Effect of intravasal copper on the fertility of rats

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Summary. Copper wire was inserted into the vas deferens and its effect on the reproductive system and fertility performance of rats was investigated. The copper wire was 100% effective as a contraceptive for up to 4 months if placed correctly, and resulted in decapitation of most of the spermatozoa. No differences between the rats with an intravasal copper wire and the sham-operated controls were found for the weights of the gonads and accessory sex glands or for protein, RNA, DNA and fructose concentrations. The intravasal copper device appears to be promising for the development of a long-term method for male contraception.

Introduction

A number of methods have been investigated to achieve a safe and effective measure for limiting male fertility. The majority of attempts have been directed towards the interruption of the passage of spermatozoa by blocking the excurrent ducts by ligation (Swanson & Hafs, 1969; Sackler, Weltman, Pandhi & Schwartz, 1973), silastic blocks (Hrdlicka, Schwartzman, Hasel & Zinsser, 1967; Laumas & Uniyal, 1967), tantalum or vanadium clips (Jhaver, Joseph, Lee, Aulka & Leight, 1971; Moss, 1971; Moss, 1972), intravasal thread (Lee, 1969), chemicals (Freeman & Coffey, 1973) or by vasectomy. Vasectomy is considered to be the most effective method for male sterilization, but the difficulties in restoration of fertility by recanalization and some of the after-effects have affected its acceptability by men. There is therefore a need for a male contraceptive which is reversible and free from after-effects. A number of investigators (Loewit, 1971; Oster, 1972; Jecht & Bernstein, 1973) have shown that copper inhibits the motility and metabolic activity of human spermatozoa *in vitro*. The adverse effects of metallic copper on the survival and ability of spermatozoa to penetrate the cervical mucus have also been demonstrated (Kesseru & Camacho-Ortega, 1972; Ullman & Hammerstein, 1972). The toxicity of copper ions to spermatozoa is believed to be responsible for the effectiveness of copper intrauterine devices (Tatum, 1973), but there has been little study of the direct contraceptive effect of copper in the male reproductive tract. We therefore investigated the contraceptive effectiveness of a metallic copper device inserted in the vas deferens of rats with a view to developing a reversible method for male contraception.

Materials and Methods

Animals

Mature male albino rats (from the Departmental colony) of proven fertility, weighing 100–150g and approximately 3 months old were used in this study. The animals were maintained under normal husbandry conditions. The rats in Group I consisted of 30 animals which were fitted with an intravasal copper device and Group II contained 20 sham-operated animals which served as controls.

For insertion of the device, the animals were anaesthetized with ether and, using sterile conditions, each vas deferens was exposed through a small midline incision in the lower abdomen. A 26-gauge hypodermic needle, loaded with copper wire (0.1 mm diameter; Phelps & Dodge Copper Products Corp., Fort Wayne, Indiana, U.S.A.), was introduced into the lumen midway along the vas and re-

moved so that a 1.5 cm length of the wire was left in the vas lumen. The protruding ends of the wire (1–2 mm) were either bent at approximately 45° to form a shape like a staple pin or coiled into two small knobs.

Fertility studies

After insertion of the wire, the males were placed with 2 or 3 pro-oestrous females for a test period of 2 weeks at a time. Separate groups of animals were allocated for mating at 4–6 weeks, 7–9 weeks, 11–13 weeks or 15–17 weeks after insertion. Mating was confirmed by finding spermatozoa in the vaginal smear or a vaginal plug, and this day was designated Day 1 of pregnancy. On Days 10 or 11 *post coitum* each female was subjected to laparotomy and the number of females with implantations was recorded. When pregnancy was confirmed in females mated with Group I males, these males were killed and examined to see whether the copper wire was still present and what its position was.

After 4 months, 10 animals from each group were killed and each testis, epididymis, vas deferens and accessory organs was dissected free of fat, and weighed on a torsion balance.

Sperm morphology. The vas deferens of one side was divided into 3 segments: (1) the part proximal to the copper wire, i.e. nearest the epididymis; (2) the segment bearing the copper wire; and (3) the portion distal to the copper wire, i.e. the urethral end of the vas. Sperm suspensions were prepared by washing each of the three segments of vas in 0.5 ml 0.9% (w/v) NaCl. Comparable portions of tubules were also taken from the cauda epididymidis, minced and then suspended in 0.5 ml 0.9% NaCl. The only abnormality found was the decapitation of spermatozoa, and the numbers of normal and decapitated spermatozoa in the four regions of the tract were estimated by using a Neubauer haemocytometer chamber.

Estimation of protein, RNA, DNA and fructose. Tissues from the testis, epididymis, vas deferens, seminal vesicle and prostate were weighed before estimation of the protein content (Lowry, Rosebrough, Farr & Randall, 1951), RNA by the orcinol colour reaction (Glick, 1958), DNA (Burton, 1956) and fructose in the coagulating gland tissue (Roe, 1934).

Results

Fertility performance

The fertility performance of rats during the test period after treatment is summarized in Table 1. The results have been pooled as the period elapsed after insertion had no effect on the frequency of mating. Live young were born to 6 of the 7 pregnant females mated with 3 Group I males. Autopsy of these males showed that in 2 the copper wire was not in the lumen but in the muscular layers of the vas, and in the third there was no wire on one side.

Table 1. Effect of intravasal copper on fertility of male rats

	No. of males	No. of known matings/females tested	No. of females with implantations	No. of infertile males	% fertility inhibition in males
Group I (intravasal copper, 1–4 months)	27	41/66	7*	24†	100
Group II (sham-operated controls)	18	34/52	29	0	0

* Females mated with 3 males in which the copper wire was found to be misplaced.

† Excluding the 3 males with misplaced devices.

Organ weights and chemical estimations

There were no differences between the two groups for any of the parameters studied.

Sperm morphology

The number of spermatozoa in the distal segment of the vas deferens was only 50% of that in controls ($41.6 \times 10^6/\text{ml}$ compared with $83.5 \times 10^6/\text{ml}$). In the cauda epididymidis of the treated rats there were 5.9% decapitated spermatozoa compared with 5.4% in the control animals.

In the epididymal portion of the vas 7.09% of spermatozoa were decapitated (5.5% in the controls), while in the middle and urethral segments incidence of decapitation was markedly increased to 87.8% and 83.6% respectively compared with 5.3% and 4.8% in the control animals.

Discussion

The results presented in this paper show that an intravasal copper wire in the rat inhibited fertility completely without producing any deleterious effects on the accessory organs of the reproductive tract during the 4-month period of study. Zbuzkova & Kincl (1971) found that a 0.5 cm loop of copper wire in the vas deferens of rats produced only a transient reduction in their fertility and that most animals were fertile 1–2 months after insertion. As in the present study, no effects on the accessory sex organs were found. The effect of the copper wire on fertility in our studies may have been because more copper was present in the vas lumen. Copper wire inserted in the testis (Zbuzkova & Kincl, 1971) and seminal vesicles (Gilmore, Hooker & Davis, 1973) of rats produced 98% inhibition of spermatogenesis and a 91% reduction in fertility, respectively. However, neither of these approaches are practical compared with insertion in the vas deferens.

The contraceptive effectiveness of intravasal copper may be due to a toxic effect on the spermatozoa (Loewit, 1971; Oster, 1972; Jecht & Bernstein, 1973). Metallic copper inhibits the motility of human spermatozoa in 20–240 min (Kessuru & Camacho-Ortega, 1972), and copper is more toxic to rat and dog epididymal spermatozoa than is cadmium (Saito, Bush & Whitmore, 1967). Maynard, Elstein & Chandler (1975) have shown that incubation of human semen with metallic copper wire causes a significant decrease in zinc content of the spermatozoa and this may be important for sperm motility. We assume that the decapitation of almost all spermatozoa in the middle and the distal segments of the vas in our experiments has occurred after immobilization and death of the spermatozoa.

The use of an intravasal silk thread for control of fertility has been investigated by Lee (1969) and appears to act by blocking sperm passage in the vas. Injections of liquid silastic (Hrdlicka *et al.*, 1967; Laumas & Uniya, 1967) and various chemicals such as ethanol and formaldehyde also result in blockage of the duct. In our experiments, the patency of the vas deferens was confirmed by the presence of spermatozoa in the vaginal smears although the number was affected. Using the present approach it may be possible to restore fertility by the simple removal of the copper wire from the duct, and studies are in progress to test this in the rat as well as the efficacy of the approach in the rhesus monkey.

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