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FINAL REPORT

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TITLE: Efficacy of a Gonadotropin Releasing Hormone Agonist as a Male Contraceptive

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Introduction

Published reports suggested that testosterone (T) supplementation to maintain ejaculatory response interfered with the induction of azoospermia in monkeys treated with a gonadotropin releasing hormone (GnRH) agonist (Ag) (Akhtar et al., 1983a). Monkeys treated with the Ag alone developed azoospermia in 8-10 wk (Akhtar et al., 1983b), while animals treated with the Ag plus T failed to become azoospermia in 22 wk. These findings had major implications relative to the possible use of GnRH analogues as a male contraceptive in humans, since androgen therapy will be essential to maintain libido in men treated with the analogues.

Interestingly, data reported for monkeys did not agree with that from rats (Heber and Swerdloff, 1980; 1981). The combination of androgen and Ag treatment in rats was more effective than Ag treatment alone in inhibiting spermatogenesis. As a result, we felt it important to re-evaluate the effect of Ag administration alone and in combination with androgen therapy on spermatogenesis in monkeys.

A second objective of this study was to determine the potential fertilizing capacity of sperm from Ag-treated oligospermic male monkeys. This aspect of the study was prompted by the possibility that sperm appearing normal in morphology and motility from these oligospermic animals may be functionally infertile. Two methods were utilized to test potential fertilizing capacity of sperm: the hamster ova penetration test and the net surface charge of sperm. Previous work has shown that alterations of motility and fertilizing capacity of sperm are associated with changes in surface composition of sperm (Acott et al., 1983; Moore et al., 1984; Naz et al., 1984) and recovered sperm from oligospermic male monkeys treated with a GnRH antagonist have a reduced ability to penetrate hamster ova (Mann et al., 1985).

Materials and Methods

Fifteen adult male monkeys ranging in weight from 6.7 to 10.9 kg were used in the study. Animals were caged in a temperature (20 - 23°C) and light (12 h light: 12 h dark) controlled environment throughout the study.

Treatment groups. The 15 monkeys were randomly assigned to one of three treatment groups of five animals each. Group 1 was treated continuously with 5 ug/day of a Ag (D-Trp^{4}-N-alpha-Me-Leu^{7}-des Gly^{10}-Pro^{9}-NH Et-GnRH; Wy-40972; Wyeth Laboratories) for 24 wk and then 10 ug/day of the Ag for an additional 20 wk using Alzet osmotic minipumps (2 ml-4; Alza Corporation, Palo Alto, CA).

These low dosages of Ag were chosen in order to prolong the oligospermic phase in the process of Ag-induced azoospermia, so that the potential fertilizing capacity of recovered sperm from these males could more fully be assessed. Animals in Group 2 were treated with 25 ug/day of Ag for 44 wk using minipumps, and those in Group 3 were treated simultaneously with 25 ug/day of the Ag and physiological doses of T. T was administered using a sc implanted 5 cm long silastic elastomere (o.d. = 0.465 cm; i.d. = 0.335 cm). One elastomere of T was implanted in each animal.

Surgical implantation of minipumps and testosterone elastomeres. Animals were anesthetized with 10 mg/kg body weight of ketamine HCl, prepared for surgery and the minipumps and T capsules implanted sc in the interscapular region using sterile technique. Minipumps containing the appropriate concentration of Ag were replaced every 28 days. T implants were replaced at 16-wk intervals.

Blood and semen sample schedule. Blood samples were taken at weekly intervals over a 5-wk pretreatment period, on treatment days 2 and 7, and then weekly throughout the treatment (44 wk) and posttreatment period (16 wk).

Semen samples were collected by penile electrostimulation. Three to five semen samples were collected from each animal during the pretreatment period, and thereafter at 2-wk intervals during the treatment and posttreatment periods. Semen samples were utilized to determine sperm counts, % motile sperm and % live sperm.

Net surface charge on sperm. Sperm recovered from all three treatment groups before and during Ag treatment were used to determine net surface charge as previously described (Gould and Martin, 1975; Gould et al., 1984). Net surface charge was measured indirectly subsequent to attachment of positively-charged colloidal iron to the surface of sperm. The amount of iron present was measured using the ISI DS 130 scanning electron microscope equipped with a Tracor Northern 5500 energy dispersive X-ray analyzer.

Hamster ova penetration test. Hamster ova penetration tests were performed on the sperm of all animals prior to treatment and during the oligospermic phase of Ag treatment as previously described (Mann et al., 1987).

Pituitary and testicular response to native GnRH. During the pretreatment period and at 4-wk intervals throughout the treatment and posttreatment periods, animals were anesthetized with 10 mg/kg body weight of ketamine HCl and were injected iv with 25 ug of GnRH. Blood samples were taken at -15, 0, +15, +30, +60 and +120 min of GnRH administration.

Testicular volumes and biopsies. Testicular volumes were determined at monthly intervals as previously described (Mann et al., 1985). Testicular needle biopsies were performed on each animal during the pretreatment period and at the termination of Ag treatment for routine histological examination as described previously (Mann et al., 1985).

Results

Serum LH and T concentration. Serum LH levels rose initially and then fell precipitously to near minimal levels of detection (0.2 ug/ml) in four of

five monkeys treated with 25 ug/day of Ag (Figure 1). In these four monkeys, serum LH values remained low throughout the remainder of Ag treatment. The fifth animal (data not included in Figure 1) was far less sensitive to the Ag. Serum LH concentrations in this animal showed similar overall changes, except that values failed to remain below pretreatment values despite a continuation of Ag administration. Serum T values in the four responsive monkeys rose from pretreatment values between 4 and 8 ng/ml to 40 ng/ml on day 2 of treatment, and then declined to near castrate values (<0.70 ng/ml) for most of the remainder of Ag administration (Figure 2). Ag treatment of the fifth animal failed to maintain suppressed values of serum T. In the monkeys treated with 25 ug/day of Ag, changes in serum LH and T were similar to those observed in the four responsive monkeys treated with only Ag except that serum T did not fall to castrate values, but was maintained at 4-5 ng/ml (Figures 3 and 4). Monkeys that received 5-10 ug/day of Ag showed qualitatively similar, but quantitatively less suppression of serum LH and T (Figures 5 and 6).

Serum LH and T concentrations quickly recovered to pretreatment values at the termination of Ag treatment (Figures 1-6). Levels of serum LH and T were comparable to pretreatment values by 4 wk of the posttreatment period. The initial phase of the recovery period (3 wk) was associated with supranormal levels of serum LH and T in the two groups that were previously treated with 25 ug/day Ag (Figures 1-4).

Pituitary and testicular response to GnRH. The serum LH and T response to 25 ug of GnRH was abolished in four of five monkeys that were treated with 25 ug/day of Ag alone and the five animals that received simultaneous Ag and T treatment (Figures 7 and 8). The remaining animal continued to show significant, but reduced serum LH and T responses to GnRH administration throughout the course of Ag treatment (data not shown). In animals that were treated with 5-10 ug/day of Ag, the serum LH and T response to GnRH was reduced to less than 50% of the pretreatment response. Within 8 wk of the termination of Ag treatment, the serum LH and T response to GnRH had recovered to the pretreatment level in all groups.

Testicular volumes. Ag administration caused an initial small increase in testicular volumes at 2 wk and then testicular volumes declined rapidly to a plateau at 10 wk in all three treatment groups (Figure 9). The % decline was less in animals that received 5-10 ug/day of Ag (44%) than in monkeys treated with 25 ug/day of Ag (71%) or 25 ug/day Ag and T (73%). Thus, T administration did not alter the decline in testicular volumes induced by the high dose of Ag. However, the data in Figure 9 do not include the monkey that was less sensitive to Ag. This monkey showed only a minimal decrease in testicular volume during Ag administration.

Semen analysis. Figures 10-12 illustrate the effect of Ag administration on mean sperm count in the three groups of monkeys. Three of five animals treated with 25 ug/day of Ag alone became azoospermic by the thirteenth wk of Ag administration (Figure 10). A fourth monkey showed azoospermia by 24 wk. The delay in this fourth monkey was related to a failure of one of the osmotic minipumps rather than any difference in the sensitivity to the Ag. The fifth animal in this group never showed a consistent azoospermic ejaculate although one azoospermic sample was collected during the recovery period. All five monkeys that received simultaneous Ag (25 ug/day) and T administration became azoospermic by 15 wk of treatment, and consistently showed azoospermic

ejaculates for the remainder of the treatment period (Figure 11). On five occasions during treatment in this group, ejaculates were recovered that had a small number of sperm. In all cases, these sperm were found in ejaculates of reduced volume (<10% normal), and in all but one case recovered sperm were dead. Figure 12 shows the effect of 5-10 ug/day Ag on sperm count. While mean numbers of sperm never achieved azoospermia, ejaculates with no sperm were recovered from all monkeys in this group especially during the late treatment and the earlier posttreatment periods.

In the two groups of monkeys that received 25 ug/day of Ag and that achieved azoospermia, ejaculates continued to be azoospermic for a 7-wk period after the termination of Ag treatment (Figures 10 and 11). After that time, sperm counts recovered quickly to pretreatment levels.

During the pretreatment period, the success rate of electroejaculation ranged between 93 and 100% for the three groups of monkeys (Table 1). This rate declined in all three groups during the treatment period. However, the decline in the success rate occurred more slowly and was always less in monkeys that were treated with T.

During the oligospermic phase of Ag-induced azoospermia, there was a steady decline in % motile (Figure 13) and % live sperm (Figure 14). Moreover, sperm recovered from all three groups of monkeys showed a significant decrease in net surface charge (Figure 15) and ability to penetrate denuded hamster ova (Figures 16-18), two indications of a reduction in potential fertilizing capacity. T therapy to maintain electroejaculatory response did not alter any of these parameters.

Summary

We have found that contrary to a previous report (Akhtar et al., 1983a), treatment of monkeys with physiological levels of T does not interfere with the induction of azoospermia with an Ag. In fact, in the present study, T administration appeared to enhance the inhibitory effect of the Ag on spermatogenesis. Thus, five of five mnkeys treated with Ag plus T became azoospermic, while only four out of the five animals treated with Ag (25 ug/day) alone became azoospermic.

Of perhaps even more importance, we found that during the early oligospermic phase of Ag treatment there is substantial reduction of potential fertilizing capacity of recovered sperm. Not only are the % motile and % live sperm recovered from ejaculates greatly reduced during this period, but the net surface charge on sperm shows a dramatic decline during this period. T administration did not alter the effect of Ag on this parameter.

Perhaps partially as a result of the change in the net surface charge on sperm, sperm from Ag-treated oligospermic monkeys showed a reduced ability to penetrate denuded hamster ova. Moreover, simultaneous administration of T did not increase the ability of these sperm to penetrate hamster ova. It should be emphasized that the number of motile sperm in this test is controlled, and that therefore any observed change in this test could not have resulted from a reduced number of sperm.

Conclusions

1. The results from this study indicate that combined GnRH analogue and T therapy is a viable option as a male contraceptive. While the present study utilized a GnRH-Ag, it is likely that with the development of more potent and less toxic GnRH-antagonists, that antagonists may represent the future hope of these compounds as male contraceptives. However, whether agonists or antagonists ultimate are of value as contraceptives, there is little doubt that if azoospermia is required as an end-point of treatment, T therapy will be required in order to maintain libido and potency.

2. On the other hand, data from this study also suggest that azoospermia may not be an essential end-point. Sperm from animals that were rendered oligospermic with an Ag have a greatly reduced fertilizing potential. These data suggest that it may not be necessary to carefully titrate every man that wishes to use this approach for contraception, in order to achieve azoospermia. In fact, the human male that is oligospermic as a result of Ag administration may already be functionally azoospermic. It must be emphasized that the parameters we used as tests for sperm fertilizing capacity have limitations. Ultimately, the best test of fertilizing capacity is the ability to impregnate the female. We believe that this is the next logical step using the nonhuman primate model. Unfortunately, this will be an expensive, but necessary undertaking.

Table 1. Effect of Ag and T administration on the electroejaculatory failure rate in male rhesus monkeys.

Treatment	% Failure				
	Pretreatment	Treatment (wk)			
		1-12	13-24	25-36	37-44
5-10 ug/day Ag (N=5)	7	11	33	35	48
25 ug/day Ag (N=5)	4	14	41	57	43
25 ug/day Ag + T (N=5)	0	7	5	24	33

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Figure 1. Mean serum LH in four monkeys treated with 25 ug/day of Ag for 44 wk.

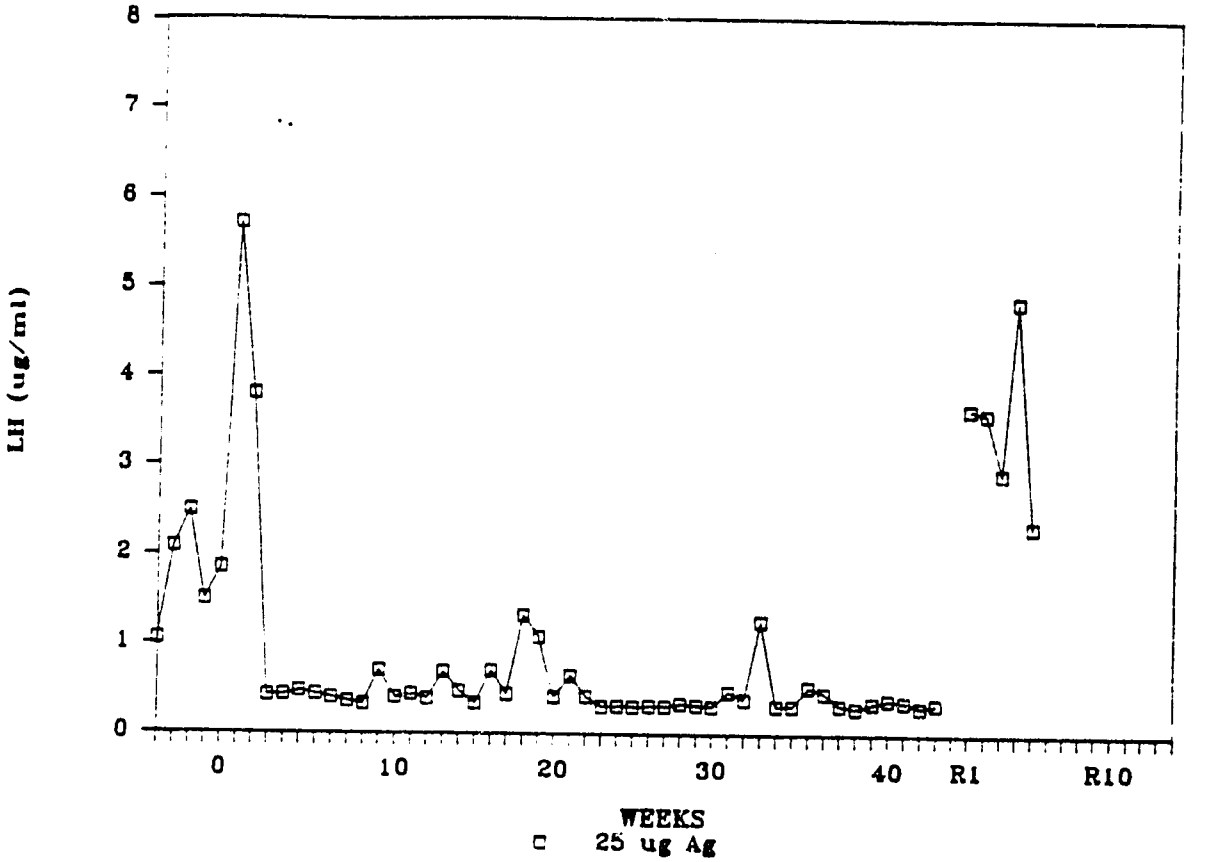

Figure 2. Mean serum T in four monkeys treated with 25 ug/day of Ag for 44 wk.

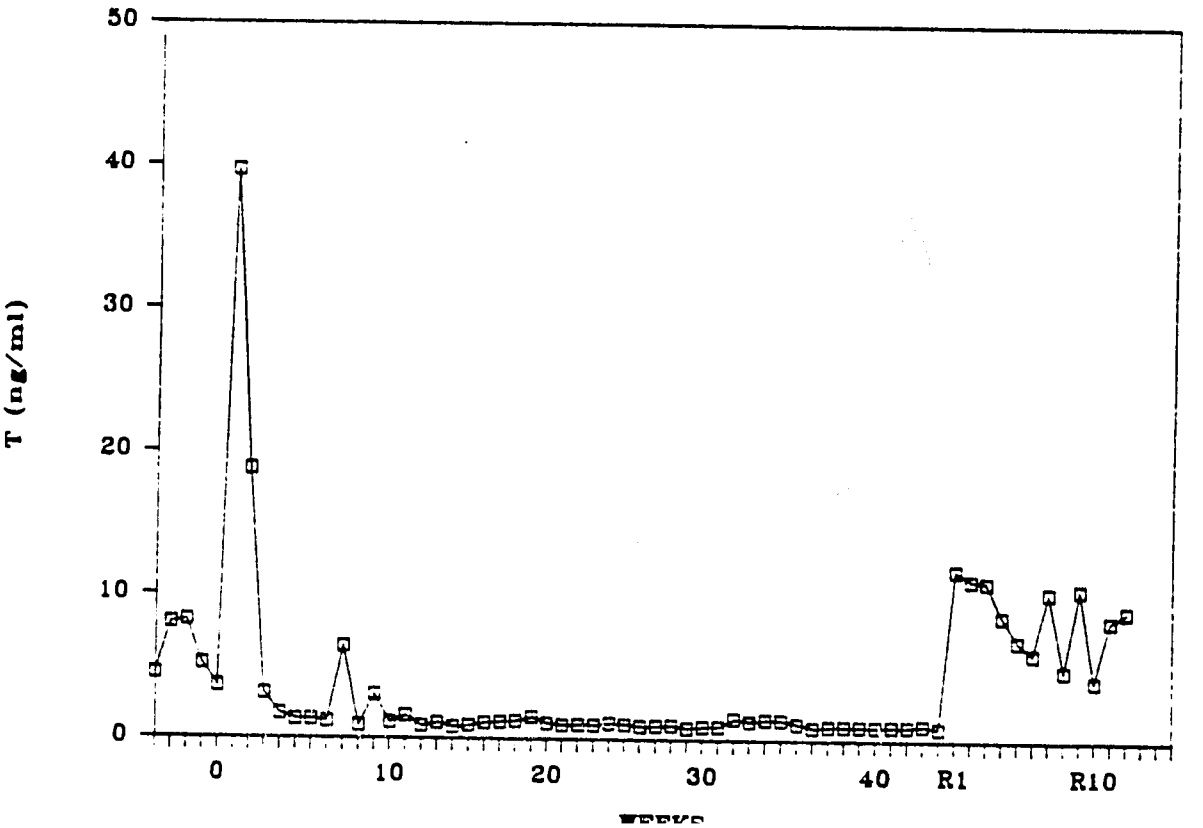

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Figure 3. Mean serum LH in five monkeys treated with 25 ug/day of Ag and T for 44 wk.

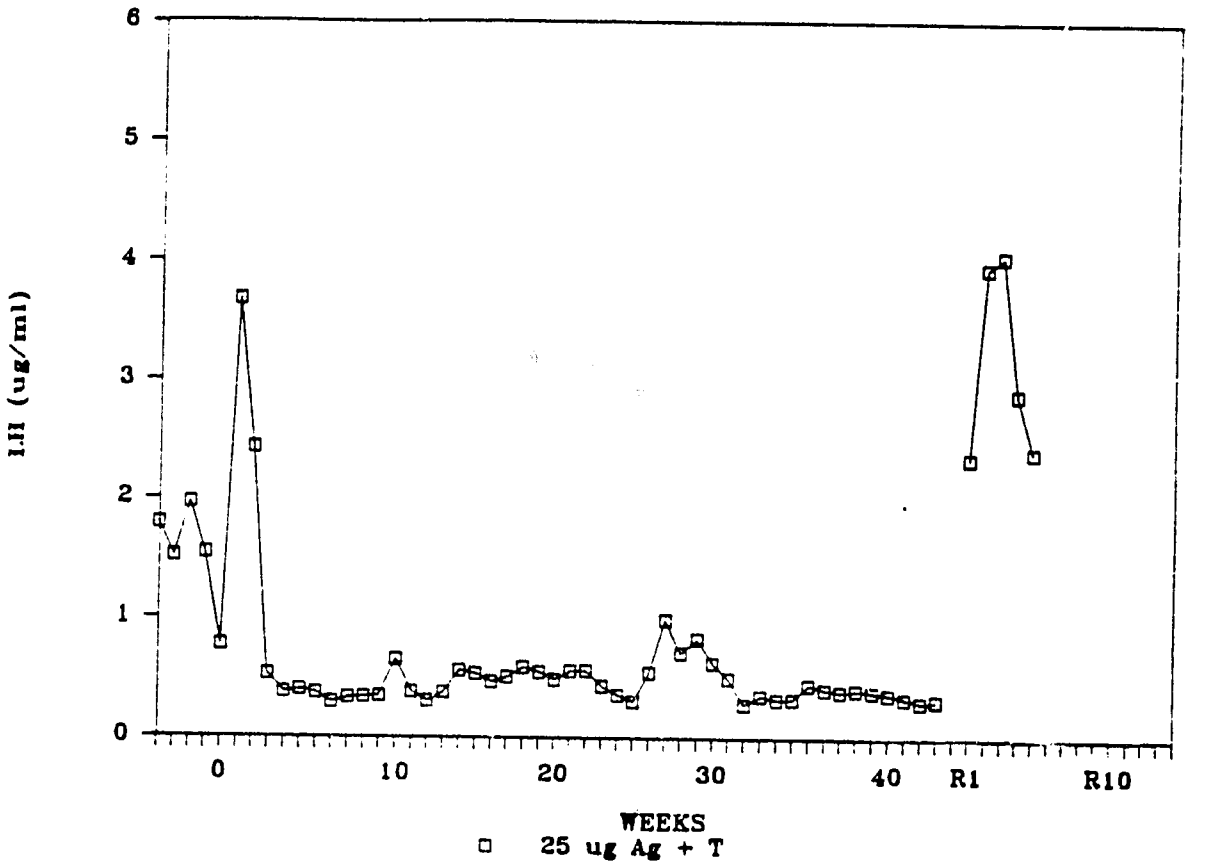
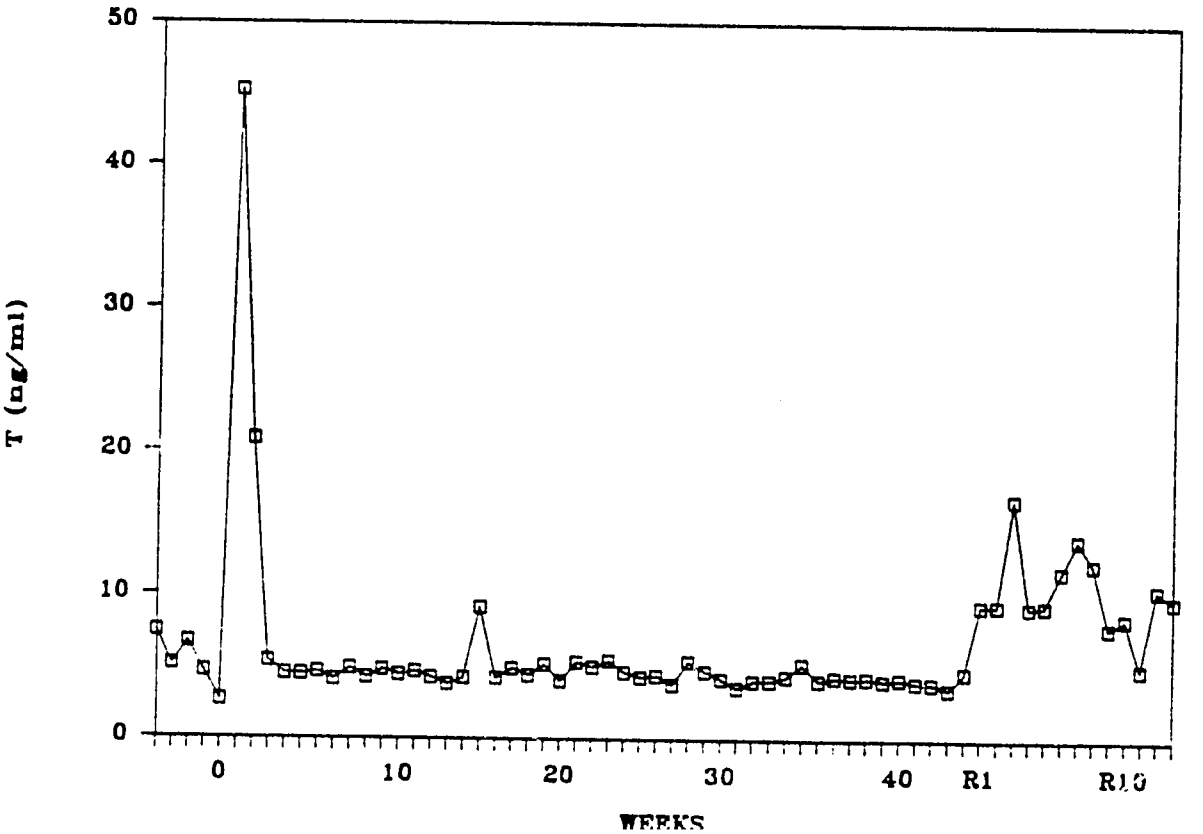


Figure 4. Mean serum T in five monkeys treated with 25 ug/day of Ag and T for 44 wk.



.gure 5. Mean serum LH in five monkeys treated with 5-10 ug/day of Ag and T for 44 wk.

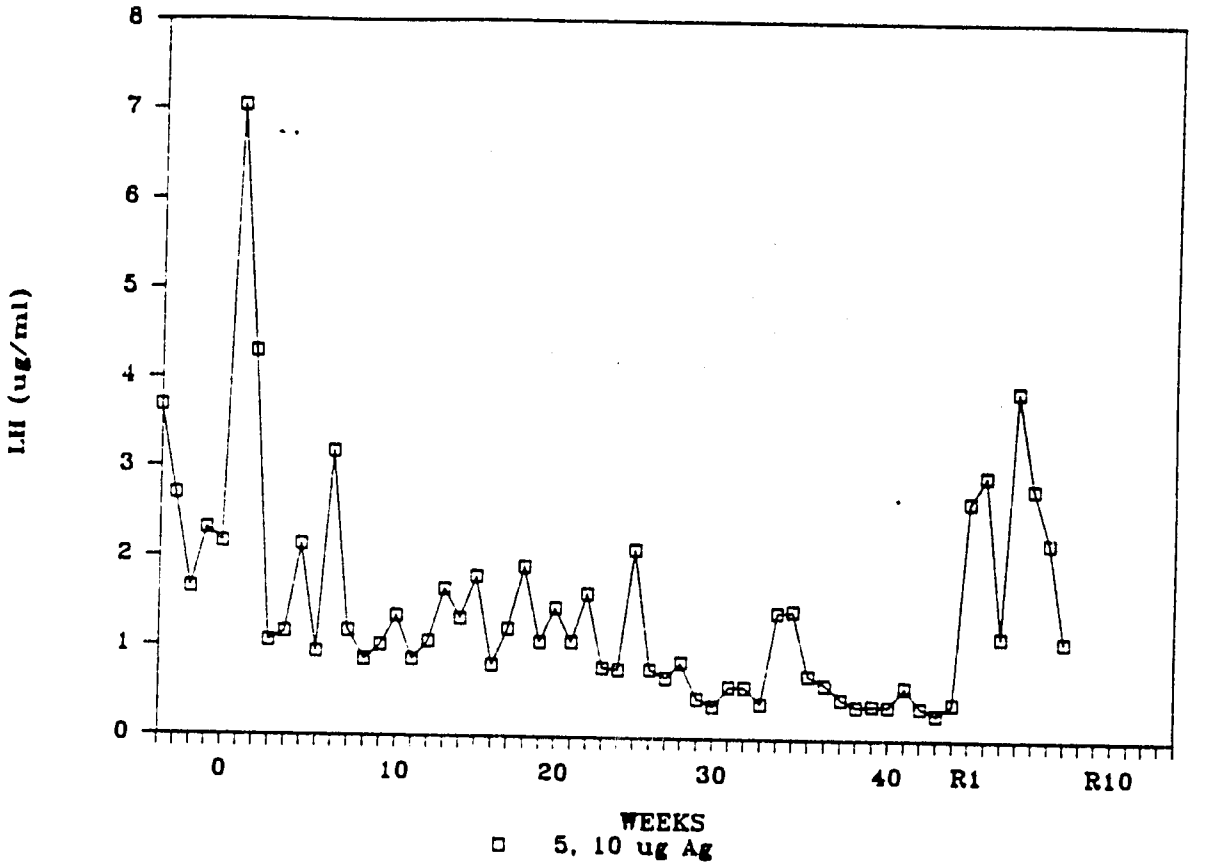
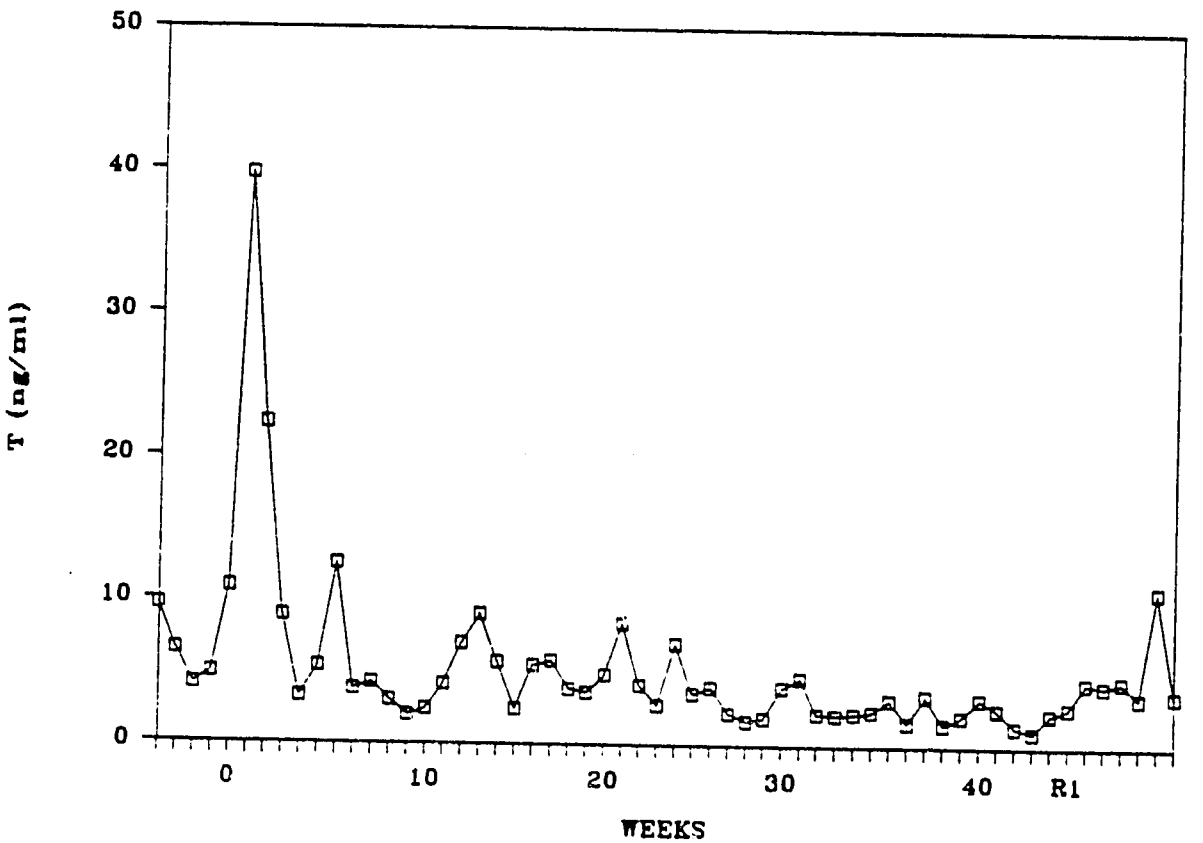


Figure 6. Mean serum T in five monkeys treated with 5-10 ug/day of Ag and T for 44 wk.



gure 7. Mean serum LH response to 25 ug of native GnRH before (0), during and after (R) treatment with 5-10 ug/day Ag, 25 ug Ag and 25 ug/day Ag and T.

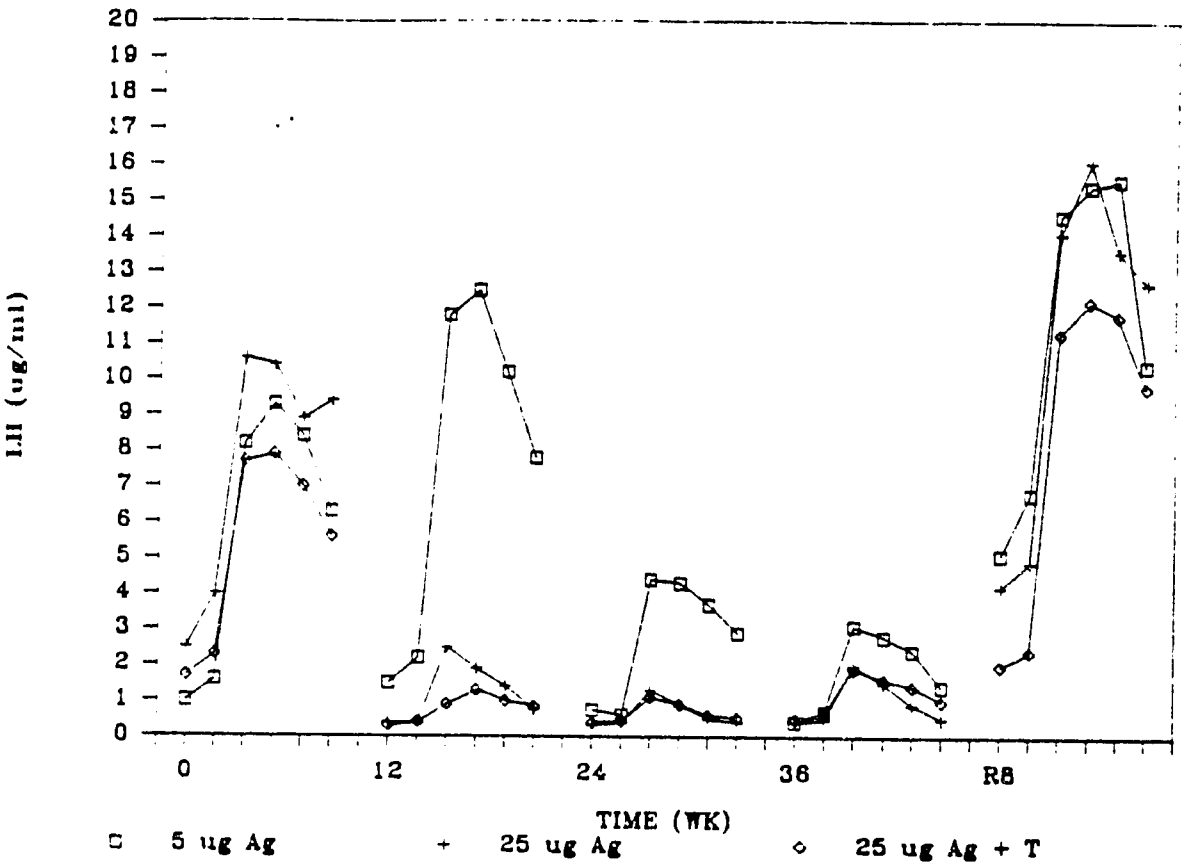


Figure 8. Mean serum T response to 25 ug of native GnRH before (0), during and after (R) treatment with 5-10 ug/day Ag, 25 ug Ag and 25 ug/day Ag and T.

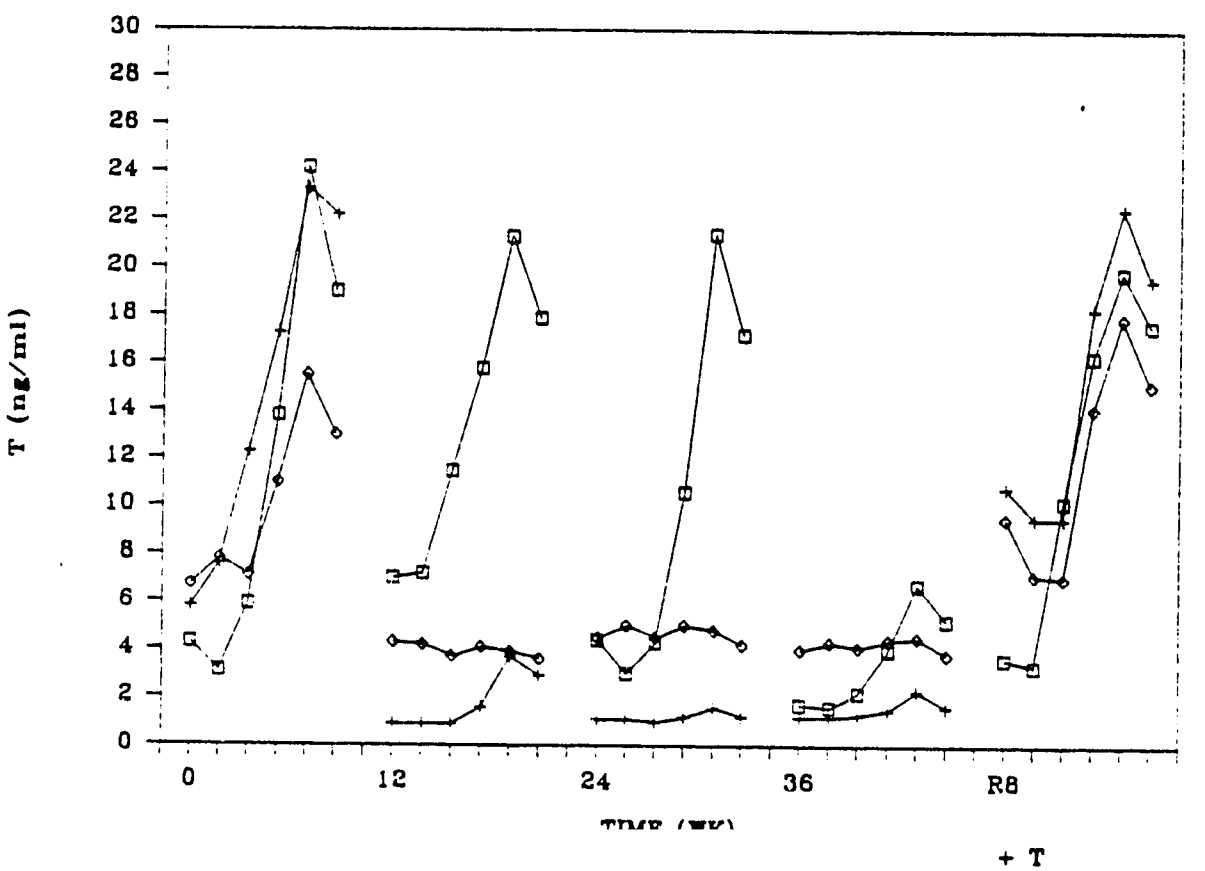
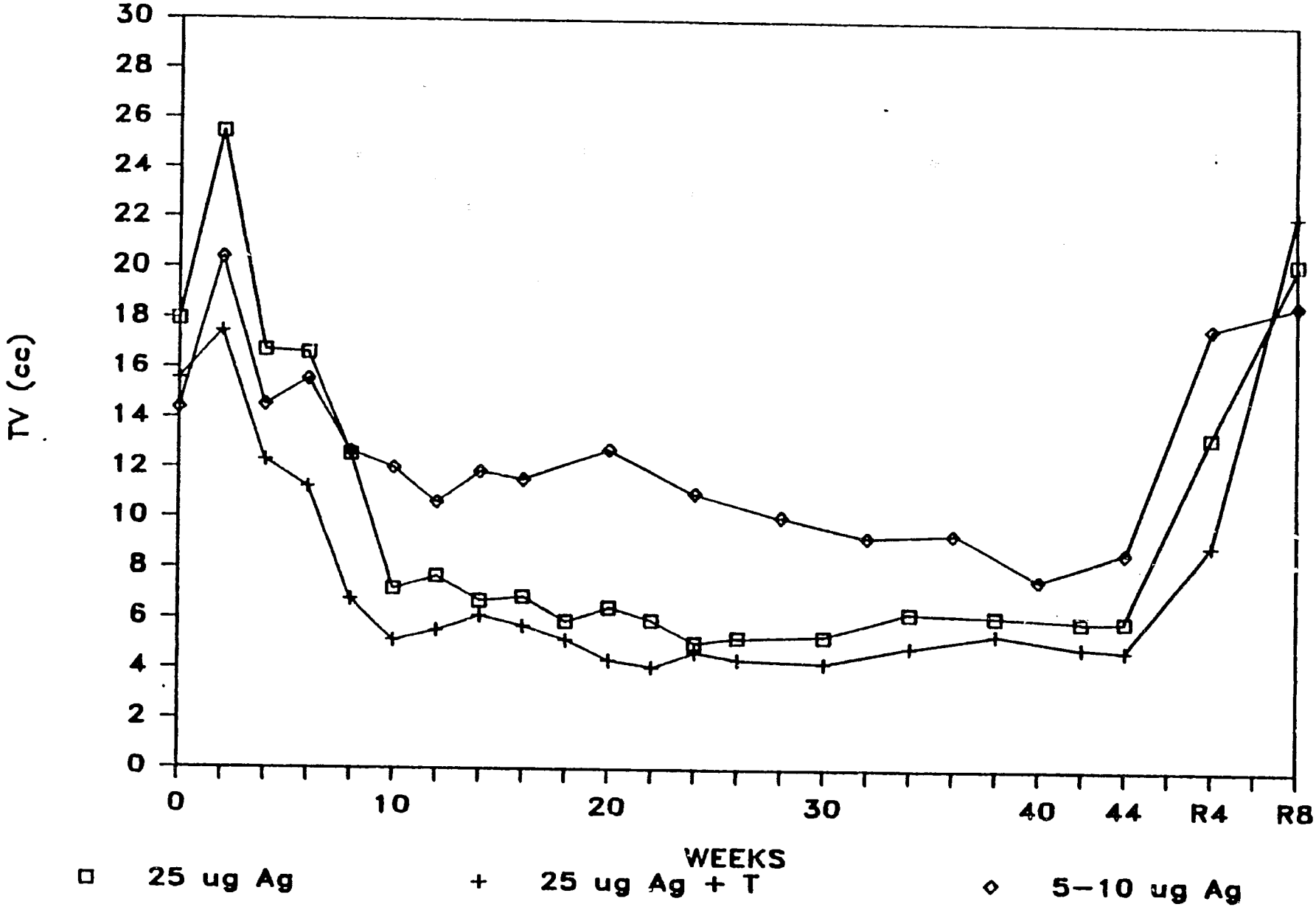


Figure 9. Mean changes in testicular volumes in monkeys treated with 25 ug/day Ag, 25 ug/day Ag and T and 5-10 ug/day Ag for 44 weeks.



gure 10. Mean sperm count in four monkeys treated with 25 ug/day of Ag for 44 wk.

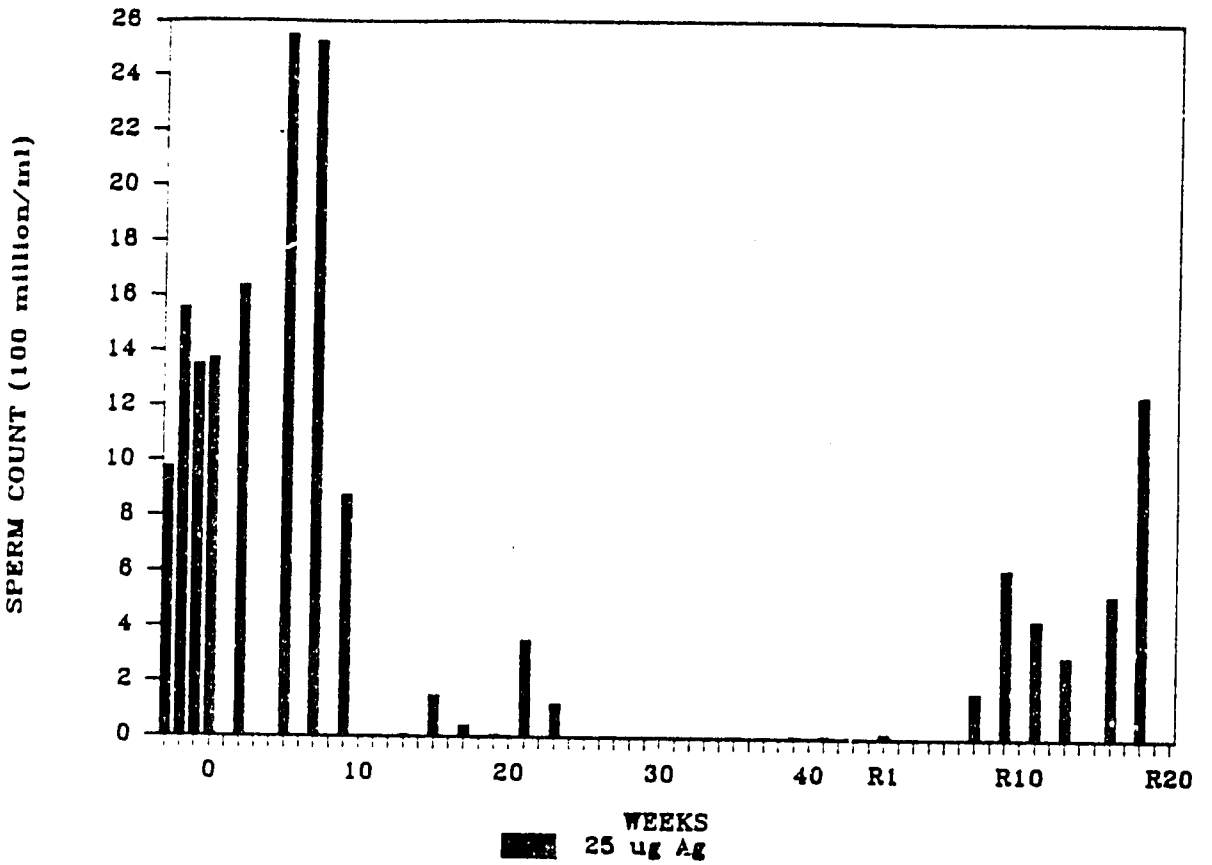
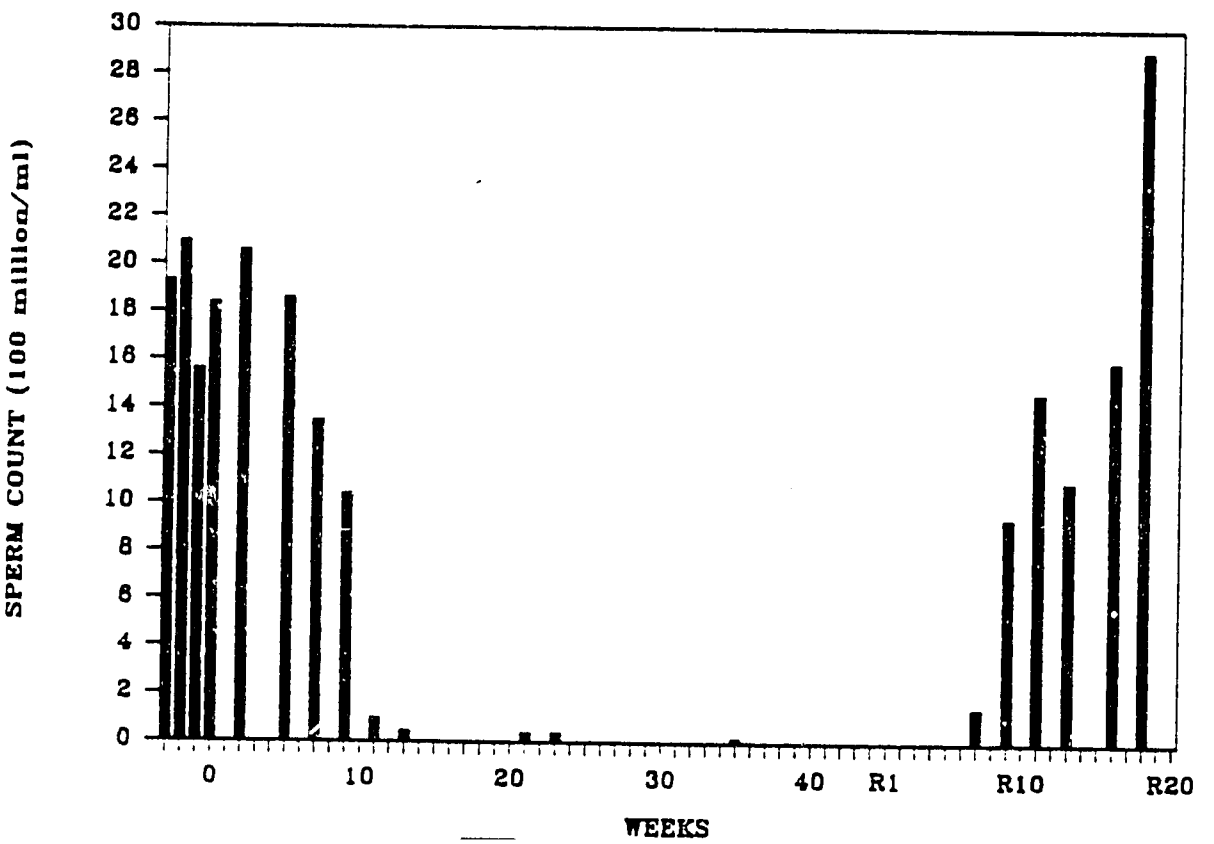


Figure 11. Mean sperm count in five monkeys treated with 25 ug Ag and T for 44 wk.



gure 12. Mean sperm count in five monkeys treated with 5-10 ug/day Ag for 44 wk.

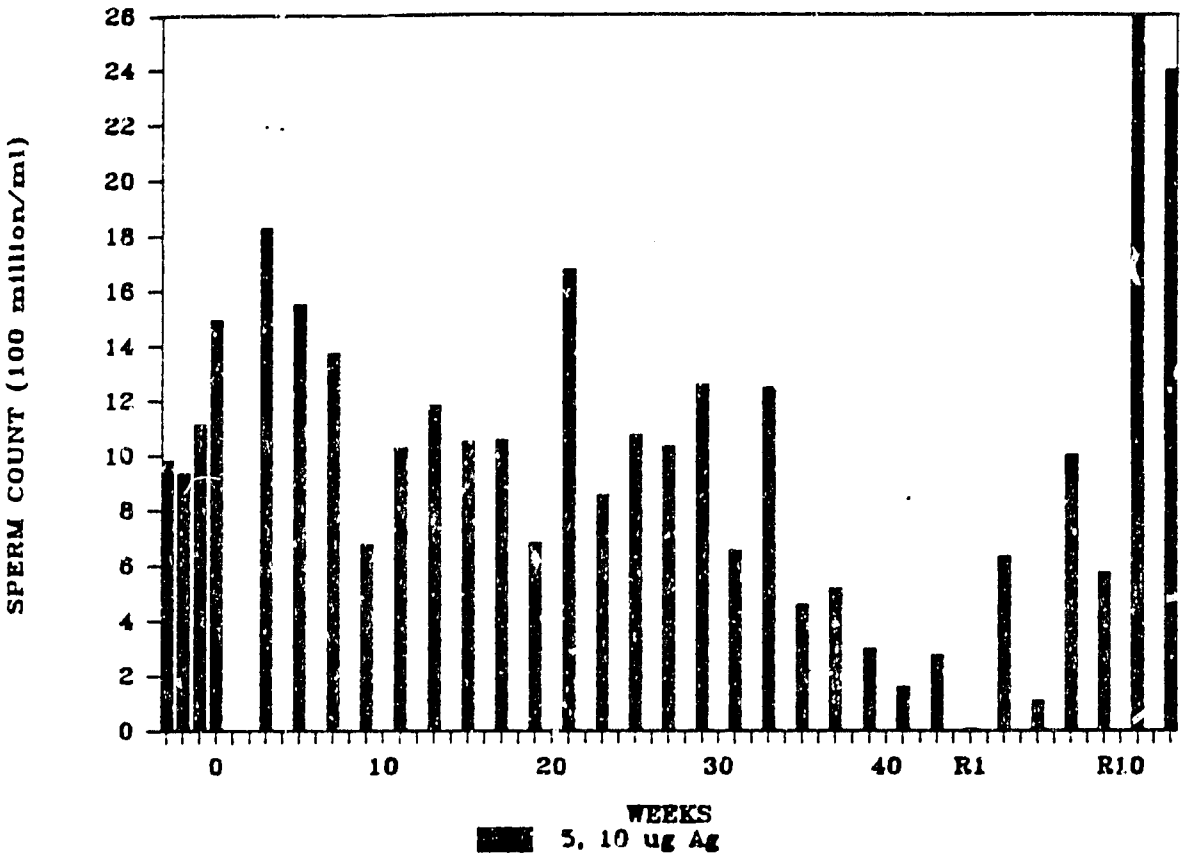


Figure 13. Mean percent motile sperm in ejaculate in monkeys treated with 5-10 ug Ag/day, 25 ug Ag/day and 25 ug Ag/day and T.

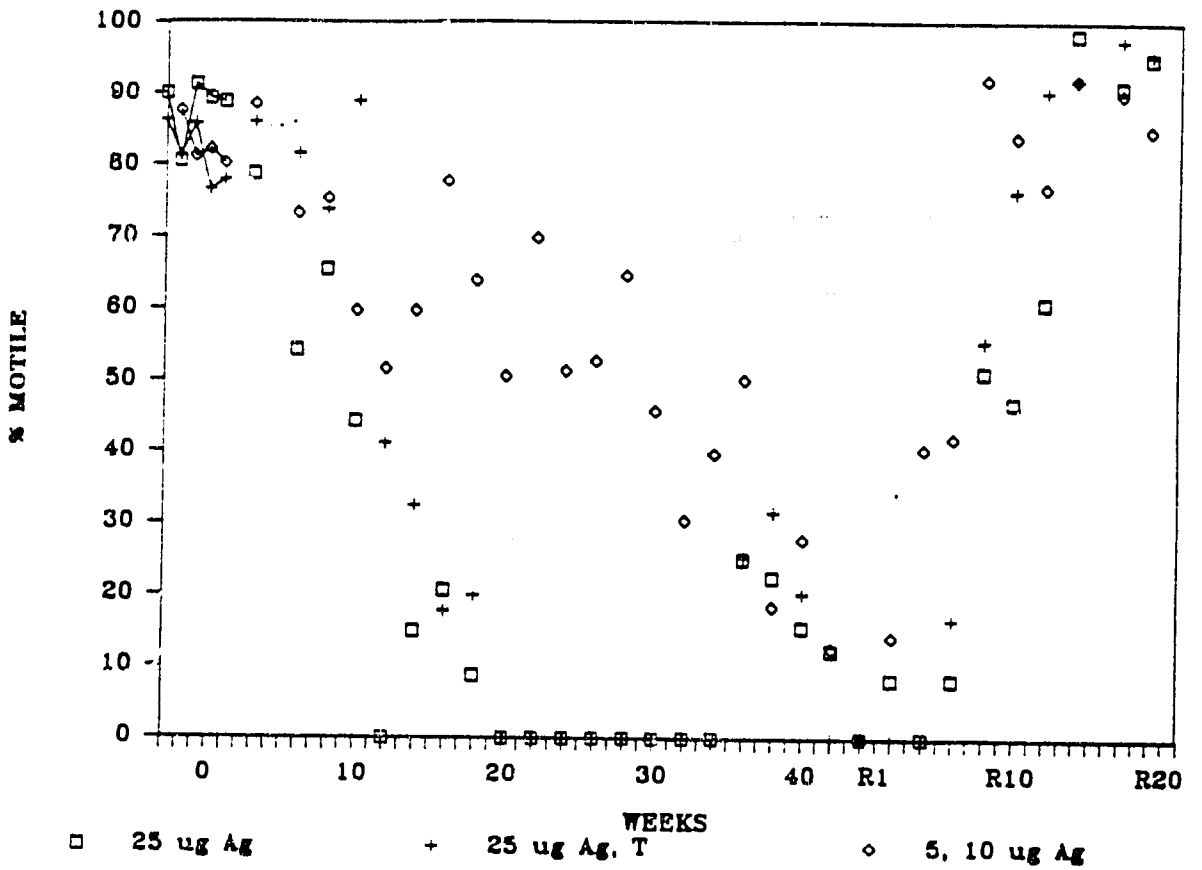


Figure 14. Mean percent live sperm in ejaculate in monkeys treated with 5-10 ug Ag/day, 25 ug Ag/day and 25 ug Ag/day and T.

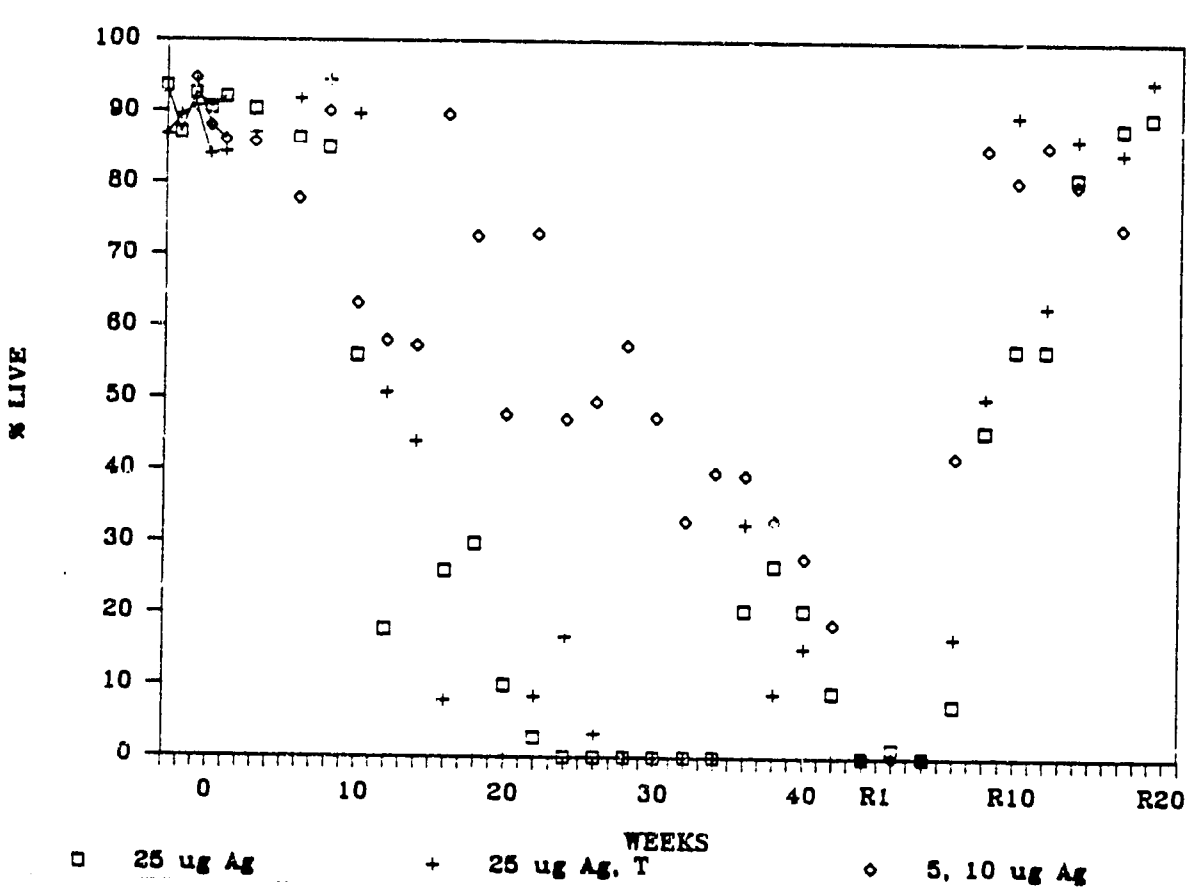


Figure 15. Influence of Ag and T treatment on the net surface charge of spermatozoa. Net surface charge was measured indirectly subsequent to attachment of positive-charged colloidal iron to the surface of the sperm.

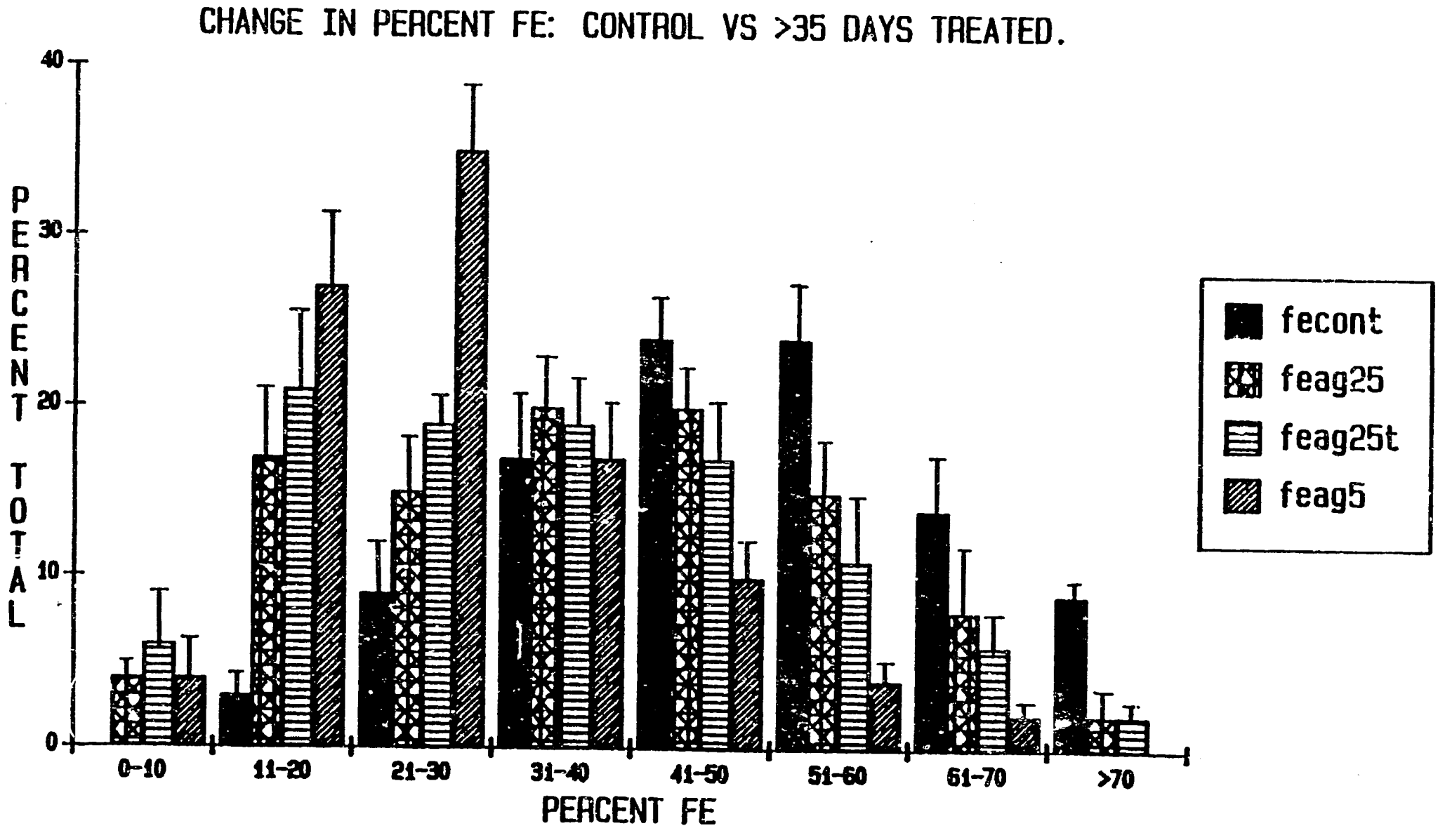


Figure 16. Percent of hamster ova penetrated by sperm from individual monkeys before, during and after treatment with 25 ug Ag/day for 44 wk.

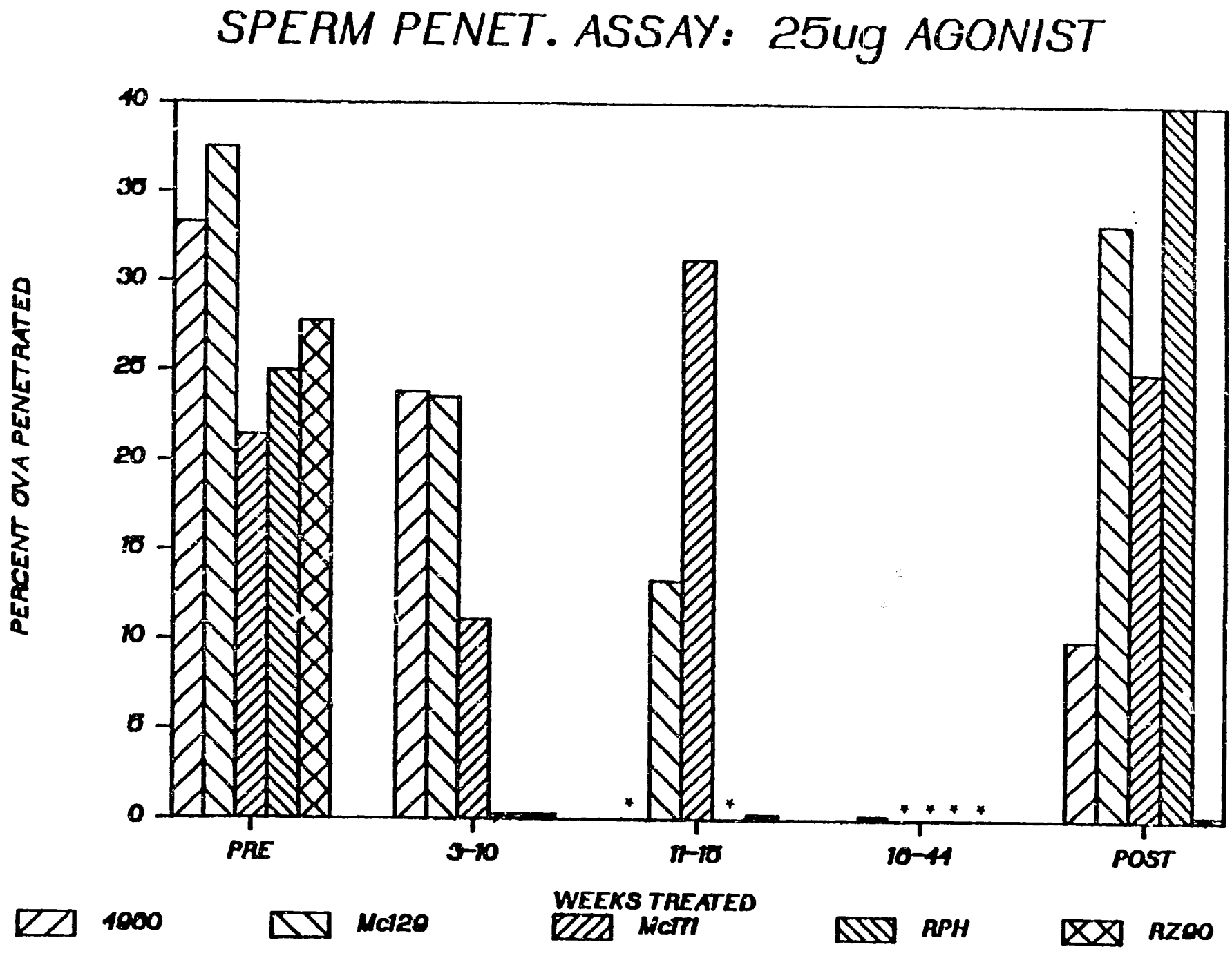


Figure 17. Percent of hamster ova penetrated by sperm from individual monkeys before, during and after treatment with 25 ug Ag/day and T for 44 wk.

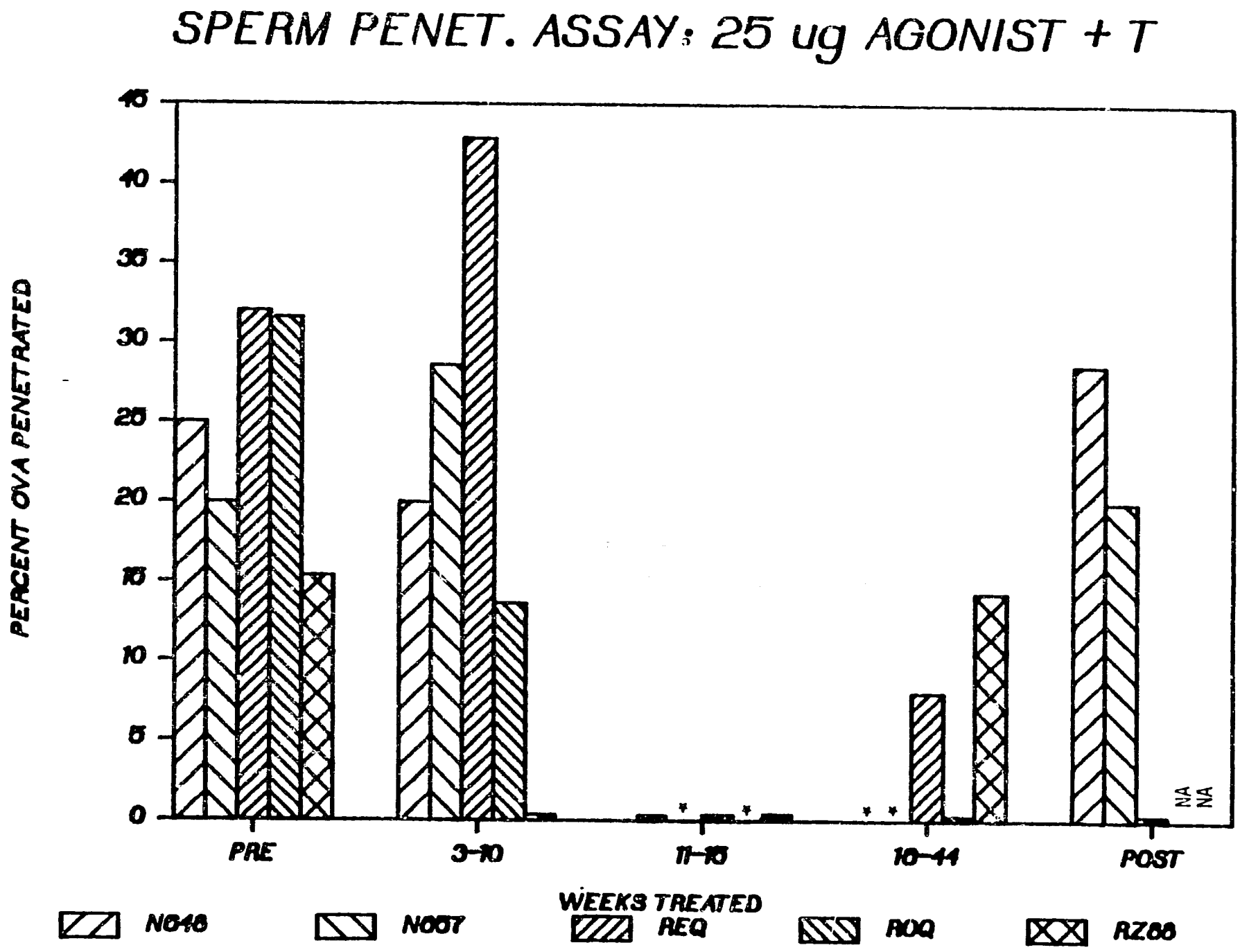
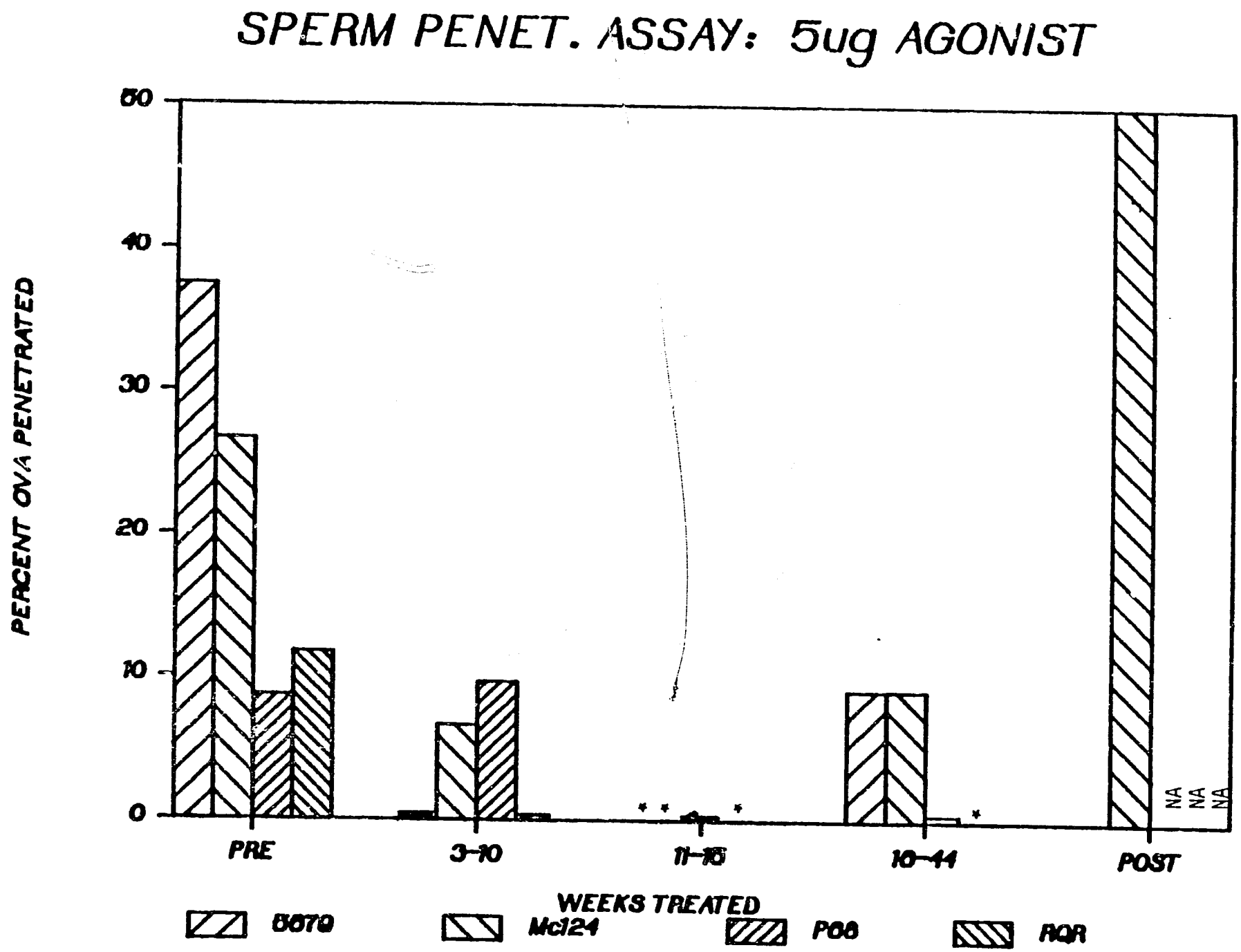



Figure 18. Percent of hamster ova penetrated by sperm from individual monkeys before, during and after treatment with 5-10 ug Ag/day for 44 wk.