

## EFFECT OF CARBACHOL ON FERTILITY OF MALE RATS WHEN ADMINISTERED LOCALLY TO EPIDIDYMIS

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### ABSTRACT

*This study examines the effect of carbachol, a potent cholinceptor agonist drug, on fertility of male rats, when administered to epididymis using silastic formulations, in the form of rods. 10% and 25% carbachol rods were found to be toxic, and resulted in death within 25 min. On the other hand, 5% rods were tolerable. These rods significantly reduced sperm numbers both in the cauda epididymis and ejaculate but failed to impair fertility. Libido (sexual desire) remained essentially unaltered. In organ bath experiments, carbachol induced rhythmic contractions in isolated vasa deferentia and cauda epididymal tubules in a dose-related manner.*

Short running head: carbachol and fertility of male rats

Key words: carbachol, silastic rods, epididymis fertility  
oligozoospermia, cholinomimetics

### INTRODUCTION

Men have only a limited choice for the regulation of their own fertility: withdrawal; the use of condoms; and sterilization by vas deferens occlusion (1). Thus, for men to have the choice as women there is clearly a need for the development of alternative methods of contraception.

There is considerable evidence to show that sperm numbers in the ejaculate is highly correlated with fertility(2). Therefore, production of severely oligozoospermic ejaculates offers a potential mechanism to suppress fertility. One way of achieving such a goal is to reduce the number of sperm stored in the cauda epididymis by inducing rhythmic contractions; majority of sperm in the ejaculate emanates from cauda epididymis(3). In addition, such a method acting via epididymis would be advantages that: spermatogenesis, libido or any other hormonally related events would not be disturbed; the effect would be rapid in onset; and on withdrawal of treatment, normal sperm would return quickly to the ejaculate (1).

The current study was initiated to test this possibility in rats using carbachol, a potent cholinceptor agonist drug(4), and local method of application to the epididymis using medical grade silastic elastomer. Similar study using acetylcholine, however, has failed to reduce fertility in rats(5). But this may be due to rapid brake down of it by enzymatic mechanisms (4).

## **2. Materials and Methods**

### **Drugs :**

Carbamylcholine chloride (carbachol) (from Sigma Chemical Co.) was used.

### **Animals :**

Healthy adult Sprague-Dawly rats of proven fertility (males weighing 200g—250g and females weighing 175g—200g) were used. They were housed under the natural photoperiod of approximately 12h light/24h at a constant temperature (28°C—30°C) with free access to food (rat pellets, Moosajees Ltd., and green leaves) and tap water.

### **Preparation of Silastic rods:**

Silastic rods (3.5mm dia. and 10—12mm length) containing 0%, 5%, 10% or 25% carbachol were constructed using appropriate weight of drug and known volume of Polysiloxane Polymer (Silastic 382, Medical Grade Elastomer: Dow Corning Corp., Midland, Michigan, U. S. A.) as described in detail elsewhere(6)

### **Insertion of rods adjacent to each epididymis :**

A single rod was placed adjacent to each epididymis using mild ether anaesthesia and aseptic precautions as described in detail elsewhere (6). The day of insertion of rod was designated as day 0. The number of rats subjected to each treatment regime were: 25% carbachol rods 6, 10% carbachol rods 6, 5% carbachol rods 10 and control rods 8.

### **Assessment of libido (sexual desire) and fertility :**

Libido and fertility of rats with 5% carbachol rods and control rods were investigated on days 3 and 7, and then approximately at weekly intervals using suitable females as described in detail elsewhere (7).

### **Ejaculated sperm count :**

Ejaculated sperm counts on day 3 and 7 estimated in 6 rats with 5% carbachol rods and in 5 rats with control rods as described in detail elsewhere(8).

### **Motility of epididymal sperm:**

Motility of epididymal sperm was assessed in 10 rats, each of which was fitted with a 5% carbachol rod adjacent to right epididymis and a control rod adjacent to left epididymis on day 3 using a subjective scale from 0 (completely immotile) to 5 (greatest motility ever observed) as described elsewhere (7).

### **Sperm counts in the male reproductive tract :**

A single 5% carbachol rod (6 rats) or a control rod (14 rats) was placed adjacent to each epididymis of 20 rats. On day 3, sperm counts on prostatic vas, epididymal vas, cauda epididymis and caput/corpus epididymis were estimated as described in detail elsewhere (9).

### **Sperm counts in the urinary bladder :**

5 rats were fitted with a single 5% carbachol rod and 3 rats with a drug free rod adjacent to each epididymis. On day 3, these rats were paired individually with a pro-oestrous female at 18.00 — 19.00h in an experimental room which was dimly lit with a 25W bulb. These paired rats were then observed continuously until ejaculation occurred. Immediately following ejaculation the males were anaesthetized with ether, urinary bladder was exposed through a pelvic incision and the entire contents of urinary bladder were withdrawn using a 1ml disposable syringe. A drop of this urine was transferred to a microscopic slide and examined for any spermatozoa.

### **Histopathology of testes :**

6 rats with a single 5% carbachol rod adjacent to each epididymis and another 3 with control rods were killed on day 14 and parts of testes were removed and fixed in Bouin's fluid. Sections (7 $\mu$ m) were stained with haematoxylin and eosin and examined microscopically at x 100 and x 400 magnifications respectively.

### **Organ bath experiments :**

20 rats were anaesthetized with ether and their vasa deferentia and epididymides were removed and placed immediately in petridishes containing fresh oxygenated (95%O<sub>2</sub> ; 5%CO<sub>2</sub>) physiological salt solution having the following composition: (mM/l): Na<sup>+</sup>, 141; K<sup>+</sup>, 5.9; Ca<sup>++</sup>, 2.6; Mg<sup>++</sup>, 1.2; Cl<sup>-</sup>, 104.8; H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 2.3; HCO<sub>3</sub><sup>-</sup>, 24.9; SO<sub>4</sub><sup>-</sup>, 1.2 and glucose 11. The vasa deferentia (length 32.5  $\pm$  2.2 mm; mean  $\pm$  s.e.m., N=15) were then dissected free from the epididymides. A small section of the cauda epididymides (length 10.6  $\pm$  0.88mm, N=18) was uncoiled and freed from the surrounding connective tissue under a magnifying lens using a fine pair of forceps and entomological pins. The tissues were suspended in 50ml organ baths at 37 $\pm$ 1 $^{\circ}$ C under a resting tension of 1.0g. After an equilibration period of 20 — 30 min acetylcholine dissolved in isotonic saline was added cumulatively to the organ bath at 10—15 min intervals. Concentrations tested were ( $\mu$ g/ml): 1, 5, 10, 25, 50 or 100. Contractions were recorded isometrically with Dynamometer UF 1 Strain gauge using Washington 400 MD 2, Polygraph.

In a separate set of experiments the effects of 25% carbachol rods on contractility (frequency and amplitude) of isolated vasa deferentia (N=12) was studied upto 45 min by suspending a rod in the organ bath, using identical procedures.

### 3. RESULTS

Insertion of 10% or 25% carbachol rods produced profuse salivation and secretion of lacrimal fluids and voidance of urine even before the suturing was completed. In addition, marked exophthalmia was evident. All the 12 treated rats, in these two groups, died within 10–25 min following insertion of the rods, perhaps due to severe bradycardia and toxicity. Bradycardia is a common response seen with the administration of cholinomimetic drugs (10). In the rats with 5% carbachol rods, the above unfavourable side effects were also evident but, appeared mild in nature. Furthermore, these rats survived throughout the period of study. Appetite, general appearance and behaviour of these rats seemed almost similar to those with control rods.

#### Assessment of libido and fertility

Precopulatory sexual behaviour pattern (nosing, genital sniffing, mounting etc.) exhibited by the rats with 5% carbachol rods were almost identical to that of rats with control rods indicating that the treatment had not caused a marked alteration in libido. Furthermore, the treatment did not significantly impair the mating performance as judged by the percentage of successful mating ( $p > 0.05$ , Fisher Exact test); the ratio of number of successful matings; number of pairings being 75:78 and 61:67 with control rods and 5% carbachol rods respectively.

The results of the fertility experiments are summarized in Table I. As shown, 5% carbachol rods did not significantly alter the fertility of the treated rats ( $p > 0.05$ , Student's t-test).

#### Ejaculated sperm counts :

At days 3 and 7, the mean ejaculated sperm count of rats with the control rods were  $97.81 \pm 4.63$  million and  $93.75 \pm 6.22$  respectively, and those with 5% carbachol rods were  $25.62 \pm 12.68$  and  $41.25 \pm 10.36$ . These reductions in mean ejaculate sperm counts were statistically significant ( $p < 0.05$ , Student's t-test).

#### Motility of epididymal sperm:

The mean motility score of spermatozoa on the control side was  $4.6 \pm 0.16$  and that on treated side was  $3.9 - 0.23$ . This effect on motility was not statistically significant ( $p > 0.05$ , Mann Whitney U-test).

TABLE I—Summarized data showing the effect of 5% carbachol rods on fertility of male rats. Fertility is expressed in terms of fertility index  $\pm$  s.e.m. Values within parenthesis=no. of successful matings: no. of pairings, N.S.—not significant (Student's t-test).

Treatment	Fertility index						
	Time after insertion of rods (in days)						
	3	7	14	21	28	35	42
Control rods (14 : 14)	8.6 $\pm$ 0.26 (14 : 14)	8.8 $\pm$ 0.29 (14 : 14)	8.7 $\pm$ 0.30 (13 : 14)	7.8 $\pm$ 0.55 (10 : 11)	8.6 $\pm$ 0.33 (6 : 6)	7.7 $\pm$ 0.45 (11 : 11)	8.0 $\pm$ 0.53 (7 : 8)
5% carbachol rods	6.8 $\pm$ 0.53 (9 : 10)	7.8 $\pm$ 0.30 (9 : 10)	6.8 $\pm$ 0.67 (8 : 10)	6.3 $\pm$ 1.00 (9 : 10)	6.6 $\pm$ 0.60 (9 : 10)	6.3 $\pm$ 0.52 (9 : 9)	7.5 $\pm$ 0.38 (8 : 8)
Significance	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

TABLE II— *Effect of 5% carbachol rods on sperm distribution in the male genital tract of rats. The results are expressed as mean  $\pm$  s.e.m. (significance tested using Student's t-test; N.S.—not significant). Values in parenthesis are the numbers of rats tested in each group.*

	Total sperm nos, ( $\times 10^6$ )			
	vas deferens	cauda	caput/corpus	Entire tract
Control rods (14)	28.2 $\pm$ 3.3	299.3 $\pm$ 19.6	153.9 $\pm$ 8.1	480.9 $\pm$ 25.0
5% carbachol rods (6)	19.7 $\pm$ 2.2	203.1 $\pm$ 19.9	140.0 $\pm$ 29.8	363.62 $\pm$ 48.4
Significance	N.S.	p<0.05	N.S.	N.S.

TABLE III — Effect of cumulative doses of carbachol on the frequency (no. of contractions/min) of contractions of the isolated vasa deferentia and cauda epididymal tubules. values are mean  $\pm$  s.e.m

	Frequency of contractions						
	Dose ( $\mu\text{g/ml}$ )						
	1	5	10	15	25	50	100
vas deferens		5.5 $\pm$ 0.67		3.2 $\pm$ 0.23	3.6 $\pm$ 0.64	3.8 $\pm$ 0.94	3.7 $\pm$ 0.98
cauda epididymal tubules	2.7 $\pm$ 0.70	3.7 $\pm$ 0.73	3.1 $\pm$ 0.54		40.0 $\pm$ 0.56	4.2 $\pm$ 0.43	4.5 $\pm$ 0.59

### **Sperm counts in the male reproductive tract :**

The results of the sperm counts in various regions of the male tract are summarized in Table II. It is seen that, 5% carbachol rods has caused a significant reduction in sperm numbers in the cauda epididymis of the treated rats ( $p < 0.05$ , Student's t-test).

### **Sperm counts in the urinary bladder :**

Urine samples collected from the bladder of any of the rats investigated had no sperm in them.

## **4. DISCUSSION**

In the current study rats were treated with three doses (5%, 10% and 25% rods) of carbachol, a potent cholinomimetic drug(4). 10% and 25% carbachol rods were toxic: produced excessive salivation, lacrimation, exophthalmia, laboured breathing and voidence of urine and died within 10—25 min subsequent to insertion of rods. The lower dose (5% rods) on the other hand, was tolerated, although the above side effects were evident to a mild degree, immediately after the insertion of the rods. In contrast, acetylcholine was reported to be less toxic, even at higher doses when applied locally to the epididymis of rats using an identical procedure(6). This may presumably due to rapid breakdown of it by the choline esterases present in blood(4).

Carbachol treatment did not impair libido (sexual desire) as evident from essentially normal precoital sexual foreplay (nosing, genital sniffing, mounting or attempts) of intromission. This was so with acetylcholine treatment (5). Since libido was uninhibited with both of these cholinomimetics it is presumed that they do not markedly alter blood testosterone or prolactin levels.

As with acetylcholine(5) carbachol rods induced a significant reduction in sperm numbers in the cauda epididymis (by 32%) and in the ejaculate (by 56—74%). However, inspite of such a marked reduction in sperm numbers the fertility remained uninhibited. This is not surprising, since in rats the ejaculates contain many times (upto 1400 fold) the number of sperms that would require to produce maximum fertility (11). Further, it has been shown in rats, that a depletion of cauda epididymal sperm number by even 90%, failed to impair fertility(12). Therefore, unless the functional competence of the residual sperm is inhibited fertility is unlikely to be depressed. In this context, it is of interest to note that even high doses of carbachol (upto 500 $\mu$ g/ml) has failed to impair motility of rat sperm *in vitro* (13), although its effect on fertilizing potential of sperm remains to be investigated using the hamster oocyte assay: However, if carbachol is capable of producing such oligozoospermic ejaculates in human



males, the fertility is likely to be suppressed even if the functional competence of sperm remained unaltered; the number of sperm per human ejaculate is typically only two to four fold higher than the number at which fertility is significantly reduced(11). By way of contrast, some studies have reported pregnancies in humans with very low sperm concentrations (14). Thus, it seems desirable, if sperm function is also simultaneously inhibited with drug induced oligozoospermia.

The exact mechanism inducing oligozoospermia in this study is not fully understood. Nevertheless, it is possible to rule out some potential mechanisms. The immediate onset of oligozoospermic ejaculates and an unimpairment of spermatogenesis rules out a testicular mechanism. Blockage of the reproductive tract is also unlikely since no granulomas were evident either in the vas deferens or epididymis. Nor could it be due to retrograde ejaculation because no sperm were detected in the urinary bladder. Since majority of sperm in normal ejaculates are known to emanate from the cauda epididymis(3) it is likely that the carbachol-induced oligozoospermia was caused from the diminished sperm stores in the cauda epididymis. This may have resulted from rhythmic contractions in vas deferens and epididymis subsequent to the implantation of carbachol rods; indeed rhythmic contractions were evident both with isolated vasa deferentia and epididymal tubules in the organ bath experiment. Other workers have also reported similar findings (15, 16). In view of the belief that the secretomotor functions of epididymis is under cholinergic innervation (17) and carbachol is a potent cholinomimetic (4) it is also possible that the sperm storage capacity of the epididymis was attenuated by the enhancement of normal sperm disposal mechanisms present in the epididymis; intraluminal dissolution (18) and intraluminal phagocytosis (18). Loss of sperm via the urine is a major pathway of sperm disposal in several animal species (19). It is possible that enhancement of this mechanism may also function as contributory factor, in reducing the sperm content of the cauda epididymis. Further experiments are however, needed to test these possibilities.

In conclusion, results of this study and others(5) indicate that cholinomimetic offer promise as a potential male contraceptive agents only if they could suppress functional competence of ejaculated sperm.

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