

# FLUID COLLECTED FROM AN EXPERIMENTALLY PRODUCED HYDROUTERUS AND SOME PROBLEMS OF REPRODUCTION<sup>1</sup>

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## INTRODUCTION

In the past two decades there has been a growing interest in the uterine secretions, their nature and their roles in reproduction. The discovery that large quantities of fluid could be collected by producing an experimental hydrouterus by ligation of the uterine cervix has greatly facilitated these studies.

There are some investigators who believe that this fluid is an ultrafiltrate of plasma supplemented by uterine secretions. (1) Others hold that this fluid is entirely a product of the secretory endometrium.

The number of reports on the composition of this fluid is increasing. Ringley (2) states that the potassium concentration was ten times greater than plasma values, that activities of beta glucuronidase and alkaline phosphatase were greater than those observed in plasma, and that acid phosphatase was absent. He also studied the protein components and observed that proteins having electrophoretic mobilities similar to beta, alpha 1 and 2 globulins and albumin of plasma were present. He also observed a protein component with a mobility exceeding that of albumin.

Albers and Castro (3), employing the immunoelectrophoretic and Ouchterlong Gel Diffusion techniques, found that proteins having mobilities of beta, alpha 1 and 2 globulins were present. They also observed a protein fraction with a mobility exceeding that of plasma albumin but noted that gamma globulin was absent.

The purposes of this experiment were four-fold: to produce a state of hydrouterus in the rat by ligating the uterine cervix; to attempt to maintain pregnancy in ovariectomized rats; to attempt to accomplish *in vitro* fertilization and transfer to recipient females; and to observe the development of the fertilized ova using uterine fluid as the medium.

## MATERIALS AND METHODS

Sexually mature male and female rats of the Sprague-Dawley strain were used. The uterine cervix was ligated in ten females and the fluid allowed to accumulate over a period of 10 days to 6 months. After this period of time, the uteri were exposed through an abdominal

incision and the fluid removed and stored at 35° C for further use.

Five female rats were mated with five male rats and copulation was determined by vaginal smears. The ovaries were removed from these animals at 2-10 days after copulation daily injections of 0.5, 0.75, 1.0, 1.5, and 1.0 cc. of uterine fluid were given intraperitoneally in two equal doses at 12 hour intervals. Vaginal smears were begun six hours after bilateral ovariectomy and continued daily. The animals were autopsied on the 5th and 10th post operative day. Portions of the uterine horns were removed and prepared for histologic study.

Ova were collected by flushing the uterine horns and tubes with 0.9% saline and later by use of uterine fluid. Sperms were obtained by cutting epididymi into small pieces in 0.9% saline and uterine fluid. The ova and sperm were incubated in uterine fluid at 37° C for 3-6 hours and transferred to the uterine horns of four recipient females in estrus and metestrus. These vaginal smears and weighings were done daily and the animals autopsied at five to ten days.

In the final phase of the experiment, ova and sperms were collected by the same methods as above but were observed at hourly intervals for a period of 6-12 hours for evidence of sperm penetration and cleavage.

## RESULTS

Of the 10 animals in which ligation of the cervix was performed, seven developed a hydrouterus. The uterine horns were markedly distended in those animals in which fluid had accumulated for 3-6 months. Three of the seven animals had developed a pyouterus, and the fluid was purulent and contained numerous leukocytes. In the remaining four, the fluid was clear and contained an occasional lymphocyte and many ova. As much as 75 cc. of fluid was removed from one animal.

In the three animals that had developed a pyouterus, tumor of the ovaries was detected. One had a serious-like cyst in one ovary which measured 5-8 mm. Another had a solid tumor of the ovary which measured 3-6 mm. Another had bilateral hemorrhagic cysts. Histologic examination of the solid tumor mass showed that this was an abscess.

Pregnancy was not maintained in any of the five ovariectomized animals receiving uterine fluid. Six hours

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after ovariectomy the vaginal smear was bloody and remained so for two days. At autopsy the fetuses were markedly reduced in size in the animals in which ovariectomy was performed 6-10 days after coitus, and only the placental site remained in others. Sections through placental sites showed a mass of necrotic tissue at microscopic examination.

The use of saline to secure motile spermatozoa was unfruitful; therefore, the use of uterine fluid was resorted to. This fluid turned out to be an excellent medium for obtaining spermatozoa from sliced epididymus.

The attempts to obtain in vitro fertilization and successful transfer to recipients were unsuccessful. Sperm penetration was observed in only one instance and this was doubtful, since it appears that after the sperm had reached the zona pellucida, it suddenly retracted its head. Cleavage was not observed.

#### DISCUSSION

Homburger et al (4) in a study of experimental hydrouterus in rats, mice and guinea pigs showed that from 33 to 50% of the animals developed hydrouterus. In a later study they showed that 98% of the mice whose cervixes were ligated developed a hydrouterus and showed that in the remainder cervical ligation was inadequate. In our experiment inadequate cervical ligation can be blamed for not producing hydrouterus in all of the animals.

The ovarian tumors that developed were probably retention cysts, due to the high pressure in the uterine

tubes and uterine horns produced by the distended uterine horns. It may be significant to note that these cysts only developed in the animals that had developed a pyouterus.

Our attempt to maintain pregnancy in castrates with uterine fluids was unsuccessful. However, we feel that the amounts of fluid given were inadequate for such an attempt, even though in all probability this fluid would not maintain pregnancy in castrates. Much larger doses should be tried, however, before we draw this conclusion.

We failed in our attempt to accomplish in vitro fertilization and to observe sperm penetration and cleavage. We do feel, however, that this fluid offers an excellent medium for such experimentation and that with better techniques and equipment some positive results might be obtained. Some observers have reported that this fluid and the condition of hydrouterus was an excellent medium for tumor transplants. Our investigation was too limited for us to draw any conclusions other than that a hydrouterus can be produced by ligation of the cervix and that this fluid offers a natural medium for further scientific investigation of the reproductive processes.

#### LITERATURE CITED

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