INTERFEMALE TRANSFERS OF OVARIES AND EGG CELLS IN THE FROG

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

James Frederick Arnold

1961

ABSTRACT

INTERFEMALE TRANSFERS OF OVARIES AND EGG CELLS IN THE FROG

By James Frederick Arnold

A procedure has been developed for the transferring of body-cavity eggs from one female to another. The first step in this procedure is to make a small incision on both sides of the female, dorsal to the front legs and poterior to the head. Through these openings it is possible to slowly pull the oviducts out, cutting the caudal ends and tearing the anterior ends. The female is then given pituitary glands and in twenty-four hours the body-cavity will be, in most cases, full of eggs. The eggs can then undergo various treatments after which they will be placed in the body-cavity of another female which has been ovarectomized. The eggs will then pass through the oviducts of the recipient female where they will be coated with jelly. Using this procedure, body-cavity eggs were treated with normal rabbit serum before being placed in the recipient female. These eggs failed to fertilize normally after being inseminated with spermatozoa. Body-cavity eggs from a Rana clamitans female were transferred to the body-cavity of a Rana pipiens female without being treated. These eggs passed through the oviducts of the recipient female into the ovisac, thereby

giving Rana clamitans eggs coated with Rana pipiens jelly. These eggs did not fertilize normally after being inseminated with spermatozoa from both species.

The operational procedure for the transferring of ovaries from one female to another was also developed. However, the ovaries at the time of transfer must be pretreated with pituitary so that they will be able to ovulate in the body-cavity of the recipient female. In some cases it was possible to achieve partial ovulation by prior injection of the donor female with pituitary glands, but it was not possible to determine the exact pituitary gland dosage and time of injection for complete ovulation.

In order to obtain egg-jelly in large amounts free from any contact with the eggs, a procedure was developed whereby small glass beads were inserted into the body-davity of pituitary-stimulated, ovarectomized females. The beads were inserted through the incision made for the removal of the ovaries. In one of the cases, the beads passed through the oviducts of the recipient female and into the ovisac. The beads were coated with jelly.

INTERFEMALE TRANSFERS OF OVARIES AND EGG CELLS IN THE FROG

By

James Frederick Arnold

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Zoology

1961

ACKNOWLEDGMENTS

The author wishes to express his sincere thanks to Dr. J. R. Shaver for his interest, direction and encouragement during the course of this investigation. Grateful thanks are also due Drs. S. H. Barch and C. A. Shivers for their assistance. Thanks are also due to Dr. A. Sawant for his interest and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

INTRODUCTION

The role of the jelly around Amphibian eggs in the fertilization process is not well understood. By analogy with the fertilizin-antifertilizin concept of fertilization that has developed from studies on Echinoderm gametes (cf. Tyler, 1949, 1959), some investigators (Glaser, 1921; Bernstein, 1952) believe that frog spermatozoa can be clumped by substances emanating from the jelly around frog eggs. The original observation of this effect by Glaser (1921) in Rana pipiens has not been confirmed by Bernstein (1952), or by Shivers and vandeBunt (personal communication); Bernstein's observations were made on Rana clamitans.

Chemical analysis of the jelly around frog eggs (Folkes, Grant, and Jones 1950; Miganti, 1955) reveals a composition very similar to that of the fertilizin of sea-urchin eggs. The gelatinous envelopes are mucopolysaccharides. Minganti (1955) estimated the composition of the jelly around toad (Bufo vulgaris) eggs as follows: 37.8 per cent; and galactose plus mannose 11.4 per cent (based on dry weight). Nothing, however, is known about the specific linkages of amino groups and sugars in the jelly.

The results of most earlier investigations of the viological role of Amphibian egg jellies agree in the observation that jelly-less eggs are not normally fertilizable.

Bataillon (1919) showed that eggs from the body-cavity of female frogs (Rana fusca) do not fertilize, even with the use of concentrated suspensions of spermatazoa; they could, however, be activated artificially by pricking with fine glass needles (parthenogenetic stimulation). Eggs which had passed through the oviduct, and consequently had been enveloped in jelly, were treated with sodium cyanide solutions, to remove the jelly, and subsequently inseminated or artificially activated. Like the body-cavity eggs, the de-jellied ova were not normally fertilized, but could be activated parthenogenetically. Kambara (1953) observed that toad (Bufo vulgaris formosus) eggs were not fertilizable after removal of the jelly coats, but could be inseminated successfully if eggs were re-enveloped in jelly. There was some indication in Kambara's studies that the outer two (of three) jelly layers were necessary for successful fertilization. This investigator concluded that the main role of the jelly-coat around toad eggs was to serve as a physical substrate for the thigmotactic response of the spermatozoön, basing his concept on the observation that de-jellied eggs covered with gelatin or agar could be fertilized. Tchou-Sou and Wang (1956) introduced toad eggs (Bufo bufo asiaticus) into isolated oviducts, and observed that eggs were fertilizable after a sojourn at any level of the oviduct.

Following the analogy of the fertilizin-antifertilizin concept of fertilization developed by Lillie (1919). Tyler

(1949) and others from experiments on Echinoderm gametes, in which the contacting of the eggs by the sperm is thought of as essentially the result of the interaction of complementary molecular configurations, Shaver, and Barch (1960) studied the effects of antisera prepared in rabbits against the jelly-coat material of frog eggs (Rana pipiens), on the fertilization process. It was found that when frog spermatozoa are treated with anti-jelly serum, the fertilization of eggs inseminated with the treated sperm cells is reduced significantly. Pre-treatment of eggs with anti-jelly serum, prior to insemination with normal spermatozoa, also resulted in some decrease in fertilizability. These investigators interpreted their results as indicative of a barrier to penetration of the spermatozoön produced by the reaction of the jelly-material and the antiserum produced against it. They felt that their data did not conform to an interpretation similar to the fertilizin-antifertilizin reaction postulated by Tyler (1949).

Shivers (1961) extended the observations of Shaver and Barch (1960) to other species of frogs, toads and Urodeles, and discovered that the jelly-envelopes around the eggs of the species studied each contained, in addition to components shared with other species, specific components. These were identified on agar-diffusion plates when antisera prepared against the jellies were reacted with homologous and heterologous antigens, producing precipitation lines. This worker

was also able to show that pre-treatment of eggs with antisera, after appropriate absorbtions, produced significant decreases in the fertilizability of the eggs due to the species-specific antibodies present in the serum.

It has been shown by means of immunological technique (Shaver and Barch, unpublished observations; Shivers, 1961) that the jelly-secreting cells in the frog are restricted to the oviduct. In view of the fact that it is possible to intervene experimentally at various stages of the reproductive cycle of the female frog, it was decided to test the feasibility of the following procedures, which, if successful, would allow further analysis of the role of the surface coats of the egg in the activation process: 1) Treatment of body-cavity eggs with antisera prepared against jelly-coat material, with subsequent introduction into the oviduct, where jelly would be deposited around the egg; 2) removal of the intact ovaries from a female frog, and transfer to a female of another related species, thereby obtaining eggs of one species coated with the jelly of another; 3) obtaining of jelly, free of contact with eggs, by means of passing glass beads through the oviducts of an ovulating female.

These purposes have been achieved in this study, with varying degrees of success, and it is hoped that the techniques developed will make possible further investigations of the actual role of the gelatinous envelopes of Amphibian eggs.

MATERIALS AND METHODS

A. General Technical Procedures

All of the frogs used in these experiments were Rana pipiens from Wisconsin or Vermont, or Rana clamitans from Wisconsin.

All operations were done under a binocular microscope. The animals used proved quite resistant to operative trauma and post-operative infection. The anesthetic used was MS 222 (tricaine methanesulfonate) at a concentration of one gram in two thousand milliliters of a one-tenth Holtfreters solution. The animals were immersed in the anesthetic solution for thirty minutes in a shallow vessel.

The females were induced to ovulate by injections of whole pituitary glands of Rana pipiens, suspended in one-tenth Holtfreters solution, into the body-cavity (Rugh, 1934). Due to seasonal variations, the number of pituitary glands needed to produce ovulation varies. In this study, all injections were of two whole pituitary glands, except where noted. The time required for the females to ovulate, at room temperature (ca. 18-20°C), was twenty-four hours.

The suture used for the closing of incisions was Ethicon 5-0, Number 692A. After the operations, the females were allowed to recover in finger-bowls containing tap water.

The operations and the post-operative care were always done at room temperature.

B. Procedure for the Transferring and Treatment of Eggs

After anesthetization of the female, a small incision (four mm. long) was made dorsal to the fore leg and posterior to the head, on both sides of the animal, (see Figure A), watchmaker's (Dumont #5) forceps were then inserted into the incision in a posterior direction and an oviduct was grasped and slowly pulled out, (see Figure B). The caudal end of the oviduct was cut off, but the anterior end was torn off. An injection of pituitary glands was made through one of the incisions. The incisions were then closed with suture. After twenty-four hours the female was reinjected with pituitary glands through the body-wall, and after forty-eight hours the body-cavity was opened and the eggs, which had been ovulated, were removed. The eggs were then transferred, without or with treatment, to the ovarectomized female. In some cases, the recipient female had had a prior injection of pituitary glands, or in the other cases, received pituitary glands at the time when the eggs were transferred. The female was then examined twenty-four and/or forty-eight hours later, to ascertain if the transplanted eggs had reached the uterus.

C. Procedure for Transferring of Ovaries

After anesthetization an incision twelve millimeters

long was made in the abdominal wall of a female frog in an antero-posterior direction, directly over the ovaries (see Figure C). An ovary was then gently pulled out with watch-maker's forceps, the blood vessel supply was tied off, and a cut made distal to the ligation, allowing the ovary to be easily removed, (see Figure D). The ovary was removed from each side in this manner. The ovary was then transferred, with or without treatment (as described below) to the body cavity of another ovarectomized female. The recipient female in some cases had received an injection of pituitary glands prior to the ovarian transplant; in other cases, the glands were introduced with the ovaries. The incision on the recipient female was closed by first sewing the abdominal musculature together, (see Figure E), and then sewing the dermis together (Figure F). The female was examined twenty-four or forty-eight hours later to determine if ovulation and transport of eggs from the transplanted ovary had occurred.

In three other experiments, the recipient's ovaries and the donor's oviducts were removed and discarded. Both females were then given injections of pituitary glands; in the case of the donor, in order to stimulate ovulation; in the recipient, to stimulate ciliary beat in the body-cavity and oviduct, and also jelly-secretion in the cells of the oviducal walls. In one experiment, the donor's ovary was inserted into the recipient's body-cavity, while still attached to the donor, the incisions were then closed by

sewing the musculature, and then the dermis, of the recipient and of the donor, together. In the two other experiments, the donor's ovaries were placed in a common body-cavity which was created by removing the abdominal walls of both, and sewing the two animals together in ventral apposition. The donors and recipients were tied together with string, in all cases, in an attempt to keep them from pulling themselves apart. They were then placed in a dilute solution of MS 222 (1; 10,000) for a twenty-four hour observation period, after which they were examined, to determine if ovulation and egg transport had occurred.

D. Procedure for Obtaining Egg-jelly Free of Any Contact with the Eggs

A female frog was ovarectomized in the manner described in the preceding section (2, C). Pituitary glands were then inserted into the body cavity, after which two types of beads were introduced, as described below. The incision was closed, as described above, and the female treated as usual.

Plate I

Photographs of the Procedure for Removal of Oviducts

Figure A. The incision made (end of arrow), posterior to the head and dorsal to the front leg, to remove the oviduct.

Figure B. The oviduct removed from the body-cavity while still attached to the ovisac and the ostium.

PLATE I

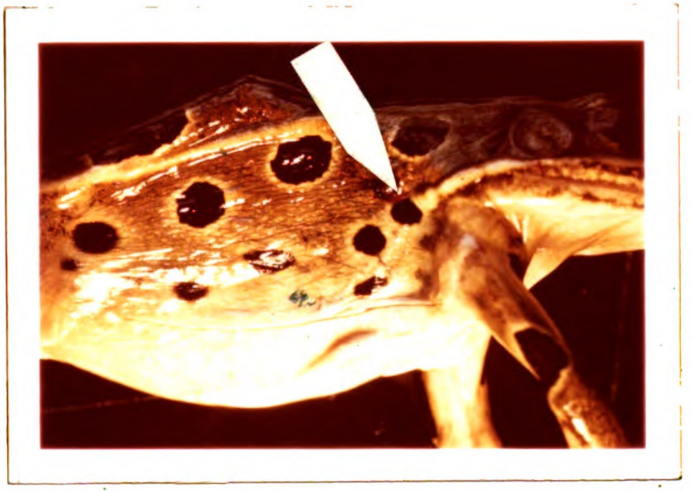

Figure A

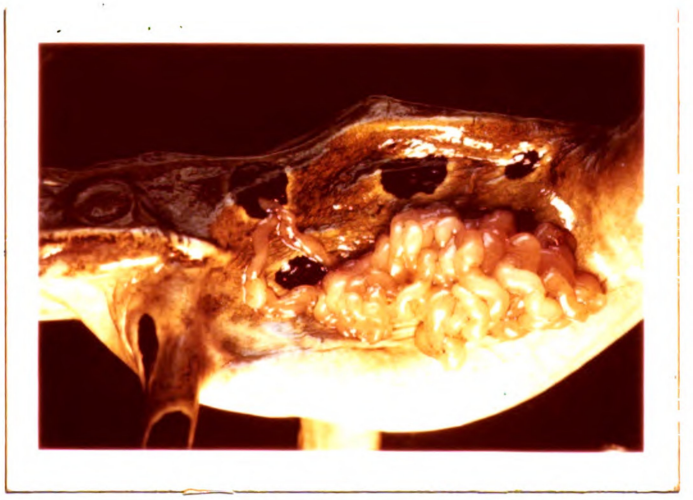

Figure B

Plate II

Photographs of the Procedure for Removal of Ovaries

Figure C. The incision made in the abdominal wall, to remove the ovary.

Figure D. The ovary prior to being cut away.

PLATE II

Figure C

Figure D

Plate III

Photographs of the Procedure for Removal of Ovaries

Figure E. The musculature sewn together.

Figure F. The dermis sewn together.

PLATE III

Figure E

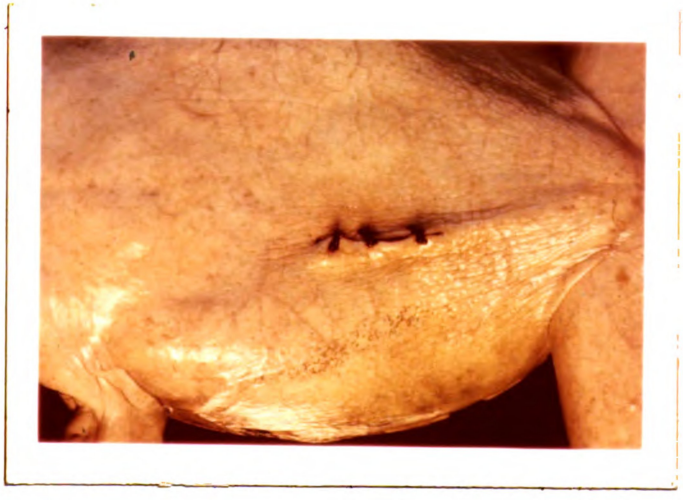

Figure F

RESULTS

A. Transfer of Eggs

The results discussed in this section are summarized in Table I.

The body-cavity eggs from six females were transferred directly to six ovarectomized females from the same stock. Examination of the ovisacs showed three of them full of eggs, two half full, and one one-quarter full. The eggs of these females were inseminated and the numbers of eggs cleaving, from the different females, ranged from 2-22 per cent.

The body-cavity eggs from one Wisconsin stock female were transferred directly to one ovarectomized female from the Vermont stock. Examination of the ovisac showed no eggs present.

The body-cavity eggs from one Vermont stock female were removed and treated for ten minutes in a one-tenths per cent Holtfreter's solution. The eggs were then transferred to an ovarectomized female of the same stock. Examination of the ovisac showed it to be full of eggs, all of which were inseminated with spermatozoa from Vermont males. There was no cleavage of these eggs.

The body-cavity eggs from two females of the Wisconsin

stock were removed and treated for ten minutes in calcium-free Ringer's solution, then transferred to two ovarectomized females of the same stock. Examination of the ovisac showed one of them to be full and the other half full of eggs, all of which were inseminated with spermatozoa from Wisconsin males. Ten per cent of the eggs from the full ovisac cleaved. The remaining eggs did not fertilize.

The body cavity eggs from three Wisconsin stock females were removed and treated for fifteen minutes in 0.1 per cent Nile Blue sulfate solution (a vital stain), then transferred to three ovarectomized females from the same stock. Examination of the ovisacs showed that three animals had ovisacs one-quarter full, and one had no eggs in the ovisac. When the eggs were inseminated with spermatozoa from Wisconsin males, none cleaved.

In the following three experiments, the eggs were all treated by immersing them in undiluted normal rabbit serum for ten minutes.

The body-cavity eggs from six Wisconsin stock females were removed, treated in the serum, and then transferred to six ovarectomized females of the same stock. The recipient females had all been injected with two pituitary glands into the body-cavity, five hours earlier. Examination of the ovisacs showed that in two females they were full of eggs, in two they were one-quarter full, and in two there were no eggs. All of the eggs were inseminated with spermatozoa from

Wisconsin males. None of the eggs cleaved.

The body-cavity eggs from one Wisconsin stock female were removed and treated in the serum, then transferred to one ovarectomized female of the same stock, which had been injected with two pituitary glands four hours earlier. Examination of the ovisac showed that it was one-quarter full of eggs, all of which were inseminated with spermatozoa from a Wisconsin male, and none cleaved.

The body-cavity eggs from two Wisconsin stock females were removed and treated in the serum, then transferred to two ovarectomized females of the same stock without prior injection of the recipient females with pituitary glands. Examination of the ovisacs showed that both of them were one-quarter full of eggs, all of which were inseminated with spermatozoa from the Wisconsin males. None of the eggs cleaved.

In the last experiment of this series an attempt was made to transfer body-cavity eggs from a female Rana clamitans to a female Rana pipiens of the Wisconsin stock. One such transfer was made. Examination of the ovisac showed Rana clamitans eggs coated with jelly deposited during the passage down the oviducts of the Rana pipiens. The ovisac was one-quarter full of eggs, half of which were inseminated with spermatozoa from Wisconsin males, and half inseminated with spermatozoa from Rana clamitans males (see Figure H). None of the eggs cleaved.

Table I--Interfemale Transfer of Body-Cavity Eggs after Various Treatments

DONORS			RECIPIENTS					
No.	Origin	Treatment of Eggs	Origin and Treatment	Number of Eggs in Ovisac				Eggs Fertilized and Cleaved
				None	¼ Full	½ Full	Full	
6	Wisc.	None	Ovarectomized Wisc.	-	1	2	3	2-22%
1	Wisc.	None	Ovarectomized Vt.	1	-	-	-	-
2	Vt.	1/10 Holtfreter's 10 minutes	Ovarectomized Vt.	1	-	-	1	0
3	Wisc.	1/10 Ringer's	Ovarectomized Wisc.	1	-	1	1	10%
4	Wisc.	0.1% Nile Blue Sulfate 15 minutes	Normal Wisc.	1	3	-	-	0
6	Wisc.	Normal Rabbit Serum 10 minutes	Ovarectomized Wisc., Injected pituitary glands 5 hours before	2	2	-	2	0
1	Wisc.	Normal Rabbit Serum 10 minutes	Ovarectomized Wisc., Injected pituitary glands 4 hours before	-	1	-	-	0

Table I--Continued

DONORS			RECIPIENTS					
No.	Origin	Treatment of Eggs	Origin and Treatment	Number of Eggs in Ovisac				Eggs Fertilized and Cleaved
				None	¼ Full	½ Full	Full	
2	Wisc.	Normal Rabbit Serum 10 minutes	Ovarectomized Wisc.	-	2	-	-	0
1	Rana clamitans	None	Ovarectomized Rana pipiens Wisc.	-	1	-	-	0

B. The Transferring of Ovaries

The results of the transfer of ovaries between females will be dealt with in three sections, and summarized in Table II.

The first section involves the direct transfer of untreated ovaries from one female to another. In all cases, the recipient females had been previously ovarectomized.

The ovaries from a female of the Vermont stock were transferred to a female from the Wisconsin stock, at the same time, two pituitary glands were administered. Eighty-five eggs, able to be stripped, were inseminated with spermatozoa from Wisconsin males. Twenty-two of these eggs cleaved normally.

The ovaries from a female of the Wisconsin stock were transferred to a female of the same stock, which had been given a pituitary gland injection twenty-four hours earlier. Twenty-four eggs were stripped, all of which were inseminated with spermatozoa from Wisconsin males. Four of these cleaved normally.

The ovaries from two females of the Wisconsin stock, which were injected with pituitary glands ten hours earlier were transferred to two females of the same stock, which had been injected with pituitary glands six hours prior to the transfer. One of the females had twenty-five eggs, all of which were inseminated with spermatozoa from Wisconsin males. Three of these cleaved normally. The other female had not

ovulated upon examination of the body-cavity.

The second section deals with the treatment and transfer of ovaries.

Ovaries from two females of the Wisconsin stock were immersed in ten cc. of a suspension of one crushed pituitary gland in one hundred cc. of Ringer's solution, for eight hours. One of these two ovaries was transferred to a female of the same stock which had been injected with pituitary glands twenty-four hours earlier; the other was transferred to a female of the same stock which had not had prior pituitary gland injections. No ovulation was observed in the body-cavity of either female.

Ovaries from two females of the Wisconsin stock were immersed in a pituitary gland suspension (1 gland in 80 cc.) for eight hours. They were then transferred to two females of the same stock, which had been injected with pituitary glands two hours earlier. Both females died, and no ovulation was observed in post-mortem.

Ovaries from two females of the Wisconsin stock immersed in a pituitary gland suspension (1 gland in 60 cc.) for eight hours were transferred to two females of the same stock. One of them had twelve eggs in the body-cavity and three in the right oviduct. The other showed no ovulation in the body-cavity.

Ovaries from two females of the Wisconsin stock immersed in a pituitary gland suspension (1 gland in 40 cc.)

for eight hours were transferred to two females of the same stock. No ovulation was observed in the body-cavity of either female.

The ovaries from two females of the Wisconsin stock immersed in a pituitary gland suspension (1 gland in 20 cc.) for eight hours, were transferred to two females from the same stock. No ovulation was observed in the body-cavity of either female.

Ovaries from a female of the Wisconsin stock immersed in a pituitary gland suspension (1 gland in 40 cc.) for ten hours, were transferred to a female of the same stock, which had been given a pituitary gland injection ten hours earlier. No ovulation was observed in the body-cavity of the female.

The ovaries from two females of the Vermont stock immersed in a pituitary gland suspension (1 gland in 10 cc.) for one minute were transferred to two females of the Wisconsin stock, which had been injected with pituitary glands two hours earlier, one receiving two ovaries, the other one ovary. The female receiving one ovary had twelve eggs stripped, all of which were inseminated with spermatozoa from Wisconsin males. None of the eggs cleaved. The other female was dead, and no ovulation was observed in post-mortem.

The third section of the results deals with the partial transfer of ovaries.

One ovary from each of two females of the Wisconsin

stock, while still attached to their own circulatory systems, were inserted into small openings on the sides of two females of the same stock, all females having been given pituitary gland injections eleven hours earlier. The donors and recipients were then sewn together at the sites of the incisions. In each pair, the donor and recipient pulled themselves apart upon awakening from the anesthesia. One pair showed slight ovulation, the other showed none. The blood vessels to the ovaries were, in both cases, stretched and full of clotted bloos, the animals were otherwise in good health.

To avoid stretching the blood vessels, the next two pairs of donors and recipients had, in the first case, half of the abdominal wall removed from both females, and in the other case the whole abdominal wall removed, before being sewn together. Both donor females had their oviducts removed, and all females were injected with pituitary glands several hours earlier. In addition to being sewn together, both pairs were also tied together with string. Neither pair pulled apart and the four animals survived, however no ovulation was observed in either case.

Table II--Interfemale Transfer of Ovaries After Treatment with Pituitary Glands

DONORS			RECIPIENTS[a]				
No.	Origin	Treatment of Ovaries	Treatment with Pituitary Glands	Degree of Ovulation	Eggs in Ovisac	Number of Eggs Stripped	Fertile and Cleaved
1	Vt.	-	At transfer	Complete	Full	85	22
1	Wisc.	-	24 hrs.earlier and at transfer	Complete	Full	24	4
2	Wisc. inj. 10 hrs. earlier	-	6 hrs. earlier	Complete	Full	25	3
2	Wisc.	1 pit./100 cc. 8 hours	1, 24 hrs.earlier 2, at transfer	None	-	-	-
2	Wisc.	1 pit./80 cc. 8 hours	8 hrs. earlier	None on post-mortem	-	-	-
2	Wisc.	1 pit./60 cc. 8 hours	At transfer	1, 12 eggs 1, none	- -	- -	- -
2	Wisc.	1 pit./40 cc. 8 hours	None	None	-	-	-
2	Wisc.	1 pit./20 cc. 8 hours	None	None	-	-	-
1	Wisc.	1 pit./40 cc. 10 hours	10 hours earlier	None	-	-	-
2	Vt.	1 pit./10 cc. 10 minutes	2 hrs. earlier	1, none on post-mortem 1, complete	- Full	- 12	- 0

[a]All recipients of Wisc. stock.

C. The Obtaining of Egg-jelly Free of Any Contact with the Eggs

The ovaries were removed from a female and pituitary glands were inserted. Then twelve doughnut-shaped plastic beads two mm. in diameter were inserted through the incision after which it was sewn closed. The body-cavity was opened twenty-four hours later and all of the beads were still at the site of the incision.

The ovaries were then removed from two females and pituitary glands were inserted. One female received approximately thirty small glass beads, varying in size from one to two mm. The other female received thirty small glass beads which had been coated with Desicote. The ovisacs of both females were full of jelly-coated glass beads (see Figure H).

The ovaries were removed from three females and pituitary glands were inserted. Thirty small glass beads were inserted into each female. After twenty-four hours the females were reinjected with pituitary glands. The females were examined after forty-eight hours and in all three cases the beads had not moved from the site of the incision, and in each case they were covered with a mucoid substance.

Plate IV

Photographs of a Glass Bead and of a Rana Clamitans Egg Coated with Rana Pipiens Jelly

Figure G. A glass bead coated with jelly, after transport through the oviduct of a Rana pipiens female; normal egg to the left.

Figure H. A Rana clamitans egg coated with jelly, after transport through the oviduct of a Rana pipiens female.

PLATE IV

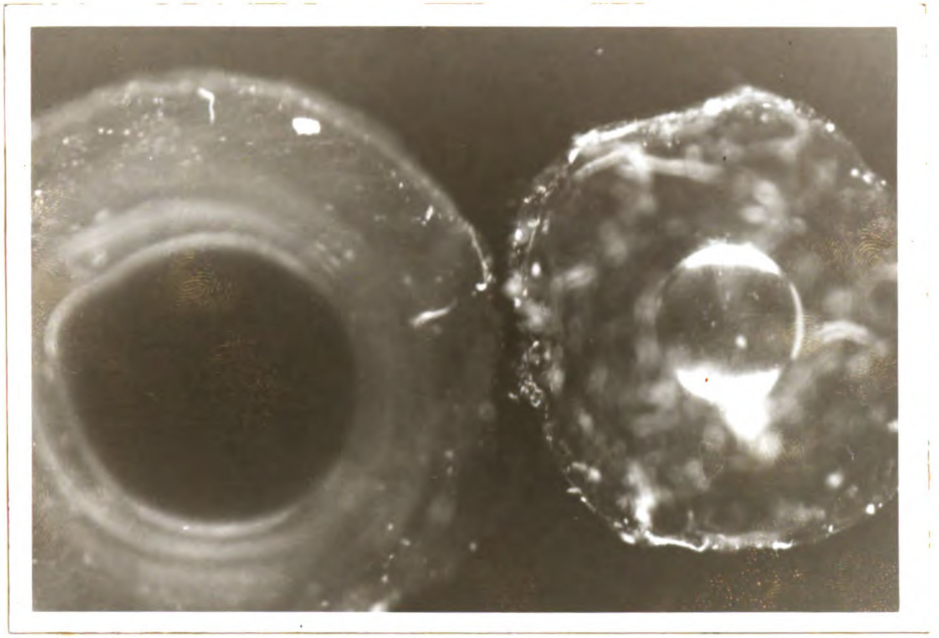

Figure G

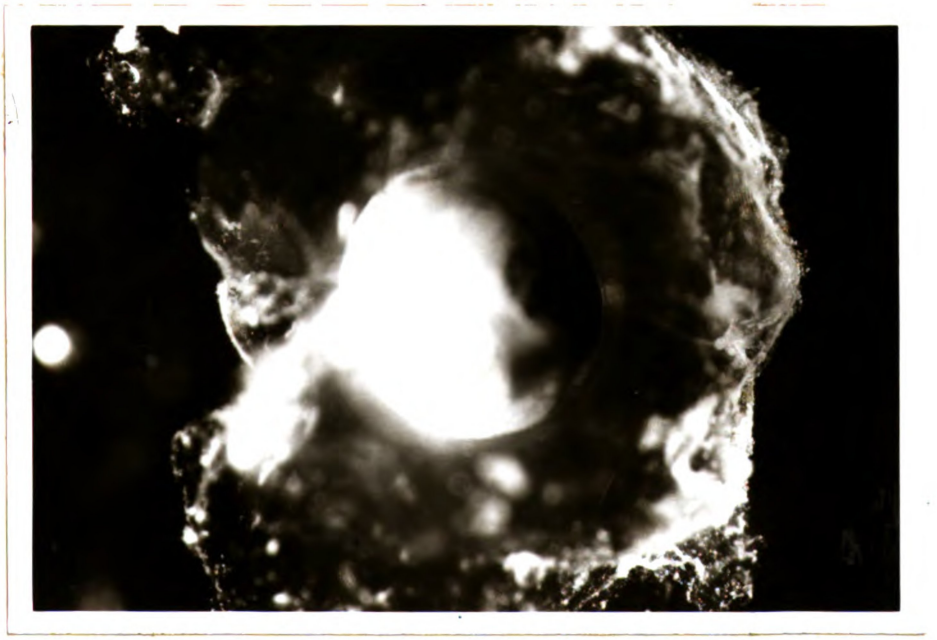

Figure H

DISCUSSION

The results of this study show the feasibility of interfemale transfer of eggs and ovaries, and, in doing so, makes available new methods for further study in the reproductive physiology and experimental embryology of Amphibia. These experiments were made in the months of March and April, the normal breeding season for Rana pipiens. The animals available at this time were very suitable for this work, because a high percentage of eggs obtained in the laboratory develop normally, and because the females survive major operations. Unfortunately, the shipments of frogs received in the latter part of May arrived in an unhealthy condition, thus precluding further study.

The work unfolds several interesting and intriguing problems. One of them would be that of the transfer of ovaries from one species to another and the subsequent insemination of the eggs by spermatozoa from males of both species. In view of Shivers' (1961) observations on the species-specificity of components in the egg-jellies of various species of frogs, the interaction of the jelly-coat material of one species with the surface of the egg of another species would be very important to analyze.

Another interesting problem would be the introduction of body-cavity eggs into different levels of the oviduct, to

ascertain, 1) at which level of the oviduct the jelly-coat renders the egg fertilizable, and, 2) the origin of the species-specific component in the layer of jelly-secreting cells lining the oviduct.

Since body-cavity eggs can be artifically activated (Bataillon, 1919; Subtelny and Bradt, 1961), it might be of interest to transfer parthenogenetically activated body-cavity eggs into an ovulating recipient female and follow the course of development. Subtelny and Bradt (1961) observed that body-cavity eggs into which nuclei of blastula cells were transferred, developed better if they were inserted into a jelly envelope, in vitro.

SUMMARY

1) It has been shown that ovulated eggs from the body-cavity of Rana pipiens can be treated in various ways and transferred to another female, where they proceed through the remaining phases of egg transport. Some of these eggs cleaved normally after insemination. In one case, many eggs of one species (Rana clamitens) were enveloped in the jelly of another species (Rana pipiens) after transfer. None of these eggs developed after insemination.

2) A procedure was developed for transferring ovaries from one female to another. Eggs which were ovulated from the transferred ovaries in some cases fertilized normally.

3) Glass beads of various diameters were introduced into the body-cavity of pituitary-stimulated females (Rana pipiens), and in a few cases, the beads were obtained from the ovisacs of the female after having been coated with jelly.

4) These results are discussed from the standpoint of further experiments which the procedures developed may make possible.

LITERATURE CITED

Bataillon, E. 1919. Analyse de l'activation par la technique des oeufs nus et la polyspermie expérimentale chez les Batraciens. Ann. Sci. Nat. et Zool. Série 1 8, pp. 1-40. (Cited in Shaver and Barch, 1960, p. 180.)

Bertstein, G. S. 1952. Sperm agglutinins in the egg jelly of the frogs, Rana pipiens Schreber and Rana clamitans Latreille. Biol. Bull., 103:285.

Folkes, B. F., Grant, R. A., and Jones, J. K. N. 1950. Frog spawn mucin. J. Chem. Soc., No. 8., 2136.

Glaser, O. 1921. Am. Nat. 55:368. (Cited by Bernstein, G. 1952.)

Kambara, S. 1953. Role of jelly envelope of toad eggs in fertilization. Annotat. Zool. Japon., 26:78-84.

Lillie, F. R. 1913. Studies of fertilization. V. The behavior of the spermatozoa of Nereis and Arbacia with special reference to egg extractives. J. Exp. Zool., 14:515-574.

Minganti, A. 1955. Chemical investigations on amphibian egg jellies. Exptl. Cell Research, Suppl. 3:248-251.

Rugh, R. 1934. Induced ovulation and artificial fertilization in the frog. Biol. Bull. 77:233-257.

Shaver, J. R., and Barch, S. H. 1960. Experiments on the jelly coat of Rana eggs. Acta Embryol. et Morphol. Exptl., 3:180-189.

Shivers, C. A. 1961. Immunobiological studies of the species-specificity of egg jellies of the frog. Unpublished Ph.D. dissertation, Michigan State University.

Subtelny, S. and Bradt. 1961. Transplantation of blastula nuclei into activated eggs from body cavity and from the uterus of Rana pipiens. Part II. Development of the recipient body cavity eggs. Develop. Biol., 2:96-114.

Tchou-Su and Wang Yu-lan. 1956. Études Expérimentales sur le Rôle du mucus des Oviductes dans la Fécondation chez le Crapaud, et le Considération Générale sur le Mécanisme de la Pénétration Spermatique. Acta Experimentali-Biologica Sinica, 5, No. 1, pp. 75-122.

Tyler, A. 1949. Properties of fertilizin and related substances of eggs and sperm of marine animals. Am. Naturalist, 83:195-219.

Tyler, A. 1959. Some immunological experiments on fertilization and early development in sea urchins. Exptl. Cell Research, Suppl. 7:183-199.

ROOM USE ONLY