BIOLOGY OF REPRODUCTION 3, 338–341 (1971)

An Examination for Sperm Capacitation in the Fowl[1]

BIRKETT HOWARTH, JR.

Department of Poultry Science, University of Georgia, Athens, Georgia 30601

Received August 17, 1970

The *in vitro* culture system developed was capable of supporting recently ovulated chicken ova in a viable state for at least 24 hr. Utilizing this system, 8 out of 10 ova obtained from the body cavity or infundibulum of unmated White Leghorn hens, were successfully fertilized *in vitro*. Microscopic examination of the eight fertile blastodiscs revealed that five had proceeded to the blastula state of development during the 24-hr culture period. The remaining three fertile ova, apparently retarded, had not progressed beyond the early cleavage stage of development. None of 10 ova recovered from unmated hens showed signs of development after culture under the same conditions but without incubation with spermatozoa. These observations afford direct evidence that avian spermatozoa do not require a period of capacitation within the hen's reproductive tract in order to fertilize ova.

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 been presented suggesting a period of capacitation to be essential for the fertilizing spermatozoa in many other mammals: rat (Austin, 1951; Austin and Braden, 1954; and Noyes, 1953), ferret (Chang and Yanagimachi, 1963), hamster (Chang and Sheaffer, 1957, and Barros, 1968a, b), and sheep (Mattner, 1963). The question arises as to whether capacitation is a general phenomenon in all vertebrates. If capacitation is a reality in the fowl, the period of time required in the female tract is of a short duration since cock spermatozoa can fertilize within a few minutes after insemination (Bobr *et al.*, 1969). Furthermore, spermatozoa introduced into the infundibulum and ovarian pocket 15 min after oviposition and just prior to ovulation produce fertile eggs (Olsen and Neher, 1948).

According to Chang (1967), the best biological method to examine sperm capacitation is the technique of *in vitro* fertilization. The purpose of the present study, therefore, was to obtain direct evidence as to whether avian spermatozoa require a period of capacitation in order to fertilize ova using an *in vitro* test system.

MATERIALS AND METHODS

For this study, recently ovulated ova were obtained from White Leghorn hens. This was accomplished by attaching microswitches to individual laying cages and recording the time of oviposition for 20 birds simultaneously, using an Esterline Angus Event Recorder Model A620T.[2] Knowing the time of oviposition, it was possible to predict with considerable accuracy the time of ovulation for the next ovum in the clutch.

[1] University of Georgia, College of Agriculture Experiment Stations, Journal Series Paper number 859, College Station, Athens, Georgia.

[2] Esterline Angus Division, Esterline Corporation, Indianapolis, Indiana.

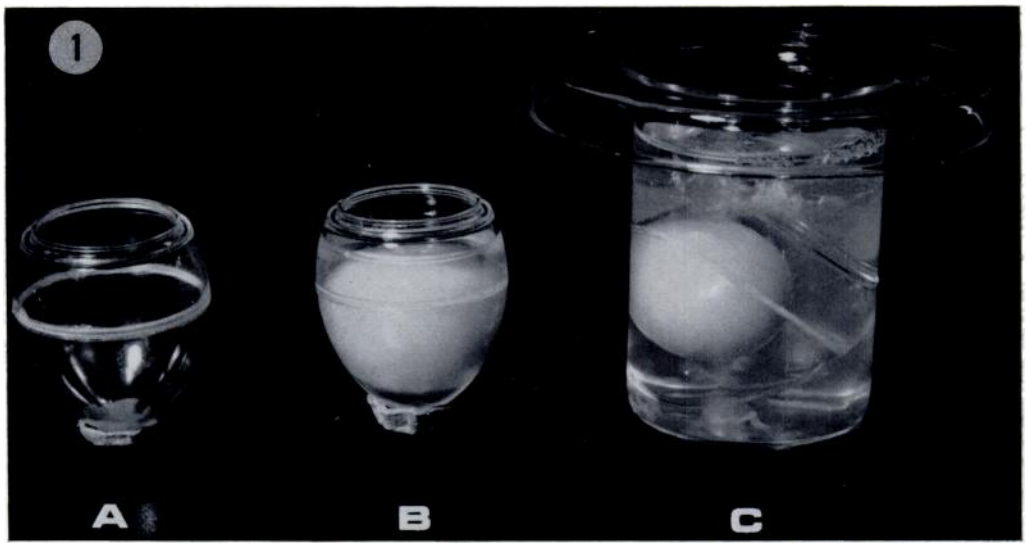

FIG. 1. A. Plastic shell container used in the *in vitro* culture technique. B. Plastic shell containing an ovum suspended in modified Ringer's solution. C. Plastic shell containing an ovum, immersed in freshly collected albumen within a 150-ml beaker. Note that the plastic shell serves to keep the ovum from floating to the surface of the albumen.

Ovulation of the succeeding ovum usually occurs on an average of 30 min after oviposition (Olsen and Neher, 1948).

Pooled semen samples were collected by the method of Burrows and Quinn (1937). Artificial insemination was accomplished utilizing the intravaginal technique of Quinn and Burrows (1936) and Burrows and Quinn (1937). The time from collection of the semen until its use either for insemination or *in vitro* fertilization never exceeded 1 hr.

Ten ova in each of three groups were evaluated for fertilization and stage of embryonic development in this study. One group of ova referred to as control ova were recovered from the infundibulum or magnum of artificially inseminated hens sacrificed 60 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* prior to recovery. Ova in this group were used to evaluate the ability of the *in vitro* culture system to support development. A second group of 10 ova served as negative controls. They were recovered from the body cavity or infundibulum of unmated hens sacrificed 30 min after oviposition and cultured *in vitro* for 24 hr. This (control) group of ova was used to determine the extent of parthenogenetic development in cultured ova from unmated hens. A third group of ova referred to as treated ova were obtained from the body cavity or infundibulum of unmated hens sacrificed 30 min after oviposition. An attempt was then made to fertilize these ova *in vitro* followed by a 24-hr *in vitro* culture period identical with that imposed upon the control ova.

After their recovery, all ova were placed individually in plastic shells (Fig. 1A and B) and suspended in modified Ringer's solution (Olsen and Neher, 1948). In addition, 0.1 cc of recently collected semen diluted 1:200 in modified Ringer's solution was placed directly over the germinal discs of all treated ova in the third group. The plastic shells containing the ova were then placed in 150-ml beakers for support and incubated at 41 C for 15 min. This period of time was considered sufficient to allow for *in vitro* fertilization, since fertilization *in vivo* usually takes place within 15 min after ovulation (Olsen, 1942, 1952). After this short incubation period, the Ringer's solution was removed

TABLE 1

RATE OF FERTILIZATION AND BLASTULA FORMATION OF RECENTLY OVULATED CHICKEN OVA AFTER 24-HR CULTURE *in Vitro*

Treatment	No. ova	No. fertile	No. blastula
Control	10	7	6
Negative control	10	—	0
Treated	10	8	5

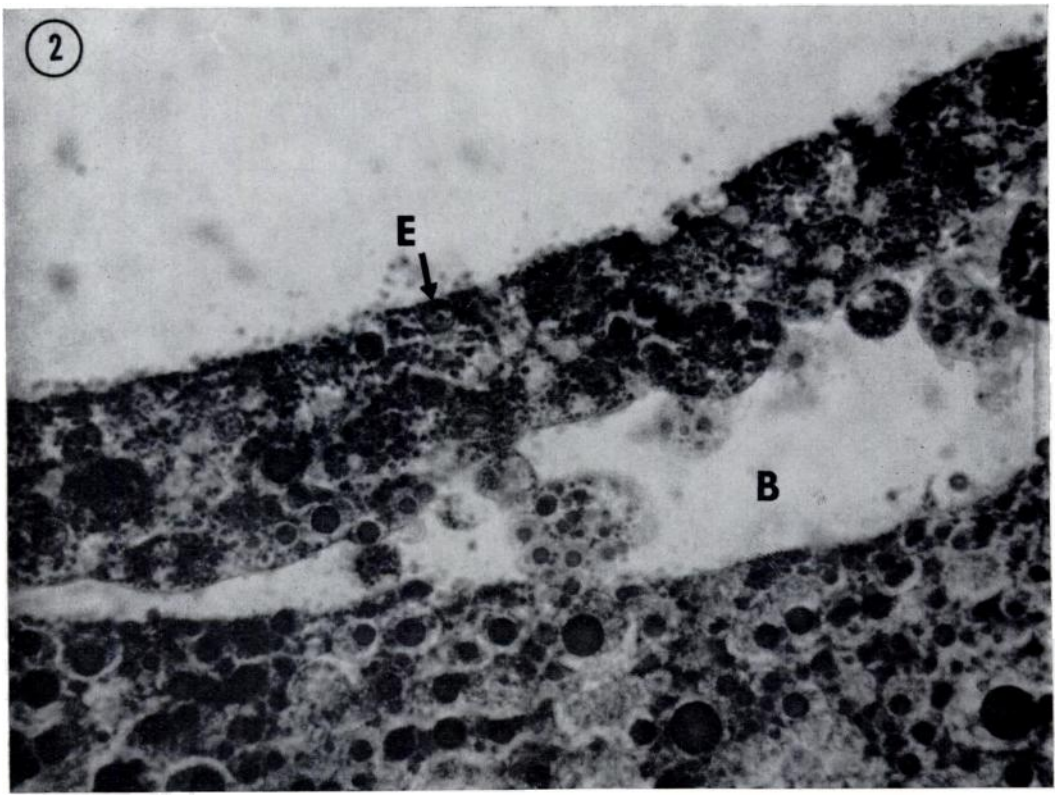

FIG. 2. Median cross-sectional view of a blastodisc removed from a treated ovum which had been fertilized *in vitro*. The photograph shows a section of the blastoderm containing epithelial-type cells (E) and the blastocoele (B) or cavity located between the blastoderm and the underlying yolk.

and freshly collected albumen was added to the beakers in an amount sufficient to cover the plastic shells containing the ova (Fig. 1C). The plastic shells served to keep the ova from floating to the surface of the albumen. An air space of approximately 15 mm was left between the albumen and the top of the beaker. A petri dish was placed on top of the beaker as a cover. The ova were then incubated for an additional 24 hr at 41 C. The time required to process each egg, i.e., from egg recovery to the beginning of the 24-hr incubation period, was approximately 25 min.

After the incubation period, the blastodiscs of all ova were removed and placed in Bouin's fixative. After a minimum of 48 hr fixation, the blastodiscs were embedded in paraffin, sectioned at 6 μ, and stained with Delafield's hematoxylin. The 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 were used as the criteria of fertilization.

RESULTS AND DISCUSSION

The results on fertilization and state of embryonic development for the three groups of ova are summarized in Table 1. An examination of the blastodiscs of control ova revealed that 7 out of 10 were fertile. Of the 7 fertile blastodiscs 6 were in the blastula stage of development. The remaining fertile blastodisc showed signs of organized cellular proliferation but was lacking a segmentation cavity or blastocoele. In contrast, the three unfertilized eggs contained numerous vacuoles in their uncleaved protoplasmic discs. These results indicate that the *in vitro* system used was capable of supporting development.

The attempt to fertilize recently ovulated

ova *in vitro* was successful. Eight of the 10 blastodiscs removed from treated ova and examined microscopically were found to be fertile. Of the eight fertile blastodiscs, five were in the blastula stage (Fig. 2), while three apparently retarded had not progressed beyond the early cleavage stage of development. The possibility of development occurring as a result of parthenogenesis was not substantiated in the present investigation as none of the ova recovered from unmated hens and cultured for 24 hr *in vitro* showed signs of development. Olsen (1966), in studying the frequency of parthenogenesis in chicken eggs, reported that only four of 5931 eggs laid by White Leghorns showed parthenogenetic development.

These observations indicate that avian spermatozoa do not require a period of capacitation within the hen's reproductive tract in order to fertilize ova. Dukelow *et al.* (1967) have examined the seminal plasma from a number of species of animals for the presence of decapacitation factor activity. Their investigations failed to demonstrate decapacitation activity in rooster semen which would support the findings of this study indicating a lack of capacitation in this species.

REFERENCES

AUSTIN, C. R. (1951). Observations on the penetration of the sperm into the mammalian egg. *Aust. J. Sci. Res.* **B4,** 581–589.

AUSTIN, C. R., AND BRADEN, A. W. H. (1954). Time relations and their significance in the ovulation and penetration of eggs in rats and rabbits. *Aust. J. Biol. Sci.* **7,** 179–194.

BARROS, C. (1968a). *In vitro* capacitation of golden hamster spermatozoa with fallopian tube fluid of the mouse and rat. *J. Reprod. Fert.* **17,** 203–206.

BARROS, C. (1968b). *In vitro* capacitation of golden hamster spermatozoa. *Anat. Rec.* **160,** 310 (Abstr.).

BOBR, W. L., OGASAWARA, F. X., AND LORENZ, F. W. (1964). Distribution of spermatozoa in the oviduct and fertility in domestic birds. II. Transport of spermatozoa in the fowl oviduct. *J. Reprod. Fert.* **8,** 49–58.

BURROWS, W. H., AND QUINN, J. P. (1937). Collection of spermatozoa from domestic fowl and turkey. *Poultry Sci.* **16,** 19–24.

CHANG, M. C. (1951). Fertilizing capacity of spermatozoa deposited into the fallopian tubes. *Nature London* **168,** 697.

CHANG, M. C. (1967). Summation of the workshop on capacitation of spermatozoa and endocrine control of spermatogenesis. *J. Reprod. Fert. Suppl.* **2:** 145–147.

CHANG, M. C., AND SHEAFFER, D. (1957). Number of spermatozoa ejaculated at copulation, transported into the female, and present in the male tract of the golden hamster. *J. Hered.* **48,** 107–109.

CHANG, M. C., AND YANAGIMACHI, R. (1963). Fertilization of ferret ova by deposition of epididymal sperm into the ovarian capsule with special reference to the fertilizable life of ova and the capacitation of sperm. *J. Exp. Zool.* **154,** 175–187.

DUKELOW, W. R., CHERNOFF, H. N., AND WILLIAMS, W. L. (1967). Properties of decapacitation factor and presence in various species. *J. Reprod. Fert.* **14,** 393–399.

MATTNER, P. E. (1963). Capacitation of ram spermatozoa and penetration of the ovine egg. *Nature London* **199,** 772–773.

NOYES, R. W. (1953). The fertilizing capacity of spermatozoa. *West. J. Surg.* **61,** 342–349.

OLSEN, M. W. (1942). Maturation, fertilization and early cleavage in the hen's egg. *J. Morphol.* **70,** 513–533.

OLSEN, M. W. (1952). Intro-ovarian insemination in the domestic fowl. *J. Exp. Zool.* **119,** 461–481.

OLSEN, M. W. (1966). Frequency of parthenogenesis in chicken eggs. *J. Hered.* **57,** 23–25.

OLSEN, M. W., AND NEHER, B. H. (1948). The site of fertilization in the domestic fowl. *J. Exp. Zool.* **109,** 355–366.

QUINN, J. P., AND BURROWS, W. H. (1936). Artificial insemination in fowls. *J. Hered.* **27,** 31–37.