

THE EFFECTS OF TETRATHIOMOLYBDATE (TTM, NSC-714598) AND COPPER SUPPLEMENTATION ON FERTILITY AND EARLY EMBRYONIC DEVELOPMENT IN RATS

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ABSTRACT

Based on its unique anti-copper action, TTM is being studied as an antiangiogenic agent and is currently in Phase II clinical trials for cancer therapy. The purpose of the present study was to evaluate the toxicity of TTM and the protection of copper supplementation on the reproductive capability of male and female CD⁰ rats. Doses of 0, 1, 4, 12 mg/kg/day (0, 6, 24, 72 mg/m²/day) and 12 mg/kg/day with copper supplementation (110 mg/kg of diet) were given orally by daily gavage to male rats for at least 59 days and to pregnant female rats for about 30 days, including 29 and 15 days, respectively, of dosing prior to cohabitation. There were no drug-related toxic effects on the estrous cycle or reproductive indices, or maternal toxicity in any female rat. Therefore, the no observed effect level (NOEL) for female reproductive toxicity was at least 12 mg/kg/day (72 mg/m²/day). Male rats given 12 mg/kg/day showed significant decreases in body weight gains and food consumption and drug-related anemia developed primarily toward the end of the study. Serum ceruloplasmin levels were dose-dependently decreased in all drug-treated male rats. At 12 mg/kg/day, rats in the copper supplemented dose group had serum ceruloplasmin levels 16 times higher than the nonsupplemented dose group. TTM given at doses of 12 mg/kg/day resulted in reduced epididymal weights, sperm counts, and sperm motility as well as sperm morphologic abnormalities including excessive sperm fragmentation and histopathologic changes in testis and epididymis. None of these drug-related effects occurred in the 1 and 4 mg/kg/day dose groups. Copper supplementation prevented the adverse sperm effects produced by 12 mg/kg/day of TTM. Therefore, the NOEL for male reproductive toxicity was 4 mg/kg/day (24 mg/m²/day). (Supported by NCI Contract No. NO1-CM-87103.)

INTRODUCTION

Ammonium tetrathiomolybdate (TTM) is a powerful copper chelating agent, which is successfully used for the treatment of the Wilson's disease for several years (Brewer *et al.*, 1991; 1994; 1999). Because copper is a required cofactor for the function of many key mediators of angiogenesis, Brewer and co-workers (Brewer *et al.*, Brewer, 2001) developed an antiangiogenic strategy of TTM usage for the treatment of cancer. It is currently in Phase II clinical trials for cancer therapy.

The objective of this study was to determine the toxic potential of TTM and the protective effect of copper supplementation on the reproductive capability of male and female CD⁰ rats. The scope of the study encompassed gonadal function, estrous cycles, mating, conception, implantation and early embryonic development.

These studies were conducted under NCI Contract NO1-CM-87103 to support the RAID application 086 entitled "Development of Tetrathiomolybdate as an Antiangiogenic Agent for Cancer Therapy and Prevention" (Principal Investigator: Dr. Sofia Merajver, University of Michigan).

STUDY DESIGN

25 male and 25 female CD⁰ Rats / dose level

Doses: 0, 1, 4, and 12 mg/kg/day (0, 6, 24, and 72 mg/m²/day) and 12 mg/kg/day with copper supplementation

Tests:

Morbidity/mortality, body weights, food consumption, hematology parameters, serum ceruloplasmin levels

Reproduction Assessment

C-Section

- Corpora Lutea
- Number of implantation sites
- Early and late resorptions
- Viable and Non-viable fetuses

Sperm Assessment

- Motility
- Sperm counts
- Morphology
- Histopathology
- Organ weights (Testes, Epididymis, Seminal vesicles and Prostate)

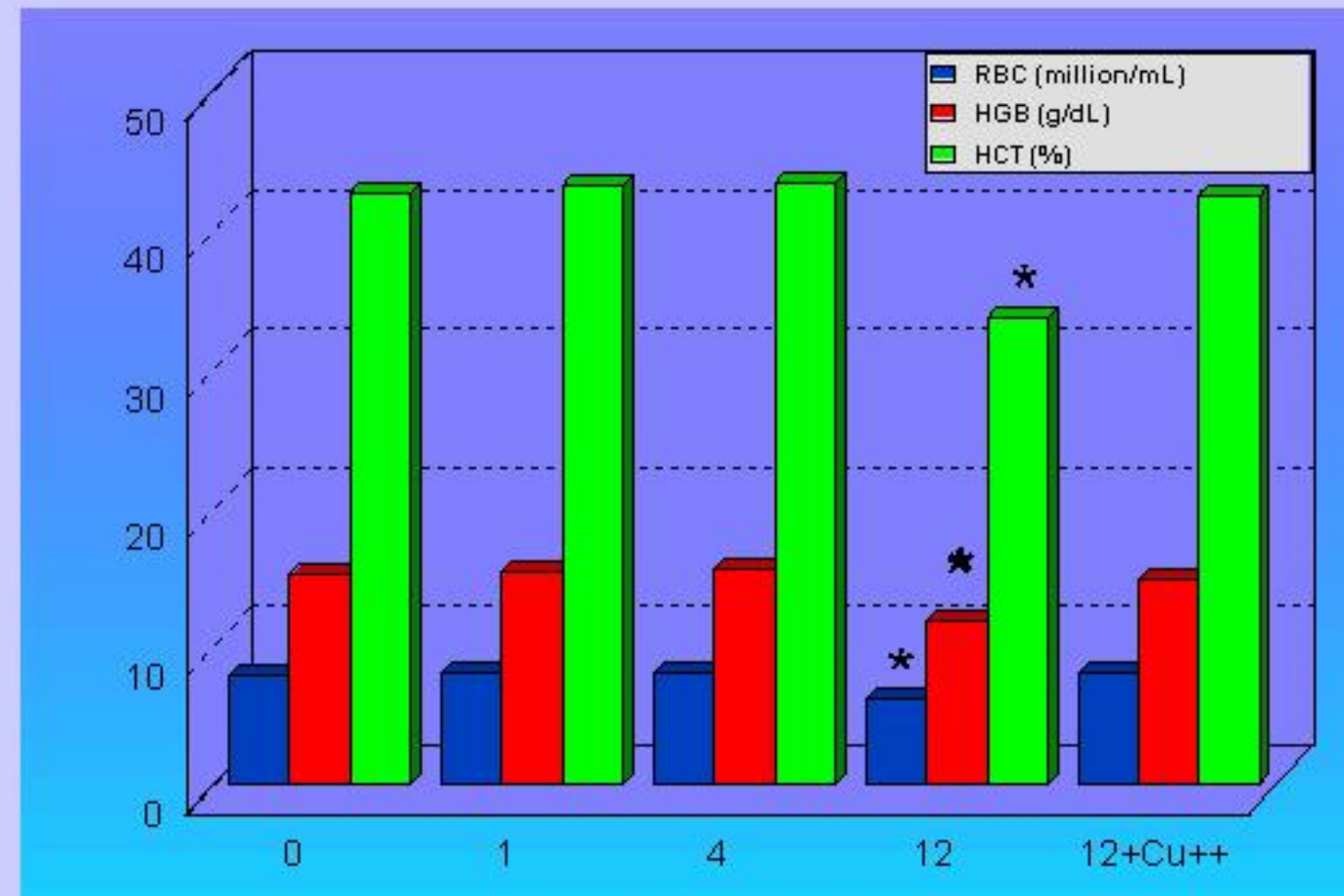
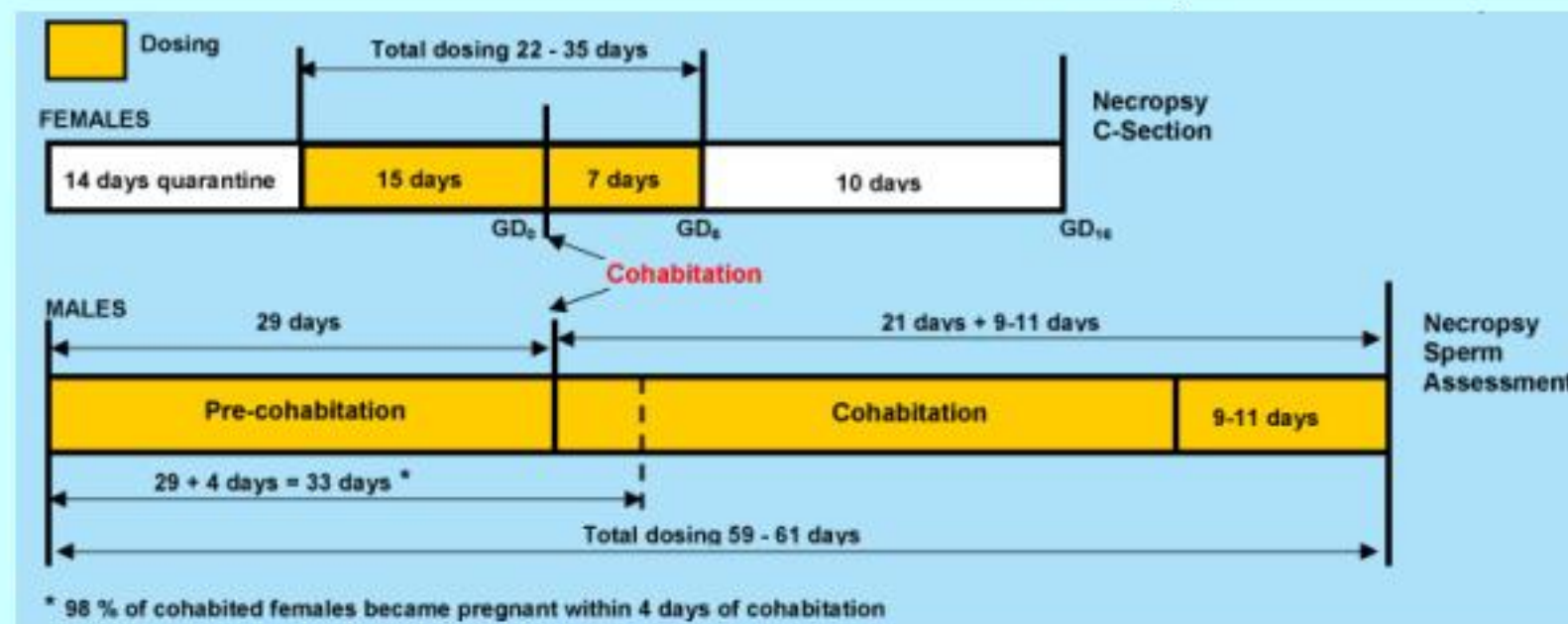


Fig. 2 Hematology Parameters of Male Rats Dosed with TTM
* Significant Difference from Control P < .05
In the 12 mg/kg/day dose group, erythrocyte counts, hemoglobin concentration and hematocrit were significantly decreased. The anemia was mild as neither MCV, MCH nor MCHC were affected. No drug-related changes in hematology parameters were seen in any other dose group, including the copper supplemented 12 mg/kg/day dose group.

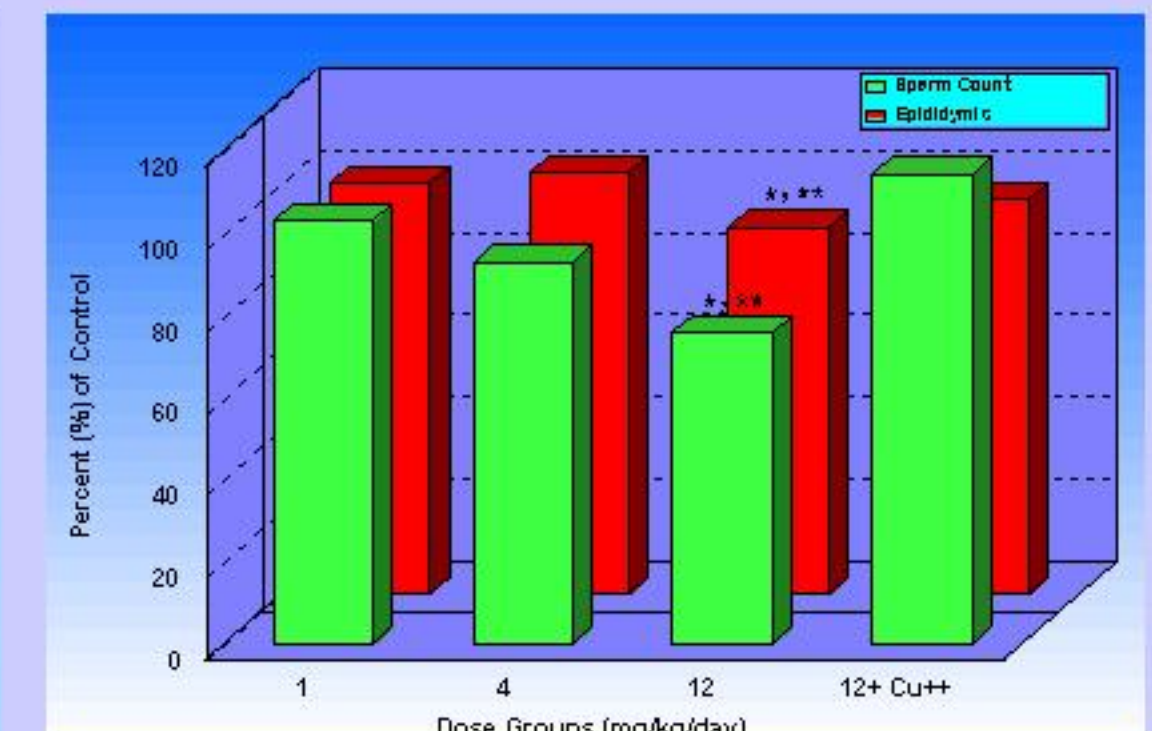


Fig. 4 Male Reproductive Parameters in Rats Dosed by Oral Gavage with TTM for 2 Months
* Significantly different from control group (p less than 0.05)
** Significantly different from the copper supplemented group (p less than 0.05)

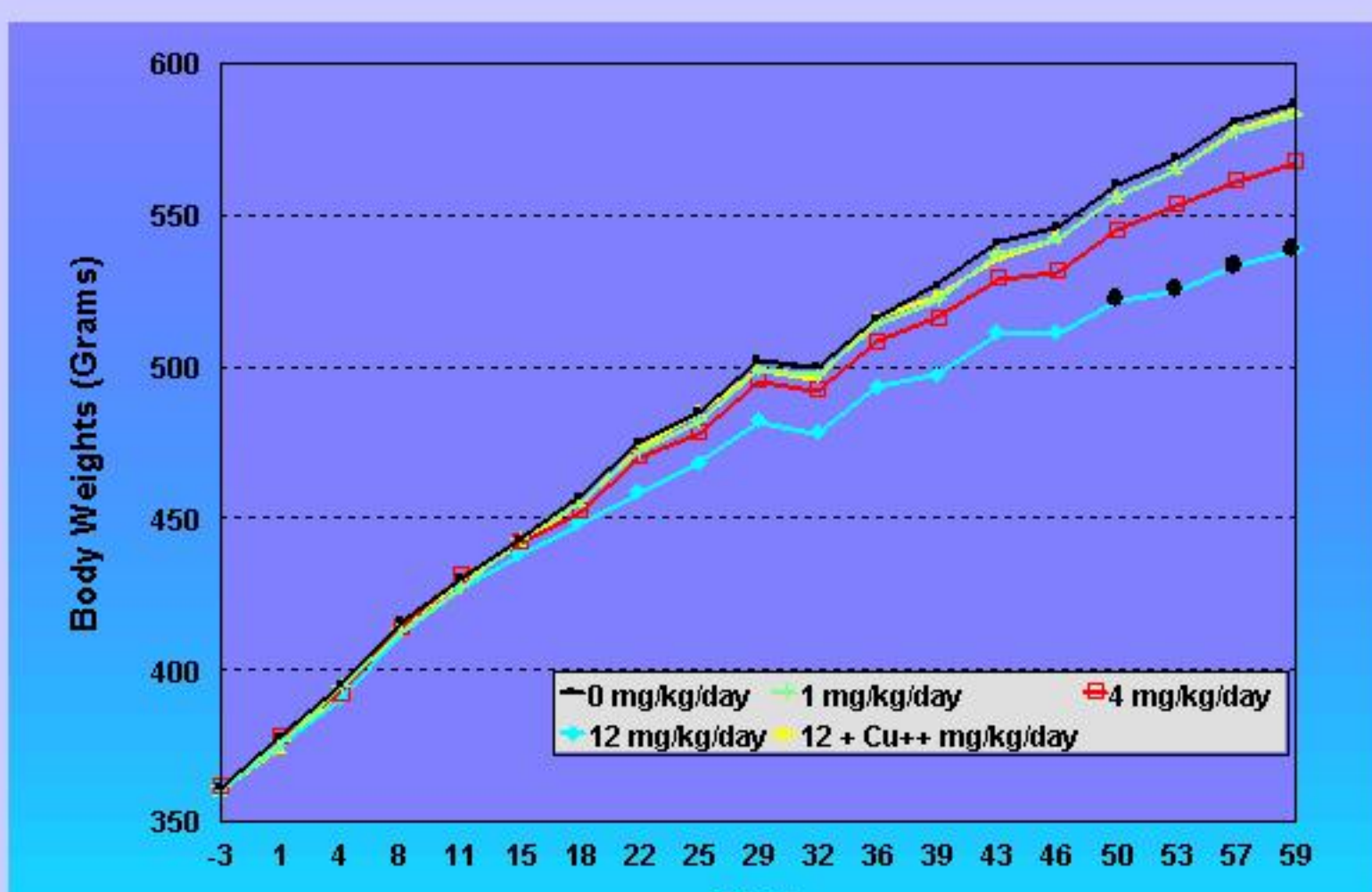
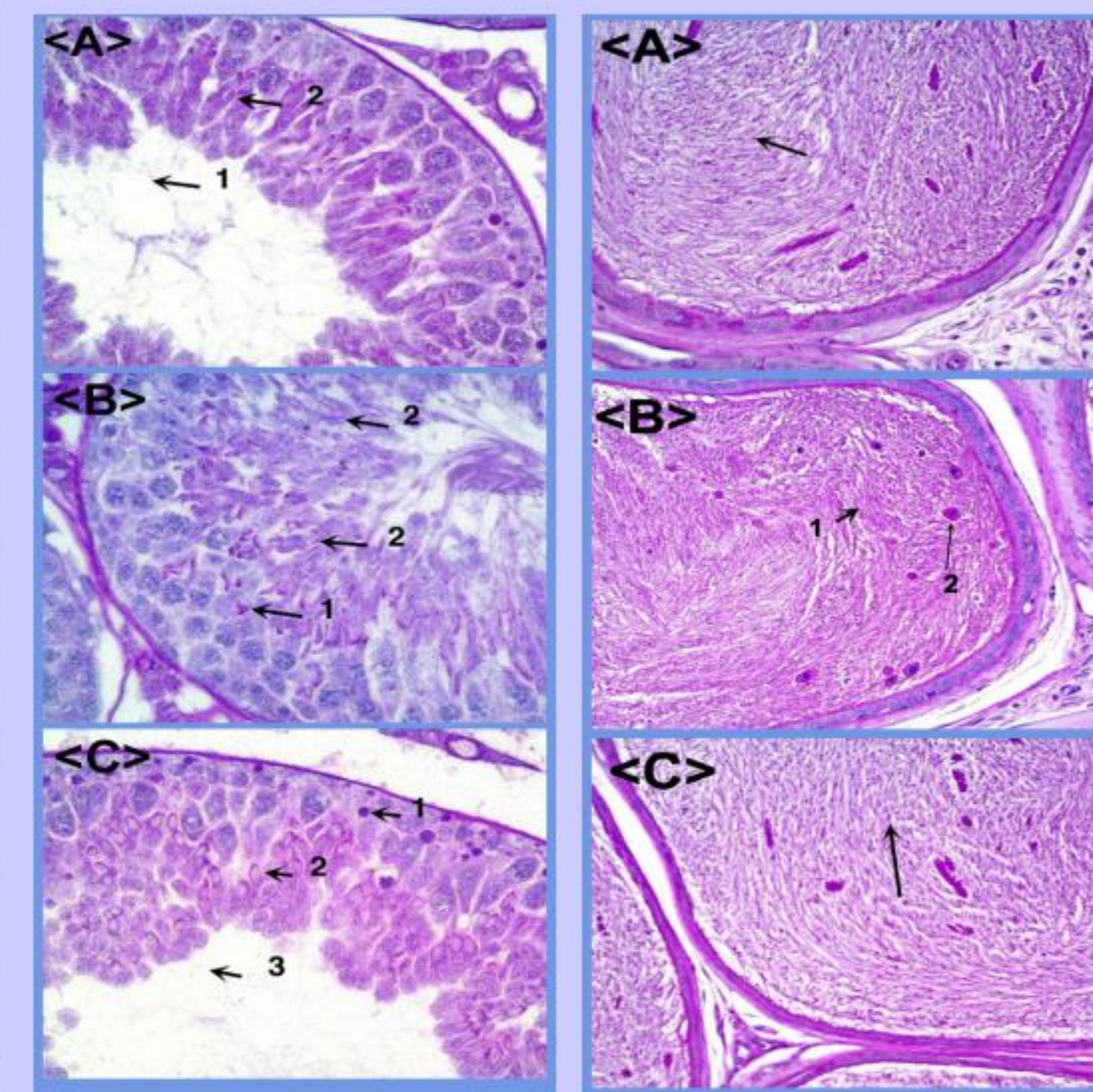


Fig. 1 Body Weights of Male Rats Dosed with TTM for 2 Months
• P less than .05

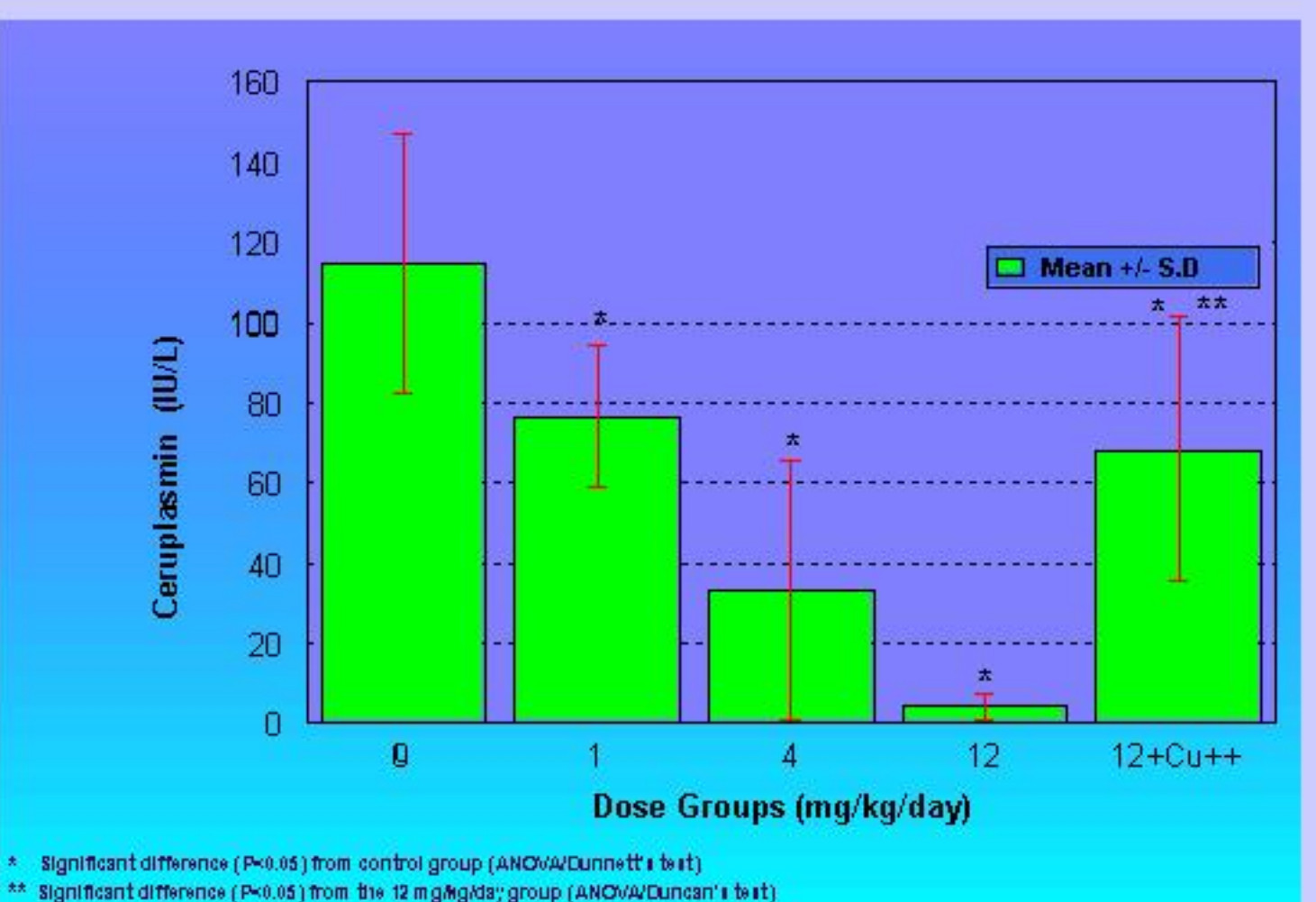


Fig. 3 Serum Ceruloplasmin Levels in Male Rats Administered TTM for 2 months
* Significant difference (P<0.05) from control group (ANOVA/Duncan's t test)
** Significant difference (P<0.05) from the 12 mg/kg/day group (ANOVA/Duncan's t test)

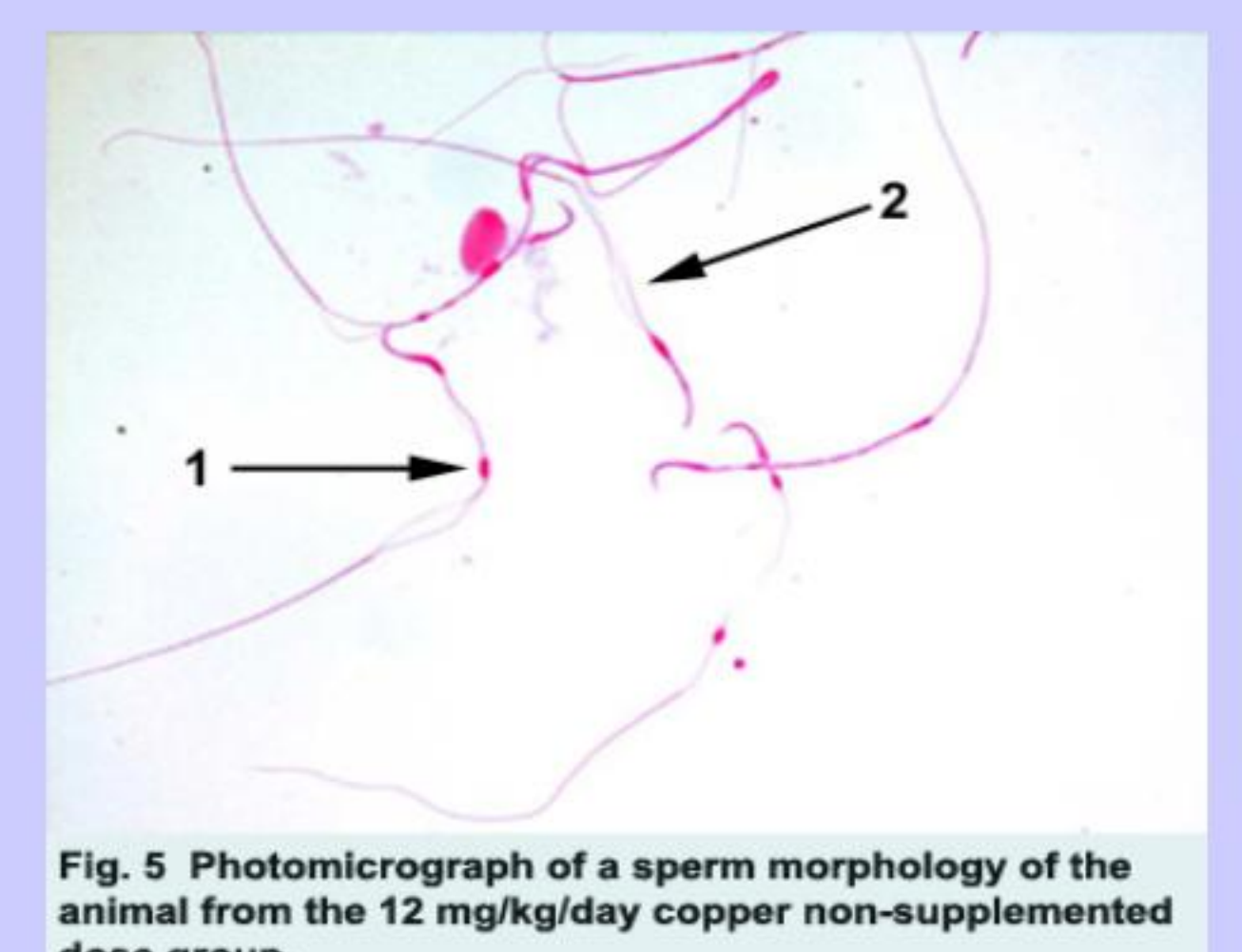


Fig. 5 Photomicrograph of a sperm morphology of the animal from the 12 mg/kg/day copper non-supplemented dose group.

SUMMARY

- ### Females
- No maternal toxic effects, effects on the estrous cycle, or effects on reproductive indices
- ### Males
- Significant toxic effects (decreases in body weight gains, food consumption, erythrocyte counts, hemoglobin concentration and hematocrit) only in the 12 mg/kg/day dose group
 - Serum ceruloplasmin levels were dose-dependently decreased in all dose groups
 - Significant reproductive toxicity at 12 mg/kg/day (reduced epididymal weights, reduced sperm counts, reduced sperm motility, specific sperm morphologic abnormalities, and histopathologic changes in testis and epididymis)
 - Copper supplementation administered with 12 mg/kg/day TTM prevented the adverse systemic and sperm effects
 - Copper plays an important role in maintenance of the sperm quality