg), methoxamine (5 mg) or ergonovine (.6 mg). Recording was continued for another 90 min. Contractions were counted and measured as described in Exp. 6: before any treatment; between 10 and 30 min, and between 60 and 80 min after injection of phenylephrine, methoxamine or ergonovine (25 to 45 and 75 to 95 min after administration of phenoxybenzamine).

Exp. 9. Ovum Fertilization. This experiment was conducted to determine whether exogenous phenylephrine or ergonovine would improve ovum fertilization rates. Estrous does were injected iv with 100 IU of human chorionic gonadotropin (hCG) to induce ovulation. Six hours later, the does were inseminated artificially with low numbers of sperm (an average of 92,000 sperm/inseminate). The aim was to cause a low rate of fertilization in control does so that potentially beneficial effects of treatment could be demonstrated. Freshly-collected semen was diluted 500- to 1,000-fold with saline solution (.9% NaCl) and insemination was performed immediately with .2 ml of diluted semen. The does were inseminated several hours before ovulation to allow time for sperm to be capacitated (Bedford, 1972) before or soon after ovulation. Does were necropsied 30 h after the injection of hCG. The oviducts were excised and ova were flushed with saline solution into watch glasses. Ova were placed in a hanging drop on a cover slip supported over a slide on pillars of vaseline and examined for cleavage and for number of blastomeres. Gentle pressure was then applied to the coverslip to rupture the zonae pellucidae and extrude the contents of the ova. Lacmoid strain was flooded under the cover slip to stain sperm heads and the number of sperm attached to each zona pellucida was counted at 200× under a phase contrast microscope.

The proportions of control and treated does with cleaved ova and the proportions of ova that were cleaved were compared by chi-square.

[7] Dibenzyline; Smith, Kline and French, Philadelphia, PA.

[8] Prostin $F_{2\alpha}$, The Upjohn Co., Kalamazoo, MI.

Results

Exp. 1. Phenylephrine. Significantly more sperm were recovered from the oviducts, uterus and cervices of phenylephrine-treated does than of control does (table 1). The magnitude of differences between control and treated does was much greater for the oviducts and uterus than for the cervix. Differences between control and treated does were not significant for either the vagina or the entire tract. The results indicated that phenylephrine either increased the transport of sperm to the cervices, uterus and oviducts or increased the retention of sperm in those sites.

Exp. 2. Phenylephrine and Methoxamine. This experiment was done to compare the effects of phenylephrine with those of a second alpha-adrenoceptor agonist, methoxamine, and to compare the effects of phenylephrine or methoxamine on sperm numbers in does inseminated artificially with about 100 million sperm to the effects in does mated naturally. About 750 million sperm were deposited in the vagina with two natural matings.

The type of insemination did not significantly affect the number of sperm in the uterus or oviducts, but more sperm were recovered from the cervices, vagina and the entire reproductive tract after natural service than after artificial insemination (table 2). Phenylephrine increased sperm numbers in the uterus and oviducts, as in Exp. 1, but methoxamine had little if any effect on sperm numbers. Differences between phenylephrine- and methoxamine-treated does in sperm numbers were significant for the oviducts, uterus and cervices (table 2), the same segments in which phenylephrine increased sperm numbers in Exp. 1.

Exp. 3. Phenylephrine Dosage. Three doses of phenylephrine, 1, 5 and 25 mg, were tested for effects on sperm numbers in the reproductive tract at 2.5 h after artificial insemination. When the variation among groups was significant, the regression of sperm number on dose of phenylephrine was partitioned into linear and nonlinear components.

Phenylephrine increased the number of sperm in the oviducts and uterus significantly, with the 5-mg dose causing the greatest increase (table 3).

The regression of sperm numbers on dose of phenylephrine had a significant nonlinear component for both the oviducts and uterus. Nonlinearity was caused by the 5-mg dose of phenylephrine having a somewhat greater stimulatory effect on sperm numbers than the 25-mg dose. Apparently, 5 mg of phenylephrine was near the optimum dose for increasing sperm numbers in the oviducts and uterus and 25 mg elicited no additional response.

Exp. 4. Ergonovine. At 2 h after natural mating, significantly more sperm were recovered from the oviducts and uterus of does treated with .6 mg of ergonovine than from the oviducts and uterus of control does (table 4). Ergonovine did not increase sperm numbers significantly in the cervices, vagina or entire reproductive tract.

Exp. 5. Ergonovine Dosage. Three doses of ergonovine were tested for effects on sperm numbers at 2.5 h after artificial insemination

TABLE 1. EFFECT OF PHENYLEPHRINE ON NUMBER OF SPERM RECOVERED FROM THE REPRODUCTIVE TRACT OF DOES 2 HOURS AFTER NATURAL MATING

Treatment[a]	Sperm recovered				
	Oviducts	Uterus	Cervices	Vagina	Entire tract
	Arithmetic means ± SE				
	(10^2)[b]	(10^3)	(10^6)	(10^6)	(10^6)
Saline	1 ± 1	94 ± 31	14 ± 3	48 ± 19	63 ± 22
Phenylephrine	60 ± 28	1,069 ± 291	31 ± 5	60 ± 15	92 ± 19
	Probability[c]				
	<.001	<.001	<.005	.20	.12

[a]Eight does/group. Saline solution or phenylephrine HCl (5 mg) was injected immediately after mating.

[b]Multiplier for each mean.

[c]Probabilities by t-test of logarithms of sperm numbers within segments of the reproductive tract.

TABLE 2. EFFECT OF PHENYLEPHRINE OR METHOXAMINE ON NUMBER OF SPERM RECOVERED FROM RABBITS 2 HOURS AFTER NATURAL MATING OR ARTIFICIAL INSEMINATION

Type of insemination and treatment[a]	Sperm recovered				
	Oviducts	Uterus	Cervices	Vagina	Entire tract
	Arithmetic means ± SE				
	(10^2)[b]	(10^3)	(10^6)	(10^6)	(10^6)
Natural service (NS)					
Saline	3 ± 1	118 ± 48	20 ± 6	47 ± 21	67 ± 27
Phenylephrine (Ph)	67 ± 38	1,232 ± 362	33 ± 6	62 ± 21	96 ± 24
Methoxamine (Me)	1 ± 1	323 ± 171	14 ± 4	43 ± 19	57 ± 22
Artificial insemination (AI)					
Saline	5 ± 2	118 ± 36	6 ± 1	11 ± 3	17 ± 4
Phenylephrine (Ph)	46 ± 14	976 ± 280	31 ± 9	23 ± 6	55 ± 14
Methoxamine (Me)	5 ± 4	418 ± 290	10 ± 5	16 ± 6	26 ± 10
	Probability[c]				
NS vs AI	>.25	>.25	<.05	<.005	.005
Saline vs Ph + Me	.25	.06	.25	>.25	>.25
Ph vs Me	<.005	<.005	<.01	.20	.07

[a]Six does/group. Natural mating to two bucks or artificial insemination with 96 ± 7 million sperm. Phenylephrine HCl (5 mg) or methoxamine HCl (5 mg) was injected im 5 min before mating or artificial insemination.

[b]Multiplier for each mean.

[c]Probabilities by analysis of variance of logarithms of sperm numbers within segments of the reproductive tract.

TABLE 3. EFFECT OF DOSE OF PHENYLEPHRINE ON NUMBER OF SPERM RECOVERED FROM THE REPRODUCTIVE TRACT OF DOES 2.5 HOURS AFTER ARTIFICIAL INSEMINATION

Dose of phenylephrine, mg[a]	Sperm recovered[b]				
	Oviducts	Uterus	Cervices	Vagina	Entire tract
	Arithmetic means ± SE				
	(10^2)[c]	(10^3)	(10^6)	(10^6)	(10^6)
0	2 ± 2	74 ± 26	6 ± 1	7 ± 2	13 ± 3
1	17 ± 6	144 ± 40	9 ± 2	6 ± 1	15 ± 3
5	50 ± 10	790 ± 182	20 ± 3	13 ± 3	33 ± 6
25	22 ± 12	465 ± 203	11 ± 5	11 ± 3	22 ± 8
	Probability[d]				
Among groups	<.01	<.05	.25	.20	.18
Linear regression	.12	.20			
Nonlinear regression	<.025	<.05			

[a]Eight does/group. Phenylephrine HCl was injected immediately after insemination.

[b]Inseminate/doe = 100 ± 7 million sperm.

[c]Multiplier for each mean.

[d]Probabilities by analysis of variance of logarithms of sperm numbers among groups and orthogonal regression comparisons of logarithms of sperm numbers on dose of phenylephrine. Regression comparisons were not made when differences among groups were not significant.

TABLE 4. EFFECT OF ERGONOVINE ON NUMBER OF SPERM RECOVERED FROM THE REPRODUCTIVE TRACT OF DOES 2 HOURS AFTER NATURAL MATING

Treatment[a]	Sperm recovered				
	Oviducts	Uterus	Cervices	Vagina	Entire tract
	Arithmetic means ± SE				
	(10^2)[b]	(10^3)	(10^6)	(10^6)	(10^6)
Saline	1 ± 1	187 ± 66	30 ± 6	61 ± 24	91 ± 33
Ergonovine	17 ± 12	956 ± 392	31 ± 5	105 ± 40	137 ± 55
	Probability[c]				
	<.005	<.025	>.25	>.25	>.25

[a]Eight does/group. Does were given .6 mg of ergonovine maleate by im injection 10 min before natural mating.

[b]Multiplier for each mean.

[c]Probabilities by t-test of logarithms of individual sperm numbers.

(table 5). As in Exp. 4, ergonovine caused significant increases in the number of sperm in the oviducts and uterus, but not in the cervices or vagina. Ergonovine caused a significant linear increase in sperm numbers in the oviducts and uterus with increasing dose (table 5). However, the similar effect of the .5- and 2.5-mg dose (table 5) suggested that .5 to 2.5 mg was probably near optimal for increasing sperm numbers.

Exp. 6. Uterine Contractions. This experiment was done to determine the effect on uterine contractions of doses of phenylephrine or ergonovine that caused the greatest increase in sperm numbers in the oviducts. Methoxamine, another alpha-adrenoceptor agonist, was also tested.

During the control recording period, charac-

TABLE 5. EFFECT OF ERGONOVINE ON NUMBER OF SPERM RECOVERED FROM THE REPRODUCTIVE TRACT OF DOES 2.5 HOURS AFTER ARTIFICIAL INSEMINATION

Dose of ergonovine[a], mg	Sperm recovered[b]				
	Oviducts	Uterus	Cervices	Vagina	Entire tract
	Arithmetic means ± SE				
	(10^2)[c]	(10^3)	(10^6)	(10^6)	(10^6)
0	2 ± 2	61 ± 11	6 ± 1	5 ± 1	10 ± 2
.1	2 ± 1	89 ± 20	4 ± 1	6 ± 2	11 ± 3
.5	22 ± 8	650 ± 264	10 ± 5	7 ± 4	18 ± 9
2.5	22 ± 9	671 ± 266	9 ± 3	6 ± 3	15 ± 6
	Probability[d]				
Among groups	<.005	<.005	>.25	>.25	>.25
Linear regression	<.005	<.005			
Nonlinear regression	>.25	>.25			

[a]Eight does/group. Ergonovine maleate was injected im 10 min before artificial insemination.

[b]Inseminate/doe = 99 ± 11 million sperm.

[c]Multiplier for each mean.

[d]Probabilities by analysis of variance of logarithms of sperm numbers among groups and orthogonal regression comparisons of logarithms of sperm numbers on dose of ergonovine. Regression comparisons were not made when differences among groups were not significant.

teristics of motility patterns varied among does, although patterns for each doe remained consistent. The most common type of motility pattern was secondary contractions approximately 30 s apart superimposed on primary contractions about 2 min apart. The number of primary contractions and the total number of contractions during the control period were reasonably consistent among does assigned to the three treatment groups (before treatment, table 6).

Uterine contractile responses to phenylephrine and methoxamine began, within 2 min after injection, with an elevated baseline, indicating an increase in uterine tone. Contractions of high frequency and amplitude were superimposed. By 10 min, the baseline had returned to normal. During the 10- to 30-min period after treatment, both phenylephrine and methoxamine caused a marked increase in the number and amplitude of primary contractions and the total number of contractions (table 6). Phenylephrine increased the number of secondary contractions but methoxamine did not. By 60 to 80 min after treatment, the characteristics of contractions in phenylephrine- and methoxamine-treated does were returning toward those of the control period. Ergonovine caused no detectable contractile response, either within the first 10 min after injection or during the 10- to 30-min and 60- to 80-min periods after treatment (table 6).

TABLE 6. EFFECT OF PHENYLEPHRINE, METHOXAMINE AND ERGONOVINE ON UTERINE CONTRACTIONS IN THE DOE

Treatment and recording period	Contractions during 20-min period[ab]			
	Primary contractions		Secondary contractions, no.	Total contractions, no.
	No.	Amplitude, mm		
Phenylephrine (4)[c]				
a. Before treatment	9 ± 1	32 ± 2	24 ± 4	33 ± 3
b. After treatment—10 to 30 min	22 ± 2	44 ± 3	75 ± 15	97 ± 16
c. After treatment—60 to 80 min	19 ± 4	41 ± 3	46 ± 7	65 ± 8
	Probability[d]			
a vs b + c	<.005	<.025	<.025	<.005
b vs c	>.25	>.25	.07	.07
Methoxamine (6)[c]				
a. Before treatment	11 ± 1	25 ± 2	27 ± 3	38 ± 2
b. After treatment—10 to 30 min	36 ± 3	39 ± 4	30 ± 5	66 ± 5
c. After treatment—60 to 80 min	30 ± 2	43 ± 7	22 ± 2	52 ± 3
	Probability[d]			
a vs b + c	<.001	<.01	>.25	<.005
b vs c	.15	>.25	.15	<.025
Ergonovine (8)[c]				
a. Before treatment	11 ± 1	37 ± 3	35 ± 10	46 ± 10
b. After treatment—10 to 30 min	12 ± 1	40 ± 3	38 ± 11	50 ± 11
c. After treatment—60 to 80 min	11 ± 2	35 ± 4	34 ± 8	45 ± 8

[a]Mean ± SE.

[b]Definitions: Primary contraction = a period of contraction consisting of either a single contraction peak or a period of increased uterine tone with secondary contraction peaks superimposed. Amplitude = distance (mm) from baseline to the highest peak in a primary contraction. Secondary contraction = a contraction superimposed on a primary contraction. Total number of contractions = primary plus secondary contractions.

[c]Number of does in the treatment group in parentheses. Amount of compound administered im: phenylephrine HCl, 5 mg; methoxamine HCl, 5 mg; ergonovine maleate, .6 mg.

[d]Probabilities by analysis of variance.

In five does given 1.2 mg of ergonovine im, no effect on uterine contractions was detectable. In four does given .6 mg of ergonovine iv, uterine tension increased immediately as indicated by a sharply elevated baseline. Tension was sustained for about 5 min and frequent contractions were superimposed. By 10 min after injection, the baseline and contraction pattern had returned to normal. This response to ergonovine given iv was similar to the responses recorded in several other studies (Saameli, 1978).

The relationship of uterine contractions to increased sperm numbers in the oviducts and uterus was not clear. Phenylephrine increased the number and amplitude of uterine contractions and, in previous experiments, increased the number of sperm in the uterus and oviducts. However, methoxamine also increased the number of uterine contractions, but had little effect on sperm numbers; ergonovine given im had no detectable effect on contractions, but consistently increased sperm numbers in the uterus and oviducts.

Exp. 7. Alpha-Adrenoceptor Blockade and Sperm Numbers. The effects of phenoxybenzamine treatment and the relevant statistical comparisons are given in table 7. Prostaglandin increased sperm numbers in each segment of the reproductive tract as compared with groups treated with saline or phenoxybenzamine alone (Groups 1 and 2 vs 3 and 4, Comparison 1, table 7). Phenoxybenzamine had little effect on sperm numbers in saline-treated or prostaglandin-treated does (Comparison 2, table 7).

As in previous experiments, phenylephrine alone (Group 5) increased sperm numbers in the oviducts, uterus and cervices, and ergonovine alone (Group 7) increased sperm numbers mainly in the oviducts and uterus. Treatment with phenoxybenzamine largely prevented the sperm-increasing effects of both phenylephrine

TABLE 7. EFFECT OF PHENOXYBENZAMINE ON THE INCREASE IN SPERM NUMBERS IN THE REPRODUCTIVE TRACT CAUSED BY PROSTAGLANDIN $F_{2\alpha}$, PHENYLEPHRINE AND ERGONOVINE (2 HOURS AFTER INSEMINATION)

Group and treatment[a]	Sperm recovered[b]				
	Oviducts	Uterus	Cervices	Vagina	Entire tract
	Arithmetic means ± SE				
	(10^2)[c]	(10^3)	(10^6)	(10^6)	(10^6)
1. Saline (S)	4 ± 2	152 ± 33	7 ± 1	5 ± 2	12 ± 3
2. Phenoxybenzamine (POBA) + S	2 ± 1	117 ± 41	4 ± 1	4 ± 1	8 ± 1
3. Prostaglandin $F_{2\alpha}$ (PGF)	24 ± 8	368 ± 61	12 ± 3	9 ± 1	21 ± 3
4. POBA + PGF	17 ± 3	524 ± 87	10 ± 1	9 ± 1	19 ± 2
5. Phenylephrine (Ph)	34 ± 8	814 ± 162	23 ± 5	13 ± 2	37 ± 7
6. POBA + Ph	4 ± 2	150 ± 58	5 ± 1	5 ± 1	10 ± 3
7. Ergonovine (Er)	26 ± 6	352 ± 70	8 ± 1	10 ± 5	19 ± 5
8. POBA + Er	9 ± 4	135 ± 60	6 ± 2	8 ± 2	15 ± 3
	Probability[d]				
Comparison					
1. S vs PGF	.0001	.0001	.02	.0008	.0005
2. S vs PGF X POBA	.78	.15	.38	.61	.50
3. Ph vs Er	.72	.19	.34	.84	.28
4. Ph vs Er X POBA	.30	.51	.02	.10	.001
5. S + PGF vs Ph + Er X POBA	.003	.002	.008	.32	.09

[a]Eight does/group. Phenoxybenzamine (20 mg) was injected im 15 min before insemination; prostaglandin $F_{2\alpha}$ (.75 mg), phenylephrine HCl (5 mg) and ergonovine maleate (.6 mg) were injected im immediately after insemination.

[b]Inseminate/doe = 99 ± 13 million sperm.

[c]Multiplier for each mean.

[d]Probabilities by analysis of variance of logarithms of sperm numbers within each segment of the reproductive tract.

and ergonovine (Groups 6 and 8). Thus, phenoxybenzamine had a significantly greater effect on sperm numbers in the oviducts, uterus and cervices of phenylephrine- and ergonovine-treated does than of saline- and prostaglandin-treated does (Comparison 5, table 7).

Phenoxybenzamine reduced sperm numbers significantly more in the cervix and in the entire tract of phenylephrine-treated does than of ergonovine-treated does (Comparison 4, table 7); these significant interactions were caused mainly by the fact that phenylephrine increased sperm numbers in the cervix but ergonovine did not.

Exp. 8. Alpha-Adrenoceptor Blockade and Uterine Contractions. The administration of phenoxybenzamine alone had little effect on spontaneous uterine contractions (Group 1, table 8). As expected from Exp. 6, phenylephrine or methoxamine alone increased the number of contractions whereas ergonovine alone had little effect (Groups 2, 4 and 6). Prior treatment of does with phenoxybenzamine antagonized the response to phenylephrine and methoxamine (Groups 3 and 5). Phenoxybenzamine given before ergonovine (Group 7) reduced the amplitude of primary contractions and the number of secondary and total contractions ("before treatment" period vs "after treatment" periods: for amplitude of primary contractions, $P<.025$; number of secondary contractions, $P<.025$; total number of contrac-

TABLE 8. EFFECT OF PHENOXYBENZAMINE ON CONTRACTIONS OF THE UTERUS IN RESPONSE TO PHENYLEPHRINE, METHOXAMINE AND ERGONOVINE

Group, treatment and recording period[a]	Contractions during 20-min period[b]			
	Primary contractions		Secondary contractions, no.	Total contractions, no.
	No.	Amplitude, mm		
1. Phnoxybenzamine (POBA; 5)				
Before treatment	11 ± 1	28 ± 2	23 ± 4	34 ± 4
After treatment—10 to 30 min	10 ± 1	26 ± 2	18 ± 5	28 ± 5
After treatment—60 to 80 min	10 ± 1	26 ± 2	22 ± 3	32 ± 3
2. Phenylephrine (Ph; 5)				
Before treatment	10 ± 1	30 ± 3	26 ± 3	36 ± 3
After treatment—10 to 30 min	25 ± 3	39 ± 4	67 ± 15	92 ± 14
After treatment—60 to 80 min	19 ± 3	35 ± 4	43 ± 8	62 ± 7
3. POBA + Ph (6)				
Before treatment	10 ± 1	31 ± 4	33 ± 5	43 ± 5
After treatment—10 to 30 min	10 ± 3	22 ± 3	20 ± 7	30 ± 9
After treatment—60 to 80 min	10 ± 2	25 ± 6	18 ± 7	28 ± 8
4. Methoxamine (Me; 5)				
Before treatment	11 ± 2	28 ± 3	28 ± 4	39 ± 4
After treatment—10 to 30 min	31 ± 4	37 ± 4	28 ± 3	59 ± 4
After treatment—60 to 80 min	23 ± 4	36 ± 4	24 ± 4	47 ± 3
5. POBA + Me (5)				
Before treatment	12 ± 2	29 ± 4	20 ± 2	32 ± 2
After treatment—10 to 30 min	14 ± 1	29 ± 2	23 ± 4	37 ± 3
After treatment—60 to 80 min	12 ± 1	24 ± 4	21 ± 3	33 ± 3
6. Ergonovine (Er; 5)				
Before treatment	10 ± 2	33 ± 5	22 ± 2	32 ± 2
After treatment—10 to 30 min	12 ± 2	33 ± 5	28 ± 5	40 ± 7
After treatment—60 to 80 min	13 ± 1	31 ± 4	26 ± 4	39 ± 5
7. POBA + Er (5)				
Before treatment	11 ± 1	27 ± 3	29 ± 7	40 ± 8
After treatment—10 to 30 min	11 ± 1	21 ± 2	17 ± 3	28 ± 4
After treatment—60 to 80 min	10 ± 1	17 ± 2	11 ± 1	21 ± 1

[a]Number of does in the group in parentheses. Amount of compound administered im: phenoxybenzamine HCl, 20 mg; phenylephrine HCl, 5 mg; methoxamine HCl, 5 mg; ergonovine maleate, .6 mg.

[b]Mean ± SE. See footnotes to table 6 for description of contraction characteristics.

tions, P<.05). Phenoxybenzamine prevents the contractile response to alpha-adrenoceptor agonists (Marshall, 1970; Fuchs, 1972) and to iv injected ergonovine (Fregnan and Glasser, 1964; Saameli, 1978). The present results confirm that phenoxybenzamine blocks that action of alpha-adrenoceptor agonists on uterine contractions. In addition, the prevention by phenoxybenzamine of phenylephrine-induced increases in uterine contractions (table 8) was associated with prevention of phenylephrine-induced increases in sperm numbers in the oviducts, uterus and cervices (table 7). Also, the reduction by phenoxybenzamine of the number and strength of uterine contractions after ergonovine treatment (Group 7, table 8) was associated with reduction of the ergonovine-induced increases in sperm numbers in the oviducts and uterus (Group 8, table 7).

Exp. 9. Ovum Fertilization. Both phenylephrine and ergonovine increased the proportion of does with cleaved ova and the proportion of ova that were cleaved (table 9). Also, more accessory sperm were counted in ova from the treated does. All accessory sperm were found in cleaved ova, and almost all cleaved ova contained four blastomeres. The results indicated that either phenylephrine or ergonovine could increase the rate of ovum fertilization, apparently by increasing the number of sperm in the oviducts around the time of ovulation, when low numbers of sperm were used for insemination.

Discussion

The results of this study indicate that both phenylephrine and ergonovine increased the number of sperm in the oviducts at 2 or 2.5 h after insemination and increased the fertilization rate.

It was not clear whether altered uterine motility mediated the increased sperm numbers in the oviducts and uterus after phenylephrine or ergonivine treatment. Phenylephrine increased both uterine contractions and sperm numbers in the cervix, uterus and oviducts. Phenoxybenzamine prevented the phenylephrine-induced increases in both uterine contractions and sperm numbers. On the other hand, methoxamine, an alpha-adrenoceptor agonist with pressor properties similar to those of phenylephrine (Innes and Nickerson, 1975), also stimulated contractions but caused little if any increase in sperm numbers in any segment of the reproductive tract. Ergonovine increased sperm numbers in the uterus and oviducts consistently but when given im, apparently had little or no effect on uterine contractions. Phenoxybenzamine prevented ergonovine-induced increases in sperm numbers and apparently caused a decrease from normal in the number and strength of uterine contractions in ergonovine-treated does.

Although sperm transport to the uterus and oviducts is believed to depend largely upon contractions of the reproductive tract, it is possible that drug-induced increases in sperm numbers in the uterus and oviducts may depend upon some contraction characteristic not identified on the contraction tracings made in these experiments. Also, contractions might have been altered at a site other than the uterus

TABLE 9. OVUM FERTILIZATION IN RABBITS AFTER INSEMINATION WITH LOW NUMBERS OF SPERM AND TREATMENT WITH PHENYLEPHRINE OR ERGONOVINE[a]

Treatment[b]	Total		Does with cleaved ova[c]	Total ova cleaved[cd]	Total accessory sperm
	Does	Ova			
	no.				
Saline	8	62	1	10 (16%)	41
Phenylephrine	8	75	6	39 (52%)	740
Ergonovine	8	75	6	47 (63%)	348

[a]Inseminate per doe = 92 ± 12 thousand sperm.

[b]Phenylephrine HCl (5 mg) or ergonovine maleate (.6 mg) was injected im immediately after insemination.

[c]Proportion of does with cleaved ova (1/8, 6/8 and 6/8), P<.02 among groups. Proportion of ova cleaved (10/62, 39/75 and 47/75), P<.005 among groups.

[d]Figures in parentheses are percentages of ova that were cleaved of the total number of ova recovered.

or noncontractile responses of some kind might have been involved.

Even though the uterine contractile responses to phenylephrine and ergonovine differed markedly, the two compounds may have increased sperm numbers in the reproductive tract at least partly through similar physiological mechanisms. Phenoxybenzamine blocked the effect of both compounds on sperm numbers (table 7). In addition, Saameli (1978) concluded that phenoxybenzamine inhibited the oxytocic action of ergonovine on the uterus by blocking alpha-adrenoceptors.

Compounds known to increase sperm numbers in the reproductive tract of the doe at about 2 h after insemination include phenylephrine, ergonovine, prostaglandin $F_{2\alpha}$ (Hawk and Cooper, 1979) and estradiol-17β (Hawk and Cooper, 1978). When given near the time of insemination, these compounds have not always increased sperm numbers in identical segments of the reproductive tract. Prostaglandin $F_{2\alpha}$ and estradiol have caused the recovery of significantly higher numbers of sperm from each segment of the reproductive tract and thus higher total numbers from the entire tract (Hawk and Cooper, 1978, 1978; Hawk et al., 1982). Phenylephrine consistently increased sperm numbers in the oviducts, uterus and cervix. Ergonovine, however, has increased sperm numbers only in the oviducts and uterus at the times observed (tables 4, 5 and 7). It is not known whether the apparent differences among compounds, particularly between ergonovine and the others, might be diminished or exaggerated at other times after insemination. Neither is it known whether the differences among compounds at about 2 h may indicate partially different sites or mechanisms of action of the compounds.

The administration of oxytocin to ovariectomized estradiol-primed does immediately after insemination increased the number of sperm to the uterus but not in the cervix 30 min later (Morton and Fitzpatrick, 1974).

Compounds that increase sperm numbers in all or some segments of the reproductive tract of the rabbit appear generally to be optimally effective over a rather narrow dose range. Such was the case with oxytocin (Morton and Fitzpatrick, 1974), estradiol (Hawk and Cooper, 1978), prostaglandin $F_{2\alpha}$ (Hawk et al., 1982) and phenylephrine and ergonovine (present experiments). Nevertheless, some of these compounds have potential usefulness in improving sperm retention and transport and ovum fertilization rates when low fertility is characterized by high rates of fertilization failure.

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