Intraperitoneal Insemination of Rabbit Doe.* (24238)

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While trying to develop a suitable technic for artificial insemination of rabbits, the intraperitoneal route offered itself as a possibility. The rabbit is a conveniently suited animal for this type of work, since ovulation takes place 10 hours after insemination(5). To check feasibility of the technic some rabbits were administered one cc India ink into the peritoneal cavity. This mostly disappeared after one hour and substantial amounts were recovered in the oviducts.

Technics. In this pilot study started in 1957, doe rabbits were mated to vasectomised bucks and 1 to 12 hours after mating they were inseminated through the linea alba with freshly ejaculated rabbit semen. The semen was collected from suitably trained bucks with the help of an artificial vagina, which in turn is a modification of Macirone-Walton's (6) old model. The semen, after having been tested for motility, was either injected immediately, or was treated in one of 3 different ways. (1) Diluted to double its amount, (2) centrifuged, the supernatant fluid discarded and the semen resuspended in 1 cc sodium citrate buffer at pH 7.4, or (3) the resuspended semen was centrifuged again and after discarding this supernatant, newly resuspended in 1 cc citrate buffer (2x washed). Only semen which had shown good motility was used. The procedure adopted was: To suspend females by their hind legs and inject the semen approximately 5 inches cranial to pelvic inlet through the linea alba. A 2-inch 14 gage hypodermic needle was used. To evaluate the result, the females were sacrificed one week to 10 days after insemination. This abdominal insemination has been performed on 30 animals.

Results. No fertilization occurred in animals which were inseminated with once washed sperms. Blastocyst formation was observed in animals which were inseminated with twice washed sperms. However, all blastocysts which were obtained from injections later than 2 hours after mating were degenerating, while those obtained as a result of insemination within 2 hours after mating were viable (Table I). These results suggest 2 important factors which influence success of intraperitoneal insemination. (1) Time elapse between mating (ovulation) and insemination, and (2) suspending media of the semen.

TABLE I. Results of Fertilization Experiments.

Exp.	No. of rabbits	Treatment of semen	Time of inj. after mating (hr)	Avg No. of blastocysts	Outcome of exp.
A	4	None	8	0	—
B	3	Diluent	8	0	—
C	6	1× washed	8	0	—
D	6	2× "	8	.5	±
E	4	*Idem*	6	2	±
F	3	"	4	2.5	±
G	4	"	1–2	6	+

—, no ovum fertilized; ±, blastocyst development stopped at very early stage; +, viable blastocysts developed.

With regard to (1), optimal result was obtained in those animals which were injected 1-2 hours after mating—8 hours before ovulation. As far as (2) is concerned, the presence of accessory genital gland secretion suggests an interference with fertilization of the ovum of the rabbit. No blastocysts were formed when accessory genital gland secretion was present.

Discussion. The experiment shows that intraperitoneal insemination in the rabbit is a possibility. Since it is more successful than vaginal insemination(3) and less cumbersome than complete laparotomy, to deposit semen in the uterus(4), it offers a few advantages.

If the present studies shed any new light on the "capacitation factor" of Austin(1) and Chang(2), it is that capacitation of intraperitoneally injected semen takes longer than those which were deposited in uterus or oviduct(2,4).

* This study was partly aided by Ortho Research Grant awarded by Am. Soc. for Study of Sterility.

While this work was in progress, 2 papers appeared whose authors proved that intraperitoneal insemination is feasible in the bovine(7), although the results were not encouraging, and in the guinea pig(8), where only epididymal semen was used. Further work on this problem is still in progress.

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Received June 4, 1958. P.S.E.B.M., 1958, v99.

Platelets XXI. Glutamic Pyruvic Transaminase Activity of Human Platelets.* (24239)

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Previous work has shown that human blood platelets represent a source of glutamic oxalacetic transaminase (GO-T)(1). Observations have now been extended to the related enzyme, glutamic pyruvic transaminase (GP-T). The study appears justified since both enzymes have been found in serum and in homogenates of many tissues with wide differences of activity from tissue to tissue and from health to disease.

Materials and methods. The activity of GO-T and GP-T was investigated in serum, platelet-rich and platelet-poor plasma, washed platelets, washings of isolated platelets and water soluble platelet extracts. All materials were of human source. Serum was obtained by centrifugation from clotted blood, collected in sterile syringes and incubated at 37° for one hour. To prepare platelet-rich and platelet-poor plasma, blood was collected in Silicone-coated test tubes, using Sequestrene as anticoagulant(2). To obtain platelet-rich plasma, the blood was centrifuged at 1,000 rpm/10′/4°C; and at 3,000 rpm/30′/4°C for platelet-poor plasma. The upper two-thirds of supernatant plasma were transferred to fresh Silicone-coated test tubes. Platelets were prepared by the technic previously described(2). They were washed 3 times with sterile isotonic saline solution using, each time, a volume identical to that of the platelet-rich plasma originally used. A discrete suspension was obtained, the platelets counted by the method previously described(3) using phase contrast microscope and their number adjusted to various levels by adding sufficient isotonic saline to the original platelet suspension. Water soluble platelet extracts were prepared exactly as described(1). Both GO-T and GP-T activity were determined within 2 hours of preparation of the reagents. The spectrophotometric technic of Karmen *et al.* (4) was used for determination of GO-T and that of Wróblewski and LaDue(5) for determination of GP-T. A minor modification was introduced since 0.03 ml of malic acid reagent was used rather than 0.1 ml as suggested in the original technics. All experiments were done in triplicate. The reacting mixture was incubated for 10 minutes and readings taken at 5, 10 and 15 minutes after adding alpha-ketoglutaric acid reagent. Therefore, the final figures reported represent the average of 9 values obtained for each sample. Duplicate determinations were also done using the colorimetric method of Reitman and Frankel(6).

* Supported by grants-in-aid from the Mass. Heart Assn. and the Nat. Inst. of Health(H-2132).

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