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Primate Model for the Evaluation of Vaginal Contraceptives

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A good animal model that simulates the human subject has not been available for the evaluation of the in vivo effectiveness of vaginal contraceptives. After careful consideration, The stumptailed macaque *(Macaca arctoides)* was studied for its applicability since it has a reproductive tract similar to that of the woman, is easy to handle, does not require tranquilization or anesthesia when the contraceptive is deposited, and breeds and conceives readily under caged conditions. The reported observations show the usefulness of this animal. Both postcoital sperm motility studies and breeding experiments were performed with the use of Delfen vaginal cream and K-Y jelly. K-Y jelly had no effect on the motility of vaginal spermatozoa or on the conception rate of the primates. Although Delfen vaginal cream consistently immobilized all spermatozoa in the postcoital test, half of the animals became pregnant within an average of 3.7 breeding cycles. These results illustrate the discrepancy between spermicidal tests and fertility measurements, and it is recommended that primate-breeding experiments be performed before a spermicide is evaluated in women as a contraceptive. (AM J. OBSTET. GYNECOL. 129:368, 1977.)

PRESENTLY AVAILABLE vaginal (chemical) contraceptives function by immobilizing spermatozoa. Normally, the sole criterion used to evaluate their contraceptive activity is a series of in vitro tests where spermatozoa are incubated with dilutions of the spermicide and observed for motility after certain periods of time.[1,2] If satisfactory immobilization rates are obtained, proper toxicologic studies are performed, including vaginal tolerance tests. The spermicides are subsequently immediately tested in women as contraceptives, notwithstanding the frequent lack of correlation between in vitro and in vivo observations, particularly regarding sperm motility and fertility.

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It has been well established that vaginal contraceptives are less effective than the hormonal contraceptives or the intrauterine contraceptive device (IUD). Failure rates have been estimated to vary from 3 to 30 per 100 woman years, depending on the spermicide and the population using it.[1,2] This variable and relatively high failure rate is usually blamed on mistakes in the application of the spermicide, i.e., too long before intercourse, not deep enough into the vagina, lack of reinsertion at repeat intercourse, etc., rather than a poor contraceptive activity of the spermicide itself. Although rules certainly have to be followed for the optimal use of the compounds, the assumption that the incorrect use of the spermicides is the major reason for their lesser contraceptive effectiveness may be unfounded.

Once spermatozoa are deposited vaginally, they pass within minutes into the cervix at midcycle and begin their transport through the female genital tract. Therefore, not much time is available in vivo for the vaginal contraceptives to exert their spermicidal activity. Further, decreased effectiveness may be due to a poor distribution of the spermicide in the vagina, removal or displacement of the spermicide by the coital act, inactivating effects of vaginal fluids on the spermicide, an intercourse position that causes rather poor spermicide-sperm contact. etc. These factors can hardly be evaluated in vitro. It would seem essential, therefore, to perform in vivo studies to determine the contraceptive activity of a vaginal contraceptive before the population at risk is subjected to undesired parenthood because for one reason or another the in vitro observations do not hold true in vivo. Additionally, it would be advantageous to know if a new contraceptive is more effective than others already available on the market, as well as its ultimate effectiveness under ideal conditions of insertion and intercourse.

Two types of in vivo tests are possible: (1) postcoital tests, and (2) breeding experiments. The human postcoital test has been used by several investigators to compare the relative effect of spermicides on the motility and cervical mucus penetrability of vaginal spermatozoa.[3-9] Although these tests are certainly more indicative for the in vivo activity of a spermicide than the in vitro tests, they are still not conclusive since the collected and observed spermatozoa may not be the ones that fertilize. Definitive statements concerning the effectiveness of spermicides can, therefore, be made only after breeding experiments.

Few authors have attempted to determine the antifertility activity of spermicides in animal

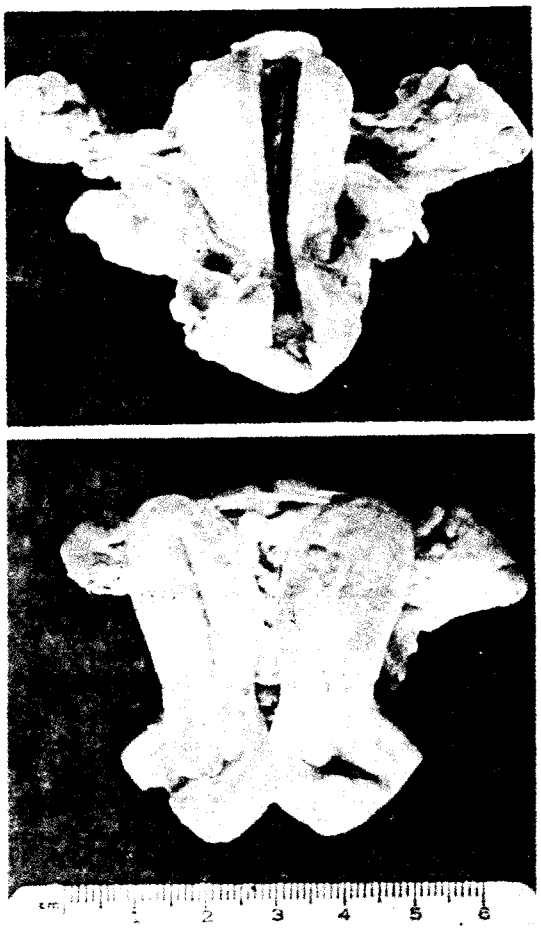

Fig. 1. The cervix, uterus, and Fallopian tubes of a stumptailed macaque *(Macaca arctoides)*. Note the relatively straight cervix.

species. Some extremely limited observations were described by Carleton and Florey,[10] Millman,[11] and Hartman,[12] who found, at best, a 50 per cent inhibition of fertilization with the dog and rabbit as experimental animals. In a short communication, Chang[13] evaluated eight spermicides in the rabbit and found that only three possessed contraceptive activity (Delfen cream,○ Ramses vaginal jelly⊕ and Lactikol vaginal jelly●). depressing fertility by approximately 40 to 50 per cent. The results with the Delfen cream in the rabbit were confirmed by Zaneveld and associates.[14] Newell and associates,[15] and Homm and associates.[16] The last group of authors also showed that Delfen foam depressed conception in the rabbit more than did Delfen cream but still did not completely prevent fertility. Both of these preparations were more effective than Preceptin gel.○ The limited effectiveness of vaginal spermicides in the rabbit has been mostly ignored because the rabbit genital tract varies from that of the woman and different mechanisms of sperm transport may take place, particularly through the cervix. A more suitable animal species is necessary, therefore, if correlations are to be made with the human subject.

The only group of animals with a reproductive tract similar to that of the woman is the nonhuman primate. Even among nonhuman primate species, only a few have a straight cervix. Further, the primate should be easy to handle so that insertion of the cream can be performed without anesthesia or tranquilization. The male should be willing to breed readily without extensive foreplay, i.e., within minutes of placing the cream into the vagina. Finally, the females should conceive readily under caged conditions. After careful considerations, only the stumptailed macaque *(Macaca arctoides)* seemed to conform

○ Ortho Pharm. Corp., Raritan, New Jersey.
⊕ Julius Schmid, Inc., New York, New York
● Durel Pharmaceutical, Inc., Mount Vernon, New York.

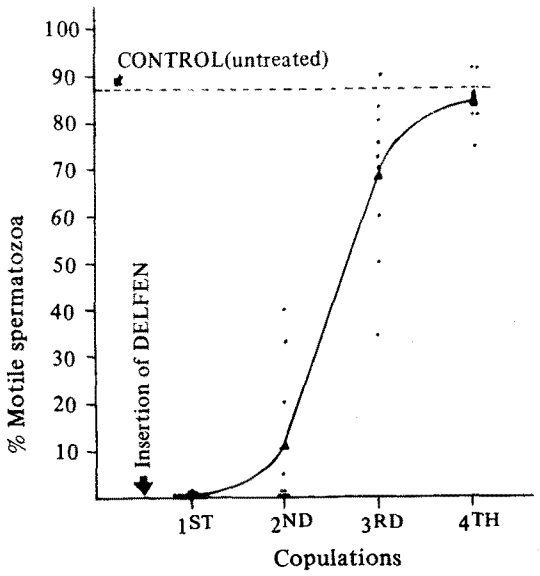

Fig. 2. The antimotility effect of Delfen vaginal cream on repeat breeding without reinsertion of the cream. Each coital event was 30 to 45 minutes apart. Spermatozoa were collected immediately after each copulation. ▲——▲, Average of nine different experiments.

Table I. Postcoital motility of spermatozoa with and without vaginal agent○

	Motile spermatozoa (%)								
	No agent			*K-Y Jelly*			*Delfen vaginal cream*		
Experiment No.	*0⊕*	*20⊕*	*45○*	*0*	*20*	*45*	*0*	*20*	*45*
1	90	90	70	90	85	55	All sper-		
2	92	90	86	95	93	75	matozoa		
3	92	84	62	92	77	52	completely		
4	89	79	70	85	70	70	immotile		
5	82	80	62	87	77	70			
6	83	80	74	83	76	75			
7	85	85	83	85	80	73			
8	80	77	50	87	75	64			
Average (to the nearest integer)	87	83	70	88	79	68	0	0	0

○ See text for experimental detail.
⊕ Minutes after coitus.

to all these requirements. Therefore, the applicability of this animal for vaginal contraceptive studies was determined by evaluating the effec-

tiveness of Delfen cream (nonylphenoxypolyethoxyethanol), the most effective vaginal cream on the market today, according to postcoital[7] and rabbit[13] studies. K-Y jelly,° a neutral vaginal depository, was used as a control.

Material and Methods

Only rarely are sufficient numbers of primates available in a colony to perform randomly designed studies. Large numbers are not needed, however, if identical test and control groups are used. By keeping detailed breeding records, the conception rate of each animal at our Primate Center could be assessed. A fertility quotient (FQ) was devised where:

$$FQ = \frac{\text{Total number of pregnancies}}{\text{Total number of breeding cycles required to obtain these pregnancies}} \times 10.$$

The FQ was calculated for each animal and was used in conjunction with the number of times that each primate had been pregnant to separate the animals into groups of equal fertility (see Tables II and III). The FQ is a good index of fertility, and we recommend its use in breeding colonies so that a uniform method is available to describe the relative breeding capacity of an animal.

Since the FQ is more accurate with increasing time that an animal has resided in a colony, the animals were divided among those who had been with the colony for four or more years and those who had been there a shorter period of time. In our study, each experimental group contained three animals from each age category; the older animals are the ones mentioned first in the tables.

A picture of an excised genital tract from a stumptailed macaque is shown in Fig. 1. The average length of the vagina was measured to be 4 to 5 cm, and the average width was 1 to 1.5cm. This is roughly 15 to 25 per cent of the human vagina, in which approximately 4 ml. of Delfen

Table II. Effect of K-Y Jelly on conception°

	Pre experimentation breeding data		*Experimental observations*	
Animal No.	*No. of pregnancies*	*FQ*	*No. of breeding cycles before pregnant (P) or not pregnant (NP)*	*FQ*
390	6	6.0	1 (P)	10
594	4	3.6	1 (P)	10
349	3	2.5	9 (NP)	1.0*
427	2	4.0	1 (P)	10
1247	2	4.0	1 (P)	10
1238	2	2.2	1 (P)	10
Group performance				
Total	19	22.3		51
Average	3.3	3.7		8.5

° See text for experimental detail

* When an animal did not become pregnant, the FQ was based on the minimal number of breedings that would have been required to obtain such a pregnancy, i.e., the number of cycles that the animal was bred + 1.

cream is deposited for contraceptive purposes. To match this size difference, a volume of 1 ml. was used in the primates. The K-Y jelly and Delfen cream were diluted 10 per cent by adding 0.1 ml. of physiologic saline to 0.9 ml. of jelly and cream because future evaluations are planned to determine if the addition of certain chemicals to already-existing vaginal spermicides enhances their contraceptive activity.

On the average, the stumptailed macaque ovulates 11 days after the onset of menstruation. Therefore, the animals were bred for five days beginning from the ninth day after the start of menstruation. Each female was bred four times each day, at least 1.5 to 2 hours apart, and the male was allowed to copulate only once each time. Six males were available for these studies and were randomly used. The vaginal agents were reinserted each time before coitus except in certain sperm motility experiments.

For the deposition of the agents, the female was taken out of her cage, placed on a table, and bent forward. A 2.5 to 3 ml. disposable syringe without a tip was filled with 1 ml. of cream or jelly and entered into the vagina as deeply as possible. The cream was deposited

while the syringe was slowly removed with care taken that the entire content remained in the vagina. The female was placed directly with the male who normally started the coital act after 30 seconds to 2 minutes of inspection. The female was removed from the male immediately after ejaculation as indicated by the cessation of penile thrusts by the male and the removal of the penis from the vagina. Pregnancy was determined by the absence of menstruation, by rectal palpation, and finally by parturition. Once the study had begun,, the females were bred during each menstrual cycle without interruption until pregnant or until the termination of the study.

Postcoital tests were performed by collecting 0.1 ml. of vaginal fluid with a syringe and plastic catheter: (1) immediately after separating the male and female, (2) 20 minutes later, and (3) 45 minutes later. The per cent motility was determined by counting 100 spermatozoa under the microscope. No differentiation was made between slow and rapid or stationary and forward movement. Besides the animals treated with K-Y jelly and Delfen cream, a control group was included for the motility studies that received no agent at all. The long-term antimotility effect of Delfen was determined by repeat breeding experiments without reinserting the cream. After the initial application of Delfen, each animal was bred four times, 30 to 45 minutes apart, and the spermatozoa were collected immediately from the vagina after each coital event.

Results and Comment

The stumptailed macaque proved to be an excellent subject for these studies. Because of its friendly personality and willingness to cooperate, it is difficult to conceive that any other primate could be as practical. The only problem that occurred occasionally was that the male did not want to let the female go after intercourse; however, since they were in a squeeze cage, separation could be readily achieved.

Table III. Effect of Delfen vaginal cream on conception○

Animal No.	*Pre-experimentation breeding data*		*Experimental observations*	
	No. of pregnancies	*FQ*	*No. of breeding cycles before pregnant (P) or not pregnant (NP)*	*FQ*
Year 1				
329	4	5.7	2 (P)	5.0
383	5	4.2	7 (NP)	1.3⊕
697	4	2.9	3 (P)	3.3
1245	3	4.3	3 (NP)	2.5⊕
1318	1	3.3	4 (P)¾	2.5
1281	2	2.5	7 (NP)	1.3⊕
Group performance				
Total	19	22.9		15.9
Average	3.3	3.8		2.7
Year 2				
590	6	6.0	2 (NP)	3.3⊕
1244	3	4.3	2 (P)	5.0
1241	2	4.0	2 (P)	5.0
1250	2	3.3	4 (P)	2.5
424	3	3.3	3 (P)	3.3
589	3	2.0	5 (NP)	1.7⊕
Group performance				
Total	19	22.9		20.8
Average	3.2	3.8		3.4

○ See text for experimental detail

⊕ See Table II for calculation of FQ when the animal did not become pregnant.

The results of the postcoital motility experiments are shown in Table I. K-Y jelly had no effect on the motility of vaginal spermatozoa as compared to that of the untreated control animals. Delfen consistently and immediately prevented the motility of all spermatozoa, clearly showing the high spermicidal activity of this cream.

The repeat breeding experiments without reinsertion of the Delfen (Fig. 2) produced approximately the same results in the stumptailed macaque as had been reported for the human subject by Johnson and Masters.[7] This adds to our confidence that information gained in the primate regarding the spermicidal activity of a compound can be applied to the human subject. Delfen was still quite effective during a second intercourse without reinsertion of the cream since only four of nine animals possessed motile

spermatozoa, and even in these the per cent motility was low. By the third coital event, however, all animals possessed motile spermatozoa, and at the fourth intercourse the spermicidal effect of Delfen had almost completely disappeared. Graphically, a sigmoid-shaped curve is obtained with characteristic slopes when the results are averaged. Such a curve is typical for the longevity of the spermicidal activity of a compound on repeat breeding and may be useful as a preliminary method to screen the comparative effectiveness of vaginal contraceptives.

K-Y jelly actually appeared to enhance the conception rate when compared to the average FQ of the animals based on their previous breeding records (Table II). All but one animal became pregnant during the first breeding cycle. The exception never became pregnant and may well have developed some sort of reproductive tract anomaly. It is highly unlikely that the improved fertility rate is due to the K-Y jelly; more likely, the improvement was caused by our breeding schedule during the experiments which differed from the usual schedule. In our colony, the males and females are normally kept together for the entire day on each of the five days during the midcycle period whereas our protocol permitted the animals to breed only intermittently. Since we found that the stump-tailed macaque will ejaculate as much as 10 to 16 times in one hour, the sperm reservoir would be entirely depleted in a short period of time. Keeping the male and female together for more than two or three ejaculates serves little in regard to conception, therfore, and intermittent separations allow the sperm reservoir to be replenished. Additionally, different males were used as much as possible for each coital event in our study.

On the basis of in vitro experiments which showed that the motility of human spermatozoa is decreased by treatment with K-Y jelly, Tagatz and associates[17] and Goldenberg and White[18] suggested that K-Y jelly should not be used in subfertile couples. However, postcoital or fertility tests were not performed by these authors and our results indicate that, at least in the primate, K-Y jelly has no detrimental effect on conception.

Two sets of Delfen experiments were performed, one year apart. The results were essentially identical in both cases (Table III). Three of the six animals became pregnant during the first year and four of the six became pregnant during the second year. As compared to K-Y jelly, Delfen had significant contraceptive properties since many more breeding cycles were required to obtain the pregnancies with Delfen. If we included all results, the average number of breeding cycles with Delfen was 3.7, although this might have been much higher if the animals had been bred for longer periods of time. By contrast only one breeding cycle was required for the K-Y jelly-treated animals to become pregnant, if the animal that did not get pregnant is omitted. Even more important and indicative is the FQ that showed distinct differences between K-Y jelly and Delfen vaginal cream (averages of 3.1 and 8.5, respectively). Similar types of evaluation can be used to compare one vaginal contraceptive to another.

The fact that a 50 per cent or higher conception rate occurred two years in a row when every precaution was made to insert the Delfen correctly and immediately before coitus is of concern. It shows that even if a compound is 100 per cent spermicidal in the postcoital test it may not prevent fertilization. This can be explained in two ways: (1) Not all the spermatozoa are immobilized by the vaginal contraceptive because of their rapid passage into the cervix, and (2) sperm motility is not essential for conception as long as "fertilizability" (ovum pernetrability) remains. The first possibility is the most likely explanation. Even though all

spermatozoa were immotile in the postcoital test, the observed spermatozoa may not have been the fertilizing ones. Therefore, our results emphasize that postcoital tests are not necessarily indicative of the conception rate even though they are much more accurate than in vitro tests. The second point should not be ignored, however, since several experiments have been published which show that particles or immotile spermatozoa can be transported from the vagina to the site of fertilization in animal species.[19-22] Some question in this regard still exists in the human subject, but it has been shown that even in this species particles can pass from the uterus to the Fallopian tubes.[23, 24] Additionally, it has been shown that the first few spermatozoa arrive in the human Fallopian tubes within five to 10 minutes after artificial insemination,[25] a faster rate of transport than one would expect based on the locomotion of the spermatozoon alone.

The discrepancy between the in vitro and postcoital tests on the one hand and the breeding experiments on the other indicates that the latter test procedure is essential before a spermicide is placed on the market as a contraceptive. Further, it would seem important to incorporate into vaginal contraceptives not only agents with spermicidal properties but also some with other antifertility mechanisms, for instance, those that prevent the fusion of spermatozoa with the ovum. The practical applicability of sperm enzyme inhibitors that prevent the penetration of the spermatozoon through the zona pellucida as vaginal contraceptives was recently established in the rabbit[14, 15] and also in the primate.[26]

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