

**ANTIREPRODUCTIVE EFFECTS
OF TAMOXIFEN WHEN APPLIED LOCALLY
TO THE EPIDIDYMISS OF RATS**

W. D. Ratnasooriya and S. Manickavasagar

*Department of Zoology,
University of Colombo,
Colombo 03, Sri Lanka.*

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Abstract

Summary: This study investigated the effects of tamoxifen, an oestrogen receptor antagonist on fertility of male rats. This was done by applying the drug directly to the epididymis through implantation of silastic rods containing 50% of drug (day 0) and subjecting these rats to serial mating on day 3, and then approximately at weekly intervals upto day 70. The tamoxifen treatment caused significant impairment in sperm numbers in the ejaculate possibly via spermatogenic arrest (between days 21-42), fertility index (on days 28 and 35), number of uterine implants (on day 35) or quantal pregnancy (on day 35) and a significant elevation in pre-implantation loss (on day 35). On the other hand, tamoxifen treatment had no significant effects on libido and in post-implantation loss. It is concluded that tamoxifen mediated its antifertility action mainly through production of oligozoospermic ejaculates possibly via the inhibition of Sertoli cell function.

Key words: Tamoxifen epididymis, spermatogenesis, fertility, sperm count, Sertoli cell, pre-implantation loss

1. Introduction

Several kinds of receptor antagonists have been tested as potential male contraceptives acting via the epididymis. These include α -adrenoceptor antagonists [Phenoxybenzamine (1) prazosin (2,3), terazosin (4) or tamsulosin (5)], B-adrenoceptor antagonists [atanolol (6)], cholinergic antagonists [atropine (7)], purinoceptor antagonists [α , B methylene ATP (8)] prostaglandin receptor antagonists [Polyphloretin phosphate (9) and Di-4 - Phloretin phosphate (10)] and testosterone receptor antagonists (cyproterone acetate (11) and flutamide (12)). However, in this regard, oestrogen receptor antagonists have not been tested as potential male contraceptives acting via epididymis although the presence of oestrogen receptors in the epididymis is well documented (13,14).

The present study was aimed at exploring the possibility of using oestrogen receptor antagonists as potential male contraceptives acting via epididymis. Tamoxifen was selected as the prototype oestrogen receptor antagonist drug which is clinically used in metastatic breast cancer and in an ovulatory infertility (15). Investigations were made in rats using a local mode of administration using a silastic formulation which has been used previously (2).

2. Materials and methods

Cross bred healthy adult albino rats of proven fertility from our colony were used (males weighing 225-250g and females 200-225g). The animals were housed in plastic cages under standardised animal house conditions (temperature: 28[±]1°C; photoperiod: approximately 12h light and 12h dark daily; relative humidity: 50-55%) with free access to food [pelleted chow (Oils and Fats Co. Ltd., Seeduwa, Sri Lanka)] and tap water.

Silastic (Silastic 382, Medical Grade Elastomer, Dow Corning Ltd., USA) rods (2mm in diameter and 8mm long) containing 0% and 50% tamoxifen were made as described previously in detail (2). A single rod (50% tamoxifen: n=6 and 0% tamoxifen n=12) was inserted adjacent to each epididymis under mild ether (BDH Chemicals Ltd., Poole, UK) anaesthesia using aseptic precautions through a midline incision made in the scrotal sac and the tunica vaginalis on each side (2). The day of insertion of the rods was designated as day 0. These rats were observed daily (between 10.00-11.00h) for mortality, general physical condition, behaviour, overt signs of toxicity, and, food and water intake. The consistency of the faeces and the colour of urine were also noted.

Libido, ejaculatory ability and fertility were evaluated at regular intervals (7-14 days prior to insertion of rods and on days 3, 7 and then at weekly intervals up to day 70) by pairing each male (between 17.00-18.00h) overnight with a prooestrous female having had a regular 4-5 day vaginal cycle. The pre-coital sexual behaviour patterns of paired rats were observed 1-2h later. Successful mating was confirmed by the presence of sperms in the vaginal smear on the following morning (7.30 - 8.30h). If sperms were present (considered as onset of pregnancy), their numbers were estimated (in duplicate) using an improved Neubauer type haemocytometer (Fisons Scientific Equipment, Loughborough, UK) after flushing the vagina with 0.05ml of normal saline solution (0.9% NaCl, w/v) and this was used as a sperm count index (10^6 ml^{-1}). Simultaneously, the gross morphology of the ejaculated sperm was noted. In the absence of sperm, daily vaginal smearing was undertaken to determine the occurrence of pregnancy or pseudopregnancy.

At day 14 **Post coitum** the mated females were subjected to laparotomy under ether anaesthesia and the number of conceptus (both viable and dead) were counted to permit analysis of fertility. The size and distribution of the fetuses were also noted. In addition, the number and the gross appearance of the corpora lutea in each ovary were recorded.

The following reproductive indices were then computed: index of libido = (number mated/number paired) x 100; quantal pregnancy = (number pregnant/number mated) x 100; fertility index = (number pregnant/number paired) 100; pre-implantation loss = [(total number of corpora lutea - total number of implantation loss) / (total number of implants - total number of viable implants) / total number of implants] x 100.

Unless otherwise noted, data are expressed as means \pm SEM. Data were statistically analysed using Mann Whitney U-test and G-test. A P value less than 0.05 was considered as statistically significant.

3. Results

Tamoxifen was well tolerated: all animals appeared normal with no treatment related overt signs of toxicity (physical and behavioural) or deaths. Compared to controls, these treated rats had apparently normal food and water intake. Further, the texture of faeces and the colour of the urine of the treated rats seemed almost the same to those of the controls.

In the mating experiments, the precoital sexual foreplay (chasing, nosing, anogenital sniffing, attempted mounts and intromissions with or without pelvic thrusting) of the treated rats was essentially similar to the controls and was within the range of the inhouse norm. Moreover, as shown in Table 1, the index of libido was not significantly inhibited ($p > 0.05$, G test) throughout the study.

Tamoxifen induced a significant ($p < 0.05$, Mann Whitney U test) impairment in the ejaculated sperm content on days 14 (by 62%) 21 (by 79%), 28 (by 88)%, 35 (by 75%) and 42 (by 83) as revealed from the vaginal sperm count index. In addition, a modest but a non significant ($p > 0.05$) reduction in ejaculate sperm numbers was evident on day 42 respectively, one or two rats became completely azoospermic. The external appearance of the ejaculated sperm was almost identical in both groups with minimal decapitation (10-15%).

Tamoxifen treatment depressed quantal pregnancy significantly ($p < 0.05$, G-test) only on day 35 although it was markedly reduced on day 21 (by 35%), 28 and 42 (by 40%). Fertility index showed a similar suppression trend to quantal pregnancy but this effect was significant ($p < 0.05$, G- test) on both days 28 and 35.

Table 1. Effect of 50% tamoxifen rods on some reproductive parameters of rats when applied locally to the epididymis (mean \pm SEM)

Parameter	Treatment	Index of libido	Vaginal sperm count index (10 ⁶ ml)	Quantal Pregnancy	Number of Implants	Fertility Index	Pre-Implantation loss	Post-Implantation loss
Pre Treatment	1x 0%rod	100%	11.3 \pm 1.6	100%	7.9 \pm 0.4	100%	22.1 \pm 3.8	6.2 \pm 3.3
	1x 50%rod	100%	11.6 \pm 2.5	100%	8.3 \pm 1.2	100%	21.6 \pm 6.0	7.5 \pm 4.1
Post Treatment in days								
3	1x 0%rod	100%	11.2 \pm 2.2	100%	7.4 \pm 0.3	100%	22.9 \pm 3.3	5.9 \pm 4.1
	1x50%rod	100%	17.3 \pm 4.2	83.33%	7.8 \pm 1.6	83.33%	33.2 \pm 14.1	8.9 \pm 5.9
7	1x 0%rod	100%	11.5 \pm 0.8	100%	6.9 \pm 0.6	100%	26.2 \pm 1.9	4.4 \pm 3.0
	1x50%rod	100%	18.7 \pm 6.2	100%	8.2 \pm 1.1	100%	23.8 \pm 11.8	21.5 \pm 12.0
14	1x 0%rod	100%	12.0 \pm 2.2	100%	7.3 \pm 0.6	100%	19.1 \pm 2.4	2.5 \pm 1.6
	1x50%rod	100%	4.5 \pm 1.2*	83.33%	7.3 \pm 2.0	83.33%	43.7 \pm 16.0	18.3 \pm 16.4
21	1x 0%rod	100%	11.5 \pm 1.3	100%	7.7 \pm 0.5	100%	23.3 \pm 3.9	3.5 \pm 2.4
	1x50%rod	83.33%	2.4 \pm 1.3**	66.67%	4.5 \pm 1.8	66.67%	59.7 \pm 15.3	20.4 \pm 11.1
28	1x 0%rod	100%	15.4 \pm 1.3	100%	7.7 \pm 0.2	100%	18.5 \pm 3.6	3.2 \pm 2.1
	1x50%rod	83.33%	1.8 \pm 1.0*	40%	4.6 \pm 2.6	50%	62.9 \pm 21.0	0.0 \pm 0.0
35	1x 0%rod	100%	13.9 \pm 1.4	100%	7.0 \pm 0.5	100%	25.6 \pm 4.7(8)	2.6 \pm 1.7
	1x50%rod	100%	3.5 \pm 2.4	60%	3.2 \pm 2.1*	40%	72.6 \pm 17.0**	0.0 \pm 0.0
42	1x 0%rod	100%	16.3 \pm 2.8	100%	6.6 \pm 0.5	100%	31.0 \pm 3.4(6)	5.7 \pm 4.5
	1x50%rod	100%	2.8 \pm 2.5**	100%	5.0 \pm 2.2	60%	56.4 \pm 19.0	4.0 \pm 4.0
49	1x 0%rod	100%	10.9 \pm 1.2(8)	100%	7.9 \pm 0.5	100%	29.7 \pm 6.0	1.9 \pm 1.9
	1x50%rod	80%	5.4 \pm 2.7	100%	8.5 \pm 1.9	80%	23.9 \pm 10.7	9.8 \pm 6.1
56	1x 0%rod	100%	15.8 \pm 4.0(6)	100%	6.6 \pm 0.7	100%	29.0 \pm 10.8(7)	1.2 \pm 1.2
	1x50%rod	80%	15.8 \pm 5.5	100%	11.5 \pm 1.8	80%	11.1 \pm 5.8	0.0 \pm 0.0
63	1x 0%rod	100%	13.0 \pm 2.7	100%	7.2 \pm 0.3	100%	23.5 \pm 5.5(7)	0.0 \pm 0.0
	1x50%rod	80%	10.2 \pm 2.7	100%	7.5 \pm 2.2	80%	31.4 \pm 19.3	16.4 \pm 13.0
70	1x 0%rod	100%	7.4 \pm 1.3(7)	100%	7.8 \pm 0.3	100%		3.2 \pm 2.9
	1x50%rod	100%	4.8 \pm 5.4	100%	7.2 \pm 1.8	100%	30.6 \pm 17.8	0.0 \pm 0.0

*P>0.05 **P>0.01

On days where tamoxifen treatment caused a significant inhibition of sperm numbers in the ejaculate there was a parallel reduction in the number of uterine implants: day 21 (by 41%), day 28 (by 40%), day 35 (by 54%) and 42 (by 24%). This effect, however, was significant ($p < 0.05$, Mann Whitney U-test) on day 35 only. In addition, the treatment rendered some mated females completely sterile on days where there was an impairment in ejaculated sperm numbers: day 21 (2 out of 6, 33%), day 28 (2 out of 5, 40%), day 35 (3 out of 5, 60%) and day 42 (2 out of 5, 40%). The size of the uterine implants (where present) in the females mated with tamoxifen-treated males seemed almost identical to that of controls.

On the days where uterine implant numbers were markedly reduced there was parallel elevation in pre-implantation losses: day 14 (by 1238%), day 21 (by 156%), day 28 (by 239%), day 35 (by 183%) and day 42 (by 82%). However, this effect was statistically significant ($p < 0.05$, Mann Whitney U-test) only on day 35. In contrast, tamoxifen treatment had no significant ($p > 0.05$, Mann-Whitney U-test) effect on the post-implantation losses at any mating during the study.

4. Discussion

The results presented herein show that chronic local application of tamoxifen, an oestrogen receptor antagonist, to the epididymis of rats can potentially impair fertility status (interms of vaginal sperm count index, fertility index, fertility index, quantal pregnancy or pre-implantation loss), in a reversible fashion, without inhibiting libido (in terms of index of libido) and masculine copulatory behaviour (measured only qualitatively). This is a novel finding which is also paradoxical since, tamoxifen is reported to improve ejaculate sperm counts in subfertile men (16). However, whether this reported improvement in sperm density here is a better fertility prognosis remains to be seen. Nevertheless, tamoxifen has been used by some for the treatment of idiopathic normogonadotropic oligozoospermia (17).

The most striking antifertility effect seen in this study was the production of oligozoospermic ejaculates, (in terms of the vaginal sperm count index) which occurred between weeks 2 and 6 of treatment. This reduced sperm output cannot result from an obstruction in the sperm passage in view of the reversibility of the effect: usually, obstructions in sperm passage leads to permanent oligo and/or azoospermia (18). Further, the delayed onset of oligozoospermia argues against an impairment of ejaculatory contracting of the epididymis and/or vas? (19) mediated via their oestrogen receptors (13, 14) by tamoxifen. Thus, it is likely that the reduced sperm output with tamoxifen results from a partial and reversible spermatogenic arrest (18) possibly at the spermatid phase of spermatogenesis (20): irreversible arrest at spermatocyst or spermatid level usually has a genetic origin (18). Further, the timing of the periods of reduced sperm output gives an indication of the stage of spermatogenesis affected via a drug or a chemical (20) and in this study, the time frame of the action coincides with the duration of the spermatid phase of the spermatogenic cycle (20). But, how could tamoxifen, an oestrogen receptor antagonist, interrupt spermatogenesis? We have no definite answer to this but some possibilities can be suggested. Oestrogens are known to suppress pituitary activity (20) and results in induced sperm counts (see ref. 21). Further, tamoxifen is known to display week agonistic properties in humans (22)

However, a suppression in spermatogenesis via this mechanism is unlikely because the libido and masculine copulatory behaviour remained essentially unaltered in this study. Evidence is accumulating that the sertoli cells are responsible for orchestrating and regulating spermatogenesis (21) and, hence sperm output, possibly via their autocrine and paracrine factors (23). Moreover, oestrogen is one such agent that is secreted by sertoli cells (23) and the presence of oestrogen receptors are well documented in the testis of mammals (24, 25). Thus, a possibility exists that tamoxifen may alter Sertoli cell function and hence sperm output. In this respect, it is of particular importance to note the recent report that a gene knock-out of the oestrogen receptor in mice impaired spermatogenesis (see 26) and to the observation that alteration in Sertoli cell numbers (and possibly its function) determines the sperm output (see 21). However, it is obvious that additional research is needed before definite conclusions are made.

Tamoxifen treatment also caused a partial but modest impairment of fertility in that the number of uterine implants was reduced by 40-50% (between days 21 and 42), fertility index by 40-60% (between days 28-42), of their respective pre-treatment levels. Since there is a powerful correlation between ejaculated sperm numbers and fertility (27) it is likely that these impairments in fertility parameters may have resulted from tamoxifen-induced oligozoospermia.

Compared to the control, tamoxifen caused a profound elevation (by 82-239%) in pre-implantation loss during the period of reduced sperm output (between days 21-42). This can provide an auxiliary mechanism for the antifertility action of tamoxifen. An elevation in pre-implantation loss is indicative of the presence of morphologically abnormal and/or functionally defective sperm in the ejaculate. However, the external characteristics of the ejaculated sperm of the tamoxifen treated rats was essentially similar to those of control rats. Thus, it is likely that the enhancement in the pre-implantation loss arose from disruption of sperm function. The presence of putative oestrogen receptors (28) and oestradiol-178 (29) has been demonstrated in sperm. Further, oestradiol-178 was shown to increase sperm motility and stimulated ova penetration (30). These evidences argue for the potential of tamoxifen to induce proposed disruption in sperm function. On the other hand, tamoxifen had no effect on post-implantation loss.

In conclusion, this study, for the first time has demonstrated that tamoxifen, can inhibit fertility in male rats, without affecting libido. This antifertility action primarily resulted from production of ejaculates with low sperm numbers possibly through impaired Sertoli cell status. Since man is

such a subfertile species with inefficient spermatogenesis when compared with other mammals (see 21) it may be possible to develop a male contraceptive based on oestrogen receptor antagonism.

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Reprints requests to: Professor W. D. Ratnasooriya,
Department of Zoology,
University of Colombo,
Colombo 03, Sri Lanka.