AN EXAMINATION OF THE NEED FOR SPERM CAPACITATION IN THE TURKEY, *MELEAGRIS* *GALLOPAVO**

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Since the recognition by Austin (1951) and Chang (1951) that rabbit spermatozoa require a period of time within the female tract before they are capable of fertilizing ova, evidence has accumulated suggesting an essential period of capacitation for spermatozoa in many other mammals: rat (Austin, 1951; Noyes, 1953; Austin & Braden, 1954), ferret (Chang & Yanagimachi, 1963), hamster (Chang & Sheaffer, 1957; Barros, 1968a, b), cat (Hamner, Jennings & Sojka, 1970) and sheep (Mattner, 1963). The question therefore arises as to whether capacitation is a general phenomenon in all vertebrates. In the fowl, *Gallus domesticus*, the period of time required by spermatozoa in the female tract is of a short duration since cock spermatozoa can fertilize eggs within a few minutes of insemination (Bobr, Ogasawara & Lorenz, 1964). Furthermore, spermatozoa introduced into the infundibulum and ovarian pocket of the fowl 15 min after oviposition and just before ovulation produce fertile eggs (Olsen & Neher, 1948). Recent observations based on successful fertilization *in vitro* of fowl ova with freshly collected spermatozoa indicate that cock spermatozoa do not require a period of capacitation within the hen's reproductive tract in order to fertilize ova (Howarth, 1970). The purpose of the present study was to determine whether turkey spermatozoa require a period of capacitation in order to fertilize ova, using the same test system *in vitro* as that used in the previous investigation dealing with the fowl (Howarth, 1970).

Recently ovulated ova were obtained from Large White turkey hens. Prediction of the time of ovulation was based on knowledge of the time of oviposition. Ovulation of the succeeding ovum in Beltsville Small White turkeys usually occurs on average 30 min after oviposition (Wolford, Ringer & Coleman, 1964). In the present study, it was found necessary to wait for approximately 45 min after oviposition to increase the likelihood of obtaining recently ovulated ova.

One group of three ova, referred to as control ova, were recovered from the magnum of artificially inseminated hens killed 85 min after oviposition and were then cultured *in vitro* for 24 hr. The time of recovery was chosen to allow sufficient time for ovulation and fertilization to occur *in vivo* before recovery. Ova in this group were used to evaluate the ability of the culturing system to support development *in vitro*. A second group of five ova served as negative controls. They were recovered from the infundibulum of unmated hens killed

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45 min after oviposition and cultured *in vitro* for 24 hr. This (negative control) group of ova was used to determine the extent of parthenogenetic development of cultured ova from unmated hens. A third group of five ova, referred to as treated ova, were obtained from the infundibulum of unmated hens killed 45 min after oviposition. An attempt was then made to fertilize these ova *in vitro* followed by a 24-hr culture period *in vitro* identical with that imposed upon the control ova. The culture procedure used in this study has been described previously (Howarth, 1970). All ova were suspended initially in modified Ringer's solution (Olsen & Neher, 1948). In addition, 0.1 ml of recently collected semen diluted 1 : 300 in modified Ringer's solution was placed directly over the germinal discs of all treated ova in the third group. All ova were then incubated at 41°C for 15 min. Following this short incubation period, which was considered sufficient to allow for fertilization *in vitro*, the Ringer's solution was removed and all ova were resuspended in freshly collected albumen. The ova were then incubated for an additional 24 hr at 41°C.

Following the incubation period, the blastodiscs of all ova were removed and placed in Bouin's fixative. The blastodiscs were then embedded in paraffin wax, sectioned at 6 µm, and stained with Delafield's haematoxylin. Stained sections were examined microscopically to determine fertilization rates and the extent to which embryonic development had progressed. The presence of darkly stained nuclei and organized cellular proliferation represented by single layered sheets of cells were used as the criteria of fertilization. By contrast, the parthenogenetic development of newly laid, unfertilized turkey eggs has been depicted by Olsen (1965) as being represented by compact unorganized masses of cells (varying greatly in size and shape) located near the centre of the protoplasmic disc.

An examination of the blastodiscs of control ova revealed that two out of three were fertile. One fertile blastodisc was in the blastula stage of development. The other fertile blastodisc showed signs of organized cellular proliferation but was lacking a segmentation cavity or blastocoele (Pl. 1, Fig. 1). By contrast, the remaining unfertilized egg contained numerous vacuoles in its uncleaved protoplasmic disc (Pl. 1, Fig. 2). These preliminary results indicate that the system used was capable of supporting development *in vitro*.

The attempt to fertilize recently ovulated ova *in vitro* was successful. Two of the five blastodiscs removed from treated ova and examined microscopically were found to be fertile. Of the two fertile blastodiscs, one was in the blastula stage (Pl. 1, Fig. 3) while the other, which was apparently retarded, had not progressed beyond the early cleavage stage of development. The possibility of

EXPLANATION OF PLATE 1

FIG. 1. Median cross-section of a fertile blastodisc removed from a control ovum. Note the presence of epithelial-type cells (E) and the absence of a well-defined segmentation cavity or blastocoele. $\times 740$.

FIG. 2. Median cross-section of an infertile disc removed from a control ovum. Note the presence of large vacuoles (V) in the uncleaved protoplasm. $\times 740$.

FIG. 3. Median cross-section of a fertile blastodisc removed from a treated ovum which had been fertilized *in vitro*. The photomicrograph shows a section of the blastoderm containing epithelial-type cells (E) and a well-defined segmentation cavity (SC) located between the blastoderm and the underlying yolk. $\times 740$.

PLATE 1

development occurring as a result of parthenogenesis was not substantiated in the present investigation as none of the five negative control ova recovered from unmated hens and cultured for 24 hr *in vitro* showed signs of organized development. Microscopic examination of one of these blastodiscs revealed what appeared to be an unorganized group of enlarged blastomeres near the centre of the protoplasmic disc, but no nuclei were present. The remaining four negative control ova contained numerous vacuoles in their uncleaved protoplasmic discs and were similar in appearance to the unfertilized control egg depicted in Pl. 1, Fig. 2.

These preliminary observations indicate that turkey spermatozoa, like cock spermatozoa, do not require a period of capacitation within the female reproductive tract in order to fertilize ova.

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