

as in the specimens from sham-operated rats, cytoplasmic ribonucleic acid (as shown by staining with methylene blue) was generally in the form of dense clumps, in 3 of the hepatectomized rats it was diffusely distributed throughout the cytoplasm as a delicate stippling. This pattern seemed to be correlated with the presence of enlarged parenchymal cells and variability in nuclear structure. Some nuclei were enlarged and often contained either multiple nucleoli or hypertrophied nucleoli of variable shape. In one case, parenchymal cells were extensively vacuolated, indicating that fat had accumulated. The glycogen content did not follow a consistent pattern, in at least 3 cases being comparable in amount to that in the sham-operated controls.

Histologic study of the livers of the rats which died or were killed before termination of the experiment confirmed the frequency of infection in these rats by the presence of abscesses in the fibrotic scarred areas and by

mononuclear cellular infiltration into the connective tissue of the portal canals of some rats. Otherwise the histologic changes varied only in degree from those described for the rats which lived throughout the experiment.

In no case was histologic evidence of neoplasia observed.<sup>†</sup>

**Summary.** The capacity of liver to regenerate was maintained in rats which were subjected to partial hepatectomy 12 times within a period of one year. At the end of the experiment, only minor cytological changes were observed in the regenerated liver and there was no neoplasia.

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## Intraperitoneal Insemination of the Heifer.\* (23372)

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Anatomically, there is no barrier to prevent sperm from reaching the ovary following intraperitoneal injection of semen in species such as the cow, ewe, mare(1), rabbit(2), and to some extent the sow(1) whereas bursae such as those of the rat(2), bitch(1), and mouse(3) could be a barrier. Physiologically, little is known of the compatibility of the peritoneal surfaces and fluid with sperm. Brown(4) found peritoneal fluid of the human being compatible with living sperm. Van Drimmelen(5) obtained fertilization in the chicken following intraperitoneal insemina-

tion. Black *et al.*(6) failed to obtain fertilization when 9 superovulated calves were inseminated intraperitoneally. Skjerven(7) recently reported conception in a heifer following intraperitoneal deposition of semen by puncturing the vaginal wall.

It is the object of this paper to report the results of intraperitoneal insemination of heifers.

**Materials and methods.** Four sexually mature Holstein-Friesian heifers were inseminated a total of 6 times near the end of estrus by injection of Holstein-Friesian semen, in some cases diluted, in other cases undiluted and of variable quality, into the peritoneal cavity by puncturing the abdominal wall in the region of the right paralumbar fossa with a 16-gage, 4 inch hypodermic needle. An in-

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TABLE I. Pregnancy in the Heifer following Intraperitoneal Insemination.

| Heifer | Day of insemination | Semen                        | AP extract (ml) | Pregnancy |
|--------|---------------------|------------------------------|-----------------|-----------|
| 146    | 4/24/53             | Undiluted, 2 ml, 12 hr old   | none            | no        |
| 145    | 4/28/53             | Diluted, 2½ ml, 1 day old    | "               | "         |
| 826    | 5/ 8/53             | Undiluted, 1½ ml, 12 hr old  | 2               | yes       |
| 148    | 5/10/53             | Undiluted, 5½ ml, 2 days old | 2               | no        |
| 146a   | 5/12/53             | Diluted, 1 ml, 1 day old     | 2               | "         |
| 145a   | 6/ 8/53             | Diluted, 2 ml, age unknown   | none            | "         |

travenous injection of anterior pituitary extract was given to 3 heifers at the time of insemination. Periodic rectal palpations of the uterus were made following insemination to determine if conception occurred. Bulls were not kept on the experimental farm or on adjacent farms during the experiment. The heifers were exercised in a small pasture and observed daily.

**Results.** One of 6 intraperitoneal inseminations was followed by pregnancy. The gestation length of 275 days is within one standard deviation of the mean gestation length for the breed(8).

**Discussion.** Experimental insemination of the mammal by the intraperitoneal route offers a method of studying the processes leading to fertilization. Chang(9) found an aging process of sperm while transgressing the female tract necessary to induce "fertilizing capacity" in rabbit sperm. In contrast to this, Runner(2) injected sperm into the ovarian bursae of mice, by-passing sperm migration through the uterus, and obtained con-

ception. Data reported herein indicate that sperm may not need to transgress the tubular genital tract of the cow in order to be capable of fertilization; although it is possible, in our experiment, that fertilization occurred in the tubular genital tract after the fertilizing capacity of the sperm had developed. The peritoneal surface and fluid permitted sperm viability in at least one animal, indicating compatibility.

Injections of anterior pituitary extract were made in order to hasten ovulation as demonstrated by Marion *et al.*(10). A shortened insemination ovulation interval should encourage fertilization if the sperm were weakened by an abnormal environment.

**Summary.** Intraperitoneal insemination of 4 heifers a total of 6 times resulted in one pregnancy. This experimental method is offered as a means of studying the physiological processes leading to fertilization.

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