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Increased Sperm Numbers in the Deferent Duct after Prostaglandin $F_{2\alpha}$ in Rabbits[1] (37964)

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Notwithstanding the observations that oxytocin increased the rate of flow of sperm from the cannulated deferent duct in rams (1) and increased sperm output in rabbits (2, 3), little is known of controls of the movement of sperm through the excurrent ducts. Prostaglandins are known to affect neurotransmission (4) and contractility of smooth muscle (5), including that of the deferent duct in the guinea pig (6, 7). The purpose of the present research was to test whether prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) affects the distribution of sperm in the epididymis and deferent duct in rabbits.

Materials and Methods. In a preliminary experiment, we anesthetized nine rabbits with nembutal (intravenously) and removed through a midventral incision one testis with its epididymis and deferent duct (control). Then, 5 mg $PGF_{2\alpha}$ Tham salt[2] in 0.2 ml saline was injected within the tunica vaginalis around the remaining testis and epididymis. The remaining testis, epididymis, and deferent duct were removed 10, 30, or 60 min (three rabbits each) later. Immediately before a side of the reproductive tract was excised, the deferent duct was ligated at the urethra and at the epididymis to prevent movement of sperm out of that organ. Sperm numbers in the deferent duct, tail epididymis, and head-body epididymis were determined (8).

In a second experiment, 16 rabbits were anesthetized with nembutal (intravenously) and one deferent duct, epididymis, and testis was removed as a control as in the first experiment. Then the remaining deferent duct was ligated at the urethra to prevent sperm leakage into the urethra, and saline (1.0 ml) was injected into eight rabbits and 5 mg $PGF_{2\alpha}$ Tham salt[2] in 1.0 ml saline was injected (subcutaneously) into eight rabbits. At 10 or 30 min later (four rabbits in each treatment), the treated deferent duct was ligated at the epididymis, that side of the reproductive tract was excised, and sperm numbers were determined as in the first experiment.

A third experiment was conducted on eight unanesthetized rabbits; four were given saline (0.7 ml, subcutaneously) three times at 20-min intervals and four were given (subcutaneously) 10 mg $PGF_{2\alpha}$ Tham salt[2] in 0.7 ml saline three times at 20-min intervals. Twenty minutes after the final injection, each rabbit was killed by cervical dislocation, the reproductive tract was removed, and the distribution of sperm was determined as in the first experiment except that testicular sperm also were determined and sperm content of right and left organs were composited within each rabbit.

The fourth experiment with 20 rabbits was similar to the third except that all rabbits were anesthetized with nembutal, 10 controls were given saline (0.2 ml, subcutaneously) five times at 12-min intervals and 10 rabbits were given (subcutaneously) 1

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mg $PGF_{2\alpha}$ Tham salt[2] in 0.2 ml saline five times at 12-min intervals. The rabbits were killed 12 min after the final injection of $PGF_{2\alpha}$, and the distribution of sperm was determined as in the third experiment.

Results and Discussion. Before $PGF_{2\alpha}$ treatment in the first experiment, deferent ducts contained 33×10^6 sperm, close to values previously reported (8). After injections of 5 mg $PGF_{2\alpha}$ around the contralateral testis, the deferent duct contained 71×10^6 sperm (Table I). Although the difference between before and after $PGF_{2\alpha}$ only approached significance ($P \simeq 0.18$), 8 of the 9 rabbits had more sperm in the deferent duct after $PGF_{2\alpha}$. Averages for sperm content of the tail and of the head-body epididymis were within ranges previously reported for rabbits (8), and not affected significantly by $PGF_{2\alpha}$.

The second experiment was designed to determine whether sperm would accumulate in the deferent duct of anesthetized rabbits with or without $PGF_{2\alpha}$ treatment. To exclude the possibility that sperm may move from the deferent duct into the urethra during anesthesia, the deferent duct on the first side was removed and the remaining deferent duct was ligated at the urethra; then saline or 5 mg $PGF_{2\alpha}$ was injected subcutaneously. In rabbits injected with saline, no significant number of sperm accumulated in the deferent duct during the 10- or 30-min intervals; an average of 28×10^6 sperm were in these deferent ducts (Table II), similar to the number of sperm in deferent ducts removed before $PGF_{2\alpha}$ in the first experiment (33×10^6, Table I) and in the second experiment (33×10^6, Table II). At 10 min after sc $PGF_{2\alpha}$, the number of deferent duct sperm was unchanged; but at 30 min after $PGF_{2\alpha}$, the number was increased ($P \simeq 0.02$) 2.5-fold to 89×10^6 (Table II). When $PGF_{2\alpha}$ was injected around the testis and epididymis in the first experiment, the number of deferent duct sperm appeared to be increased faster (Table I) than after sc $PGF_{2\alpha}$ in the second experiment. Increased sperm in the deferent duct 30 min after sc $PGF_{2\alpha}$ was not due simply to an accumulation during 30-min anesthesia, because the number of deferent duct sperm was unaltered during 30 min after injection of saline. As in the first experiment, the numbers of head-body or tail epididymidal sperm (Table II) were not altered significantly by $PGF_{2\alpha}$ treatment.

The third experiment was conducted to determine the distribution of sperm after sc $PGF_{2\alpha}$ without anesthesia. Since each rabbit could not serve as its own control as in the first two experiments, to minimize the variation among rabbits we calculated (a) the percent of the total excurrent duct sperm in each portion of the duct and (b) the number of ductal sperm per 100×10^6 testicular sperm. During the three sc injections of 10 mg $PGF_{2\alpha}$, all four rabbits de-

TABLE I. Distribution of Sperm in Rabbits Before and After Peritesticular Injection of 5 mg $PGF_{2\alpha}$.

Interval of exposure to $PGF_{2\alpha}$ (min)	Before $PGF_{2\alpha}$			After $PGF_{2\alpha}$		
	Deferent duct	Epididymis		Deferent duct	Epididymis	
		Tail	Head-body		Tail	Head-body
	($\times 10^6$)					
10	33[a]	1340	313	70	1578	263
30	47	1040	274	53	1240	271
60	18	1433	339	89	1513	288
Average	33 ± 13[b]	1271 ± 130	309 ± 16	71 ± 13[b]	1444 ± 130	274 ± 16

[a] Each value is the average for one side of each of three rabbits. The control side was removed immediately before injection of $PGF_{2\alpha}$.

[b] Based on pooled subclass variance.

TABLE II. Distribution of Sperm in Rabbits Before and After sc Injection of Saline or 5 mg $PGF_{2\alpha}$.

Treatment	Interval of exposure to saline or $PGF_{2\alpha}$ (min)	Before saline or $PGF_{2\alpha}$			After saline or $PGF_{2\alpha}$		
		Deferent duct	Epididymis		Deferent duct	Epididymis	
			Tail	Head-body		Tail	Head-body
		($\times 10^6$)					
Saline	10	19[a]	555	110	16	710	120
	30	33	775	162	41	745	162
	Average	26 ± 8[b]	665 ± 89	136 ± 17	28 ± 8	728 ± 89	141 ± 17
5 mg $PGF_{2\alpha}$	10	30	695	135	26	630	140
	30	36	1015	152	89	1035	152
	Average	33 ± 8[b]	855 ± 89	144 ± 17	57 ± 8	832 ± 89	146 ± 17

[a] Each value is the average for one side of each of four rabbits. The control side was removed and the deferent duct was ligated at the urethra on the treated side immediately before saline or $PGF_{2\alpha}$ injection (subcutaneously).

[b] Based on pooled subclass variance.

veloped diarrhea. The number of sperm in the deferent duct of rabbits treated with $PGF_{2\alpha}$ was nearly threefold greater than that in the controls ($P \simeq 0.12$, Table III). $PGF_{2\alpha}$ increased ($P \simeq 0.25$) the percentage of sperm in the deferent duct, and more than doubled ($P \simeq 0.06$) the number of deferent duct sperm per 100×10^6 testicular sperm. Numbers of tail epididymidal sperm were not altered significantly after $PGF_{2\alpha}$. Similarly, numbers of head-body epididymidal sperm and head-body epididymidal sperm per 100×10^6 testicular sperm were not affected significantly by $PGF_{2\alpha}$. However, the percent of excurrent duct sperm found in the head-body epididymis was reduced ($P \simeq 0.03$) by $PGF_{2\alpha}$. The results from this experiment suggested that $PGF_{2\alpha}$ increased ductus deferens sperm numbers at the expense of head-body epididymidal sperm numbers. Numbers of testicular sperm did not differ significantly between controls and $PGF_{2\alpha}$-treated rabbits (517 vs 536×10^6).

In the fourth experiment, we chose a smaller dose of $PGF_{2\alpha}$ (5 mg total) in an

TABLE III. Distribution of Sperm in Rabbits After sc Injection of Saline or 10 mg $PGF_{2\alpha}$ Three Times at 20-min Intervals.

Duct	Treatment	
	Saline	$PGF_{2\alpha}$
Deferent duct sperm ($\times 10^6$)	74 ± 19[a]	203 ± 68
(% of total sperm)	4.8 ± .3	9.4 ± 1.0
(per 100×10^6 testes sperm)	18 ± 6	41 ± 16
Tail epididymis sperm ($\times 10^6$)	1,163 ± 172	1,465 ± 364
(% of total sperm)	69 ± 4	72 ± 4
(per million testes sperm)	2.4 ± .3	2.8 ± .7
Head-body epididymis sperm ($\times 10^6$)	426 ± 44	348 ± 62
(% of total sperm)	26 ± 3	18 ± 2
(per 100×10^6 testes sperm)	94 ± 23	70 ± 18

[a] Each value is the average ± the standard error for the paired ducts from each of four rabbits.

TABLE IV. Distribution of Sperm Rabbits After sc Injection of Saline or 1 mg $PGF_{2\alpha}$ Five Times at 12-min Intervals.

Duct	Treatment	
	Saline	$PGF_{2\alpha}$
Deferent duct sperm ($\times 10^6$)	70 ± 14[a]	224 ± 41
(% of total sperm)	3.6 ± 0.4	10.3 ± 2.0
(per 100 × 10^6 testes sperm)	18 ± 3	58 ± 13
Tail epididymis sperm ($\times 10^6$)	1,480 ± 268	1,612 ± 239
(% of total sperm)	69 ± 4	65 ± 4
(per million testes sperm)	3.6 ± 0.4	3.7 ± 0.4
Head-body epididymis sperm ($\times 10^6$)	437 ± 56	541 ± 45
(% of total sperm)	24 ± 3	25 ± 3
(per 100 × 10^6 testes sperm)	116 ± 12	131 ± 13

[a] Each value is the average ± the standard error for the paired ducts from each of ten rabbits.

effort to obviate the diarrhea observed in the third experiment, and we anesthetized the rabbits to nullify any stress associated with sc injections. We observed no diarrhea. The increases in deferent duct sperm after $PGF_{2\alpha}$ treatment in this experiment (Table IV) were remarkably similar to those in the third trial (Table III); numbers of deferent duct sperm were increased ($P < 0.01$) about threefold by each of the three methods of calculation. However, $PGF_{2\alpha}$ failed to alter significantly the numbers of tail or head-body epididymidal sperm. Similar to the third experiment, numbers of testicular sperm did not differ significantly between controls and $PGF_{2\alpha}$-treated rabbits (397 vs 429 × 10^6).

The results of these four experiments demonstrate that $PGF_{2\alpha}$ results in increased numbers of sperm in the deferent duct of rabbits. Presumably, the extra sperm in the deferent duct after $PGF_{2\alpha}$ originated in the epididymis, but we failed to show this unequivocally. We speculate that $PGF_{2\alpha}$ causes increased contractility of the excurrent sperm ducts, moving epididymidal sperm into the deferent duct. In support of this notion, (a) prostaglandins evidently control neuromuscular transmission in the guinea-pig deferent duct (6, 7) and (b) Hunt and Nicholson (9) published that daily injections of $PGF_{2\alpha}$ into rabbits for 2 months may hasten the transit of sperm through the excurrent ducts without altering spermatogenesis.

Summary. Four experiments were conducted to test the influence of $PGF_{2\alpha}$ on the distribution of sperm in male rabbits. In the first, nine rabbits were anesthetized, one side of the reproductive tract was removed, and 5 mg $PGF_{2\alpha}$ was injected around the remaining testis and epididymis. Relative to the controls, the deferent duct contained more than twice as many sperm at 10, 30, or 60 min after $PGF_{2\alpha}$. Results from the second trial showed that the deferent duct of rabbits did not accumulate sperm during 30 min under anesthesia without $PGF_{2\alpha}$ treatment, but deferent duct sperm were more than doubled at 30 min after 5 mg $PGF_{2\alpha}$ injected subcutaneously. In the third experiment, four unanesthetized rabbits injected subcutaneously three times (at 20-min intervals) with 10 mg $PGF_{2\alpha}$ had about threefold more deferent duct sperm and significantly fewer sperm in the head-body epididymis than four saline-injected control rabbits. Relative to ten controls in the final experiment, after five sc injections of 1 mg $PGF_{2\alpha}$ at 12-min intervals, ten rabbits had more sperm in the paired deferent ducts (70 vs 224 × 10^6, $P < 0.01$), a greater percentage of the total excurrent duct sperm in the deferent duct (3.6 vs 10.3%, $P < 0.01$), and more deferent duct sperm per 100 × 10^6 testicular sperm (18 vs 58, $P < 0.01$). We conclude that $PGF_{2\alpha}$ causes increased numbers of sperm in the deferent duct, possibly by increasing the rate of movement of sperm out of the epididymis.

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