CAN MAMMALIAN EGGS UNDERGO NORMAL DEVELOPMENT IN VITRO?*

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Communicated January 4, 1934

The certain test of the viability and normal development of mammalian ova subject to experimental manipulation in vitro may be made by transplanting these ova into a properly prepared female and obtaining young which have developed from the transplanted ova. Heape,[1] Biedl[2] and Pincus[3] long ago demonstrated that rabbit eggs in the early cleavage stages may be successfully transplanted from one female to another (cf. Nicholas[4]). Pincus[3] had no success in obtaining young from culture-grown rabbit ova, and the conclusion was that either greater refinement of technique was required or that apparently "normal" development in vitro was not attained. Thus the regular sequence of cleavage divisions observed in fertilized ova placed in appropriate culture media may be abnormal in the sense that ova which so cleave might never give rise to rabbits. Similarly the observation of apparently successful fertilization of mammalian ova in vitro (Long,[5] Pincus,[3] Yamane[6]) is also open to question. For it has been demonstrated (Yamane,[6] Gilchrist and Pincus[7]) that mammalian eggs placed with dead spermatozoa will exhibit the usual signs of fertilization, e.g., shrinkage of the vitellus and formation of the second polar body. In addition, the parthenogenetic cleavage of rabbit ova occurs readily under certain culture conditions (Pincus[3]).

We have in two instances obtained young from ova subjected to experimental manipulation in vitro.

In one case ten ova were recovered from the fallopian tubes of an agouti doe mated to a vasectomized English-spotted buck. These ova were obtained 13 hours after copulation and were not fertilized since vasectomy had rendered the male sterile. Evidence of the fact that no fertilization had taken place was had by the presence of typical follicle cell masses about each ovum (see Yamane,[6] Pincus,[3] Pincus and Enzmann[8]). Sperm obtained from the vas deferens of a self-colored non-agouti black male were placed with those ova for a period of twenty minutes. During this period the events ordinarily occurring during insemination were observed, namely, the falling away of the surrounding granulosa mass and the slight shrinkage of the vitellus. The eggs freed of surrounding spermatozoa were taken up by pipette in a small amount of Ringer's solution and transferred to the right fallopian tube of a New Zealand Red doe rendered pseudopregnant by mating 48 hours previously with a vasectomized English-spotted male. Thirty-three days later the New Zealand doe

produced seven dark gray young. If, by some chance, the vasectomized male had produced spermatozoa these young would have been English spotted. Since the ova of pseudopregnant does are certainly not fertilizable by twenty-four hours after copulation (Hammond,[9] Pincus[3]), and since the New Zealand Red foster mother was mated not twenty-four but forty-eight hours previous to the transfer of ova, it is obvious that the young could not have arisen from the adventitious fertilization of her ova by a few spermatozoa that may have been accidentally introduced into the tubes with the transplanted ova. We believe, therefore, that this is the first certain demonstration that mammalian eggs can be fertilized in vitro.

In a second case a pseudopregnant albino doe (rendered pseudopregnant by copulation with a vasectomized black male) received five ova which had been cultured in Carrel flasks for twenty hours before their transplantation to the left fallopian tube of the pseudopregnant doe. These ova had been obtained in the one cell stage from the fallopian tubes of an English doe mated to a fertile agouti male. Two gray English-spotted young were obtained thirty-one days after the transplantation.

We believe that the successful recovery of young in these cases (in contrast to the negative results previously reported) is due to careful refinements of the operative procedure which will be described in detail elsewhere.

Incidentally, these experiments demonstrate that the corpora lutea of pseudopregnancy are fully functional.

* The experiments here reported have been supported in part by a grant from the NATIONAL RESEARCH COUNCIL Committee for Research in Problems of Sex.

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