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Foreign Gamete Survival in the Rabbit

Coggins

TRANSPORT, SURVIVAL AND UNION OF FOREIGN GAMETES IN THE GENITAL TRACT OF THE RABBIT

by

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TABLE OF CONTENTS

TABLE OF CONTENTS

ABSTRACT

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TRANSPORT, SURVIVAL AND UNION OF FOREIGN GAMETES IN THE GENITAL TRACT OF THE RABBIT

Forty virgin does were surgically inseminated with rabbit, boar, ram, bull or human semen in Experiment I. Transport and survival of spermatozoa were evaluated 12 hours later.

Rabbit, boar, ram, bull and human spermatozoa were recovered from 16, 3, 14, 5 and 5 oviducts, respectively. Greater numbers of rabbit and ram spermatozoa were transported than boar, bull or human spermatozoa. Greater proportions of rabbit, ram and human spermatozoa survived than boar or bull spermatozoa.

In Experiment II, 1,112 ovulated and follicular ova from rabbits, pigs or sheep were transferred into 67 does previously inseminated with homologous semen. Location and condition of gametes were observed 24 hours later.

More ovulated ova than follicular ova were recovered and recovery of rabbit and sheep ova was greater than that of pig ova. The presence of homologous ova did not affect spermatozoan transport. Results indicated that capacitation of pig and sheep spermatozoa did not occur in the genital tract of the rabbit for although normal appearing fertilized pig ova were recovered, no foreign spermatozoa were observed in the zona pellucida or perivitelline space of any ova in the project.

LIST OF TABLES

INTRODUCTION

Under natural conditions, fertilization of mammalian ova can occur if capacitated spermatozoa meet fertilizable ova in the ampulla of the oviduct. Mechanisms whereby spermatozoa are transported to the site of fertilization and become capable of penetrating viable ova have not been clearly defined.

Up to 20 billion or more spermatozoa are deposited in the mammalian genital tract during mating but relatively few reach the ampulla. Although it has been demonstrated that the female genital tract is primarily responsible for spermatozoan transport, the mechanisms involved, other than muscular contractions of the tract, are not known.

Transport of spermatozoa to the ampulla is relatively rapid but no ova can be fertilized until the spermatozoa have acquired the ability to penetrate ova. This process is referred to as capacitation and generally requires a greater length of time than transport of spermatozoa to the site of fertilization.

Spermatozoa can be transported up the oviduct of an unrelated species and can fertilize the ova of closely related species. The resulting heterologous zygotes will continue development to various stages. Similarly, zygotes from union of homologous gametes can develop to blastocysts in the genital tract of unrelated species. Therefore, the mechanisms involved in fertilization and further development do not appear to be species specific.

Thus, if gametes of domestic species could survive and unite in the genital tract of a laboratory animal, investigations concerned with

the process of fertilization in those species could be conducted under more controlled laboratory conditions.

To test this hypothesis, spermatozoa and ova from domestic animals were introduced into the genital tract of the rabbit. In Experiment I, does were inseminated with rabbit, boar, ram, bull and human semen to observe transport and survival. In Experiment II, rabbit, pig and sheep ova were transferred into the oviducts of does previously inseminated with homologous semen. The transport, survival and union of gametes was observed to evaluate the specificity of mechanisms involved in fertilization.

REVIEW OF LITERATURE

Transport of Spermatozoa in the Genital Tract

It has been known for sometime (Heape, 1898) that muscular contractions of the female genital tract of the mammal were largely responsible for transport of spermatozoa. Of the 0.05 to 20 billion spermatozoa deposited in the genital tract during mating, only a very small proportion reach the ampulla or site of fertilization. Four regions of the genital tract have been demonstrated to cause this decline in numbers of spermatozoa reaching the ampulla. They are the cervix, uterine horns, uterotubal junction and isthmus. The relative importance of each anatomical area varies with species. In the rabbit, the cervix and uterotubal junction were most predominate from this point of view, as only one out of 40 inseminated spermatozoa passed through the cervix and of these, only one-third reached the uterotubal junction. One out of 160 spermatozoa reaching the junction was transported into the oviduct and of these, one-fourth reached the ampulla (Braden, 1953). The mechanisms involved in reducing numbers of spermatozoa transported through these four areas are not known. However, Braden and Austin (1954) demonstrated that numbers of spermatozoa at the site of fertilization tended to be related to the size of the site. Braden and Austin (1954) recovered a mean of 90 spermatozoa from the ampullas of rats 12 hours after mating. At similar times after mating, Austin (1948), Chang (1951) and Braden (1953) recovered 5,400, 5,100 and 4,500 spermatozoa, respectively, from the fallopian tubes of does. Between 5,000 and 15,000 spermatozoa were recovered from the oviducts of ewes 11 to 48 hours after coitus (Braden and Austin, 1954; Mattner, 1963a). Rigby (1965) recovered a mean of 20,000 spermatozoa from the oviducts of sows 12 to 24 hours after insemination. Thus, concentration of spermatozoa at the site of fertilization remained fairly constant throughout the various species.

The number of spermatozoa in the ampulla did not appear to b affected by the number of spermatozoa inseminated. Chang (1951) demonstrated that if 10 x 10^6 or more spermatozoa were inseminated, the number of spermatozoa transported into the oviducts of does would be relatively constant and fertility would not be affected unless two million or less spermatozoa were introduced.

Van Demark (1953) and Mattner (1963b) demonstrated that spermatozoan motility is not essential for transport. However, immotile spermatozoa were not transported as rapidly or in as great a number as motile spermatozoa.

The female genital tract appeared to be primarily responsible for spermatozoan transport as the spermatozoa seemed to play only a passive role.

Species Specificity of Transport of Spermatozoa and Fertilization

Evidence that spermatozoa could be transported up the genital tract and fertilize ova in another species has been present for many years (Ewart, 1898). Only recently has the problem been examined in any detail. Hammond and Walton (1929) inseminated rabbits with hare semen but did not get birth of living young, whereas Adams (1957) found that 97 percent of rabbit ova were fertilized when does were inseminated with hare semen, indicating that the lack of young was due to incompatability at a later stage of development. The domestic rabbit has also been inseminated with semen from snowshoe rabbits (Chang and McDonough, 1955) and snowshoe hare (Chang et al., 1964) yielding 30 and 96 percent fertilized ova, respectively. Similarly, when ferrets and goats were inseminated with mink (Chang, 1956) and sheep semen (Warwick et al., 1932), respectively, fertilized ova were recovered. Thus, spermatozoa can penetrate and fertilize ova of

a closely related species, indicating that transport of spermatozoa and capacitation are not species specific.

Transport of spermatozoa has been investigated in less closely related species. Yochem (1929) found that rat and guinea pig spermatozoa were transported up the oviducts of guinea pigs and rats, respectively. Phillips and Andrews (1937) demonstrated that rat spermatozoa can be transported through the uterotubal junction of the ewe. Leonard and Perlman (1949) surgically inseminated rats with live mouse, guinea pig, bull, rat and dead rat spermatozoa. Only live rat and rarely foreign spermatozoa passed through the uterotubal junction. In contrast, Howe and Black (1963) introduced immotile rat, rabbit, bull and human spermatozoa into ligated or unligated uteri of rats. The immotile spermatozoa of all four species were transported into the oviduct, although to a lesser degree when uteri were unligated.

Thus, work to date has shown that foreign spermatozoa can be transported through the uterotubal junction and up the oviducts of unrelated species but penetration and fertilization of ova has not been investigated.

Survival of Ova in a Foreign Genital Tract

Information on survival of ova in a foreign tract is almost entirely limited to fertilized ova; although Dickman (1963) did transfer unfertilized rat ova into mated rabbits to investigate the affinity between homologous gametes or chemotaxis. He observed a mean of 20 rabbit spermatozoa on each rabbit ovum but only a mean of 0.6 rabbit spermatozoa on four rat ova.

Fertilized rat, mouse, sheep, goat and sheep ova survived and cleaved to the blastocyst stage in the uteri of mice, rats, (Tarkowski, 1962) goats, sheep (Warwick and Barry, 1949) and rabbits

(Averill et al., 1955), respectively. As a further test of survival Adams et al. (1961) transferred sheep ova, which had cleaved to the blastocyst stage in a rabbit uterus, back into ewes and got birth of living young. Ferret ova survived and developed in rabbits' oviducts but not in the uteri; but rabbit ova did not survive in any portion of the genital tract of the ferret (Chang, 1966). Hafez and Sugie (1965) found that fertilized rabbit ova developed to the blastocyst stage in bovine uteri but most cattle embryos failed to cleave in the uteri of pseudopregnant does. In contrast, Sreenan and Scanlon (1968) found that 90 percent of the cattle embryos cleaved in the uteri of rabbits when bovine follicular fluid was used as the transfer medium.

Only fertilized ova have been shown able to survive and remain viable in a foreign genital tract. The status of an unfertilized ovum in a foreign tract has not been investigated.

In summary, many of the mechanisms involved in fertilization and subsequent development did not have species specific requirements. Transport and capacitation of spermatozoa occurred in the female genital tract of closely related species but only transport has been investigated in non-related species. Fertilized ova survived in a foreign genital tract but viability of unfertilized ova has not been investigated.

MATERIALS AND METHODS

EXPERIMENT I: Transport and Survival of Foreign Spermatozoa in the Genital Tract of the Rabbit

Forty virgin, New Zealand White does, weighing from 2.8 to 4.2 Kg were each surgically inseminated with 0.5 ml of fresh whole semen from rabbits, boars, rams, bulls or humans, six hours after an ovulating dose of Human Chorionic Gonadotrophin (HCG, Ayerst Laboratories, Montreal, Quebec). Twelve hours after insemination the does were killed and their reproductive tracts removed. The oviducts were flushed with physiological saline. The number and condition of the spermatozoa and ova recovered were recorded.

Collection and Insemination of Semen

Semen was collected from mature males of proven fertility. Immediately after collection, all semen was placed in an insulated, cork-stoppered, warmed, 10 ml glass vessel. No attempt was made to maintain the temperature of semen above room temperature other than the insulation around the vessel.

Percent and rate of motility of the spermatozoa were checked on warmed glass slides immediately after collection and insemination. The concentration of spermatozoa in each semen sample was determined by the hemacytometer method (Appendix Table 1). The inseminating equipment, surgical instruments and gloves, were thoroughly cleaned and autoclaved between inseminations to prevent possible contamination.

Four to six hours prior to insemination, the does were injected intravenously with 50 I.U. of HCG in 0.5 ml of physiological saline. Following sodium pentobarbitol anaesthesia (1 grain/2.28 Kg of body weight, Haver Lockhart Laboratories, Brampton, Ontario), a five cm midventral incision was made in the lower abdomen. Upon exposing the uterine horns,

a ligature was placed loosely around the horns one cm anterior to the cervix (Appendix, Figure 1). The inseminating equipment consisted of a blunt 22 gauge needle attached to a 0.25 ml glass syringe. The tip of the needle was forced through the wall of the uterus posterior to the ligature and then eased along in the lumen until the ligature could be tightened around it. The 0.25 ml of semen was then slowly expelled into the horn. As the needle was withdrawn, the ligature was further tightened to preven semen loss.

Recovery of Spermatozoa and Ova

The does were killed 12 hours after insemination. Their reproductive tracts were removed and dissected free of connective tissue. The uterine horns and oviducts were separated at the cervix and uterotubal junction and placed in four separate petri dishes. A sample of fluid from each uterine horn was placed on a warmed slide and examined for percent and rate of motility of the spermatozoa.

Each oviduct was flushed from the infundibulum to the uterotubal junction with five ml of physiological saline, then in the opposite direction with five ml to yield a total of 10 ml of flushings from each oviduct. The ova were removed from the flushings with the aid of a stereoscopic microscope and placed in a drop of saline on a slide between two ridges of paraffin-stopcock grease mixture. A coverslip was pressed down on the ridges until contact was made with the ova. The ova were then examined microscopically for the number of spermatozoa in the cumulus cells, in the mucin layer, attached to the zona pellucida and in the perivitelline space. Fixing for 24 to 48 hours in 25 percent acetic acid - 75 percent alcohol and staining with 0.5 percent orcein in 40 percent acetic acid (Chang and Polge, 1964) was used to determine the presence of spermatozoa in the ooplasm, metaphase plates, pronuclei and polar bodies.

After removing the ova, the flushing fluid was poured into a 30 ml centrifuge tubes. Then the petri dishes were rinsed with five to 10 ml of distilled water which was added to the contents of the centrifuge tubes. After centrifugation, for 20 minutes at 800 G's, all of the supernatant was siphoned off. The remaining 0.1 to 0.2 ml of sediment were picked up in a Pasteur pipette and expelled inside a rectangular ridge of vaseline, slightly smaller than a 22 x 50 mm coverslip, on a slide. Approximately 0.2 ml of five percent sodium citrate was used to rinse the centrifuge tube and pipette. A 22 x 50 mm coverslip was compressed on the vaseline ridge to make a complete seal around the sediment and the rinsings. A spermatozoan count was then done on the contents of the slide.

Due to the non-random distribution of spermatozoa numbers, data was analysed by analysis of variance (one way classification) following an $x = x + \frac{1}{2}$ transformation. Data involving only those animals showing transport were analysed by analysis of variance (uneven replication) without transformation.

EXPERIMENT II: Transfer of Ovulated and Follicular Rabbit, Pig, and Sheep Ova into the Oviduct of the Rabbit

General

A total of 67 virgin, New Zealand White does, weighing from 3.0 to 4.5 Kg, were surgically inseminated with fresh whole semen from rabbits, pigs or sheep. Rabbit, pig or sheep ova were transferred into the oviducts two to four hours after insemination. Totals of 84 ovulated rabbit ova, 121 follicular rabbit oocytes, 672 ovulated pig ova, 174 follicular pig oocytes, 16 ovulated sheep ova and 45 follicular sheep oocytes were transferred into 14, 14, 70, 16, 3 and 7 oviducts, respectively. The does were killed 24 hours after transfer and their reproductive tracts were removed. The ova were recovered and their location and condition recorded.

All manipulation of ova was done in a heat-treated ten percent homologous serum-acidic saline medium (Chang, 1959). The complete medium including 100 I.U. of penicillin/ml was passed through a Millipore filter, Type H.A. with a pore size of 45 u, prior to use. Flushing of ova from either oviducts or follicles was accomplished with warm (32-35°C) medium. With the aid of a stereoscopic microscope, a micromanipulator with a fine glass tube tip was used to pick up and transfer ova (Appendix, Figure 2).

The recipient does were anaesthetized with sodium pentobarbitol or Fluothane (Halothane, Ayerst Laboratories, Montreal, Quebec) and a high lumbar incision was made in each side to expose the oviducts. The glass tip of the micromanipulator was inserted 1.0 to 1.5 cm into the ovarian end of the oviduct and the ova gently expelled in approximately 0.1 ml of medium. The incisions were then closed with suture.

Twenty-four hours after transfer, the recipient does were killed and their reproductive tracts were removed. Following removal of connective tissue, the uteri and oviducts were separated at the uterotubal junction and cervix. A sample of uterine contents was placed on a warm slide; and percent of motile spermatozoa and rate of motility observed. The oviducts were thoroughly flushed with physiological saline into a gridded petri dish. If all of the ova were not recovered, the uterine horns were also flushed. The ova were mounted, examined, fixed and stained as in Experiment I. The presence of normal nuclei in blastomeres was also determined.

The percentage values in Experiment II were analysed with contingency tests (X^2).

Trial 1

Fourteen virgin, New Zealand White does were inseminated with 0.5 ml of fresh whole rabbit semen as in Experiment I. Two to four hours later, 6 to 12 follicular oocytes and 2 to 10 ovulated ova were transferred into opposite oviducts of each doe. Fourteen virgin, New Zealand White does were killed 12 hours after intravenous injection of 100 I.U. of HCG to serve as a source of ovulated ova. Follicular oocytes were recovered from 14 untreated does.

Trial 2

Five does were inseminated with ram semen as in Experiment I and four to six hours later, three to seven ovulated or four to eight follicular ova were transferred into opposite oviducts. The source of ova was 12 cull ewes synchronized with Melanogosterol Acetate (British Drug Houses Ltd., Toronto, Ontario). On the last day or day 17 of treatment, each ewe was injected with 1,000 I.U. of Pregnant Mare's Serum (PMS. Ayerst Laboratories, Montreal, Quebec). Forty-eight hours later, 500 I.U. of HCG were administered to the ewes. Animals were slaughtered 36 and 44 hours after HCG to yield follicular and ovulated ova, respectively.

Trial 3

Two to six hours after surgical insemination of 0.5 ml of boar semen, 3 to 12 ovulated and 3 to 12 follicular ova were transferred into 16 and 16 oviducts, respectively. Porcine reproductive tracts were collected at an abattoir. Fourteen tracts whose ovaries contained normal recent ovulations and 30 tracts whose ovaries had a minimum of four to six mm follicular development supplied ovulated and follicular ova, respectively.

Trial 4

Thirteen does were inseminated with boar semen, as in Experiment I and four to six hours later, 5 to 16 ovulated ova were transferred into

each oviduct. In order to supply freshly ovulated ova, 25 prepuberal gilts, weighing from 80 to 85 Kg were superovulated by injections of 2,000 I.U. of PMS and 500 I.U. of HCG (Baker and Coggins, 1968) and killed 44 hours after HCG to supply freshly ovulated ova.

Trial 5

A procedure similar to that of Trial 3 was used, with the exception that the recipient does were injected with 100 I.U. of HCG seven to eight hours before transfer. Seven to sixteen ovulated pig ova were transferred into each oviduct of four does previously inseminated with boar semen and injected with HCG.

Trial 6

The procedure involved was similar to Trial 5 with the exception that the uterine horns were not ligated following insemination. Six to twenty ovulated pig ova were transferred into each oviduct of four does previously inseminated with boar semen and injected with HCG.

Trial 7

The substitution of rabbit semen for boar semen was the only difference between Trial 7 and Trial 6. Three to twenty ovulated pig ova were transferred into each oviduct of six does previously injected with HCG and inseminated with rabbit semen.

EXPERIMENTS AND RESULTS

EXPERIMENT I: Transport and Survival of Foreign Spermatozoa in the Genital Tract of the Rabbit

General Response

In Experiment I, 250 normal ovulations were counted in the 40 does injected with Human Chorionic Gonadotrophin (HCG) for a mean ovulation rate of 6.3 ovulations per doe. A total of 220 or 88 percent of the ova were recovered. At least one ovum was recovered from each oviduct. Examination of uterine horns and their contents at the time of recovery indicated that few, if any, spermatozoa had escaped through the ligated end of the uterus.

Experimental Response

As native species, rabbit spermatozoa were transported in highest numbers but not in significantly ($P > 0.5$) greater numbers than ram spermatozoa.

Total numbers of rabbit and ram spermatozoa transported and numbers of those spermatozoa in the oviduct fluids were significantly greater ($P < 0.01$) than that of boar, bull and human spermatozoa (Table 1). Rabbit spermatozoa were present in 16 of 16 oviducts whereas, foreign spermatozoa were recovered in only 27 (42%) of 64 oviducts. Numbers of oviducts containing boar, ram, bull and human spermatozoa were 3, 14, 5 and 5, respectively.

Statistical analysis of total numbers of spermatozoa transported was done on only the data from those animals in which spermatozoa were transported. No significant ($P > 0.5$) difference was found between any of the species.

Table 1. NUMBERS OF RABBIT, BOAR, RAM, BULL AND HUMAN SPERMATOZOA RECOVERED FROM THE RABBIT OVIDUCTS

Species of Sperm Inseminated	No. of Animals Inseminated	Sperm Associated with Ova			Sperm in Oviduct Fluids			Total No. of Sperm	Sperm in Animals Showing Transport		
		No. of Animals	No. of Oviducts	No. of Sperm/Ani.	No. of Animals	No. of Oviducts	No. of Sperm/Ani.		No. On Ova	No. in Fluid	Total No.
Rabbit	8	8	16	121	8	16	569	691	121	569	691
Boar	8	3	3	51	3	3	291	342	105	742	880
Ram	8	3	4	46	7	14	478	524	159	440	599
Bull	8	2	2	27	3	5	56	83	107	148	330
Human	8	3	3	71	3	5	271	330	227	690	915

The criteria for transport of spermatozoa to the ampulla was presence of spermatozoa on the ova or in the ova. Numbers of rabbit spermatozoa associated with ova were significantly greater ($P < 0.01$) than numbers of foreign spermatozoa (Table 1). All 56 ova recovered from does inseminated with rabbit semen had one or more spermatozoa associated with them. A total of 47 (84%) of these ova were fertilized, whereas, foreign spermatozoa were attached to rabbit ova from 12 (44%) of 27 oviducts shown to contain spermatozoa. When foreign spermatozoa were not observed in the oviduct fluids, no spermatozoa were seen on the ova.

Evaluation of attachment to or penetration of rabbit ova by spermatozoa was done at a time when preliminary work indicated that 50 percent or more of the ova were nude and not surrounded by mucin. No foreign spermatozoa were observed attached to the zona pellucida of rabbit ova but appeared to be either stuck in the cumulus cells or in the mucin. The lack of ability of foreign spermatozoa to attach to rabbit ova was most noticeable in does inseminated with ram semen. A high proportion of the ova recovered were completely nude without mucin and due to this, only 4 of 14 oviducts contained rabbit ova with ram spermatozoa associated with them. Foreign spermatozoa were not observed either in the zona pellucida or perivitelline space of the rabbit ova.

Examination of uterine contents showed that ram and human spermatozoa survived as well as rabbit spermatozoa (Table 2). On the basis of percent loss of motility, significantly ($P < 0.01$) more rabbit, ram and human spermatozoa remained motile than bull or boar spermatozoa.

The same species also survived well from the point of view of number of horns containing motile spermatozoa.

Table 2. SURVIVAL OF FOREIGN SPERMATOZOA IN THE RABBIT UTERUS.

Species of Sperm Inseminated	% Motile Sperm		Rate of Motility		No. of Horns Containing Motile Sperm
	Initial	Final	Initial	Final	
Rabbit	61	32	2.3	1.6	16
Boar	57	2	2.0	0.2	4
Ram	46	17	1.8	1.2	14
Bull	62	0	1.9	0	1
Human	61	40	2.0	1.8	15

Results demonstrated that boar, ram and human spermatozoa can survive in the uterus and that all four foreign species of spermatozoa can be transported through the uterotubal junction of the doe. All four species of foreign spermatozoa can be transported up the oviduct to the ampulla but did not appear able to attach to or penetrate the zona pellucida of rabbit ova.

EXPERIMENT II: Transport, Survival and Union of Gametes from Rabbits, Pigs and Sheep in the Genital Tract of the Rabbit

General Response

Totals of 504 (65%) ovulated and 170 (50%) follicular ova were recovered from the 67 does in the seven trials. Of these ova, 375 (74%) ovulated and 103 (61%) follicular ova were recovered in the oviduct. In trials five to seven the 14 recipient does injected with 100 I.U. of HCG ovulated a mean of 7.7 ova of which 66 percent were recovered. This recovery rate was significantly correlated with the recovery rate of transferred pig ova in the same trials ($r = 0.55$). Examination of the uterine contents of the 57 does whose uteri were ligated following insemination, indicated that few, if any, spermatozoa had escaped through the ligated end of the uterine horns. The presence of spermatozoa was demonstrated in nine of ten does whose uteri were

not ligated. Only when rabbit spermatozoa came in contact with rabbit ova were normal appearing attachment or penetration of the zona pellucida observed (Appendix, Figure 3). In all other cases, the spermatozoa appeared to be either stuck in any remaining cumulus cells or in the mucin layer (Appendix, Figures 4 and 5).

Experimental Response

Trial 1:- Comparison of Ovulated and Follicular Rabbit Ova Transferred into Does

Ovulated rabbit ova were transferred into one oviduct and follicular rabbit ova into the other oviduct of does, four to six hours after surgical insemination with rabbit semen (Table 3). Percent of

Table 3. COMPARISON OF TRANSFERRED OVULATED AND FOLLICULAR RABBIT OVA.

Type of Ova	Total No. Oviducts	Total No. Ova Transferred	% Total Ova Recovered	% Ova Recovered from Oviduct	% Total Ova Fertilized
Ovulated	14	84	83	73[a]	60
Follicular	14	121	54	48[a]	17

a. Number of ova recovered in the oviduct divided by the total number of ova recovered.

ovulated ova recovered was significantly greater ($P < 0.01$) than that of follicular ova. Similarly, percent of ovulated ova recovered in the oviduct was significantly greater ($P < 0.01$) than that of follicular ova. Significantly more ($P < 0.01$), ($P < 0.05$) fertilized ovulated (93%) and follicular ova (85%) were recovered in the oviducts (Appendix, Figure 6). Fertilization rates for ovulated and follicular ova were 60 and 20 percent, respectively. Generally, greater quantities of mucin were found on ovulated ova than on follicular ova (Appendix,

Figures 7 and 8). Similarly, no mucin was observed on ovulated or follicular ova recovered in the uterus (Appendix, Figure 6).

As the amount of mucin surrounding the ova increased, the number of spermatozoa associated with them also appeared to increase. The mean numbers of spermatozoa associated with ovulated and follicular ova recovered from the oviducts were 54 and 41 spermatozoa, respectively. The higher mean number of spermatozoa associated with ovulated ova would appear to be due to the greater quantity of mucin but the mean number of spermatozoa attached to the zona pellucida, or in the perivitelline space of ovulated ova (18) was also greater than that of follicular ova (5).

Trial 2:- Comparison of Transferred Ovulated and Follicular Sheep Ova

Ovulated and follicular sheep ova were transferred into does previously inseminated surgically with ram semen.

Table 4. COMPARISON OF TRANSFERRED OVULATED AND FOLLICULAR SHEEP OVA

Type of Ova	Total No. Oviducts	Total No. Ova Transferred	% Total Ova Recovered	% Ova Recovered From Oviduct
Ovulated	3	16	88	86[a]
Follicular	7	45	78	71[a]

a. Number of ova recovered in the oviduct divided by the total number of ova recovered.

Due to the low numbers of ovulated ova involved, no statistical differences could be demonstrated between ovulated and follicular ova on the basis of trasnport of ova or ova recovery (Table 4). There

appeared to be a difference in number of ova with spermatozoa attached but 12 of the 13 follicular ova came from one animal in which extremely high numbers of spermatozoa were transported. No differences were observed in the amount of cumulus or mucin on the ova although the amount of mucin appeared to be less than that observed on rabbit ova in Trial 1.

Trial 3:- Comparison of Transferred Ovulated and Follicular Pig Ova From an Abattoir

In the present trial, ovulated and follicular pig ova were recovered at a local abattoir and were then transferred into does previously inseminated with boar semen. Significantly ($P < 0.01$) more ovulated ova were recovered than follicular ova (Table 5). Fourteen

Table 5. COMPARISON OF TRANSFERRED AND FOLLICULAR PIG OVA (ABATTOIR SOURCE)

Type of Ova	Total No. Oviducts	Total No. Ova Transferred	% Total Ova Recovered	% Ova Recovered From Oviduct
Ovulated	16	97	62	53[a]
Follicular	16	174	40	67[a]

a. Number of ova recovered in the oviduct divided by the total number of ova recovered.

(23%) normal appearing fertilized ovulated ova were recovered (Appendix, Figures 9 and 10). Only three of these ova had spermatozoa associated with them. Estimated age of the ova did not appear to affect cleavage. No spermatozoa were observed in the uterine smears or on control ova from ovulated ova donors. Fifty-one (73%) recovered follicular ova appeared to be undergoing normal maturation whereas 59 (81%) of the control follicular ova contained normal appearing vesicular nucleii (Appendix, Figure 11). Ovulated ova were surrounded by more mucin than follicular

ova when recovered 24 hours after transfer but did not have as much mucin on them as the rabbit ova in Trial 1 (Appendix, Figures 12, 7).

Trial 4:- Effect of Source of Ova

In an attempt to eliminate the problems involved in using ova obtained at an abattoir, prepuberal gilts were superovulated with exogenous gonadotrophins. Gilts were killed 44 hours after HCG and the genital tracts removed immediately upon death of the animals. Thus, length of time between death of the animal and ova recovery was reduced, excessive heating of the abattoir eliminated, age of the ova predetermined and possible contact with a boar avoided. The ova were then transferred into does previously inseminated with boar semen. A total of 145 (54%) ova recovered of which 67 (46%) were in the oviduct (Table 6). Only three (3%) ova appeared to be fertilized but spermatozoa were not present on the zona pellucida of any of these ova. However, spermatozoa were found associated with ova in eight other oviducts. The condition of recovered ova was similar to that of ovulated ova in Trial 3 with respect to amount of cumulus and mucin.

Trial 5:- Effect of Ovulating Recipient Does

Administration of gonadotrophin to recipient does such that ovulation occurred two hours after transfer yielded 72 percent ova recovery of which 97 percent were in the oviduct (Table 6). The pig ova were surrounded by more mucin as compared to previous trials (Appendix, Figures 12, 13) but the rabbit ova were surrounded by considerably more mucin than the transferred ova. Transport of spermatozoa appeared to be adversely affected in that spermatozoa were observed on the ova in one animal (Appendix Table 2).

Table 6. EFFECT OF OVULATION OF RECIPIENTS, NONLIGATION OF UTERI AND SPECIES OF SPERMATOZOA ON GAMETE TRANSPORT AND UNION

Trial No.	Total No. Oviducts	Total No. Ova Transferred	% Total Ova Recovered	% Ova Recovered From Oviduct
4	26	269	53	46[a]
5	8	88	72	96[a]
6	8	89	67	100[a]
7	12	129	65	100[a]

a. Number of ova recovered in the oviduct divided by the total number of ova recovered.

Trial 6:- Effect of Surgical Insemination Without Ligation

Surgical insemination without ligation had no apparent effect upon transport of ova or spermatozoa. Sixty (67%) out of 89 ova were recovered all of which were in the oviduct (Table 6). Only two of eight oviducts contained ova with spermatozoa on them. Six pig ova and four rabbit ova were recovered with means of three and two spermatozoa attached, respectively. Spermatozoa were present in seven uterine smears. Survival of spermatozoa was improved as five of eight uteri contained motile spermatozoa (Appendix Table 2).

Trial 7:- Effect of Species of Spermatozoa

Substitution of rabbit semen for boar semen in this trial yielded an opportunity to observe attachment of spermatozoa. All 66 percent of ova recovered were in the oviduct of which, 80 percent were fertilized (Table 6). A mean of 48 rabbit spermatozoa were observed on recovered rabbit ova as compared with a mean of 14 on pig ova. Totals of 59 (74%) pig ova and 44 (100%) rabbit ova had spermatozoa associated with them. The rabbit spermatozoa on pig ova appeared to be either stuck in the mucin layer or at least held on to the zona pellucida by the mucin.

DISCUSSION

Mammalian spermatozoa can be transported up the genital tract of unrelated species and can be capacitated and fertilize ova in a closely related species. However, the degree of relationship between species appears to have influenced the detail as well as the end point of many investigations.

Neither the quantitative aspects of foreign spermatozoa transport, total numbers of spermatozoa transported, nor the presence of spermatozoa in the ampulla of an unrelated species have been reported. Experiment I was designed to determine these two factors.

Boar, ram, bull and human spermatozoa were observed in the oviducts of at least three of the eight does in each insemination group. These results are somewhat lower than those reported by Howe and Black (1963). Immotile rat, rabbit, bull and human spermatozoa were inseminated into the uterus of oestrous rats and then their cervix was ligated. Foreign spermatozoa were observed in atleast one oviduct of each animal. It would appear that the uterotubal junction of the rabbit is more selective than that of the rat. The basis for selection did not appear to be any physical characteristic of the foreign spermatozoa.

Motility did not affect transport as ram spermatozoa had the lowest initial motility rating but was transported in seven of eight does as compared to only three of eight does for the other foreign spermatozoa. Previous work in other species of mammals has shown that motility is not essential. Van Denmark (1953) reported that immotile bull spermatozoa were transported up the bovine genital tract almost as well as motile spermatozoa. Howe and Black (1963) also demonstrated that motility is not necessary for transport in the rat.

As in the work of Howe and Black (1963) and Phillips and Andrews (1937), the latter authors, having observed passage of rat spermatozoa through the uterotubal junction of the ewe, gross spermatozoan morphology did not influence the selectivity of the uterotubal junction. Human spermatozoa were different morphologically, particularly head shape and size, from all other species but were transported into the oviducts of as many animals as boar or bull spermatozoa.

Chang (1951) concluded that when does were inseminated with a minimum of 10×10^6 spermatozoa the number of spermatozoa transported into the oviduct was relatively constant. Similarly, in the present project, number of foreign spermatozoa inseminated had no apparent effect on the number of oviducts containing foreign spermatozoa or number of spermatozoa transported both within and between species.

Transport of any foreign spermatozoa resulted in transport similar in number to that of rabbit spermatozoa. Numbers of rabbit spermatozoa transported were much lower than that previously reported. Austin (1948), Chang (1951) and Braden (1953) recovered 4,500 to 5,400 rabbit spermatozoa from the oviducts of does 10 to 14 hours after mating or insemination. In contrast, 691 rabbit spermatozoa were recovered from the oviducts of does 12 hours after surgical insemination in the present study. The spermatozoan recovery technique, although similar to previous work (Braden, 1953), may have accounted for the low numbers of spermatozoa observed. Although cervical ligation improved spermatozoan transport in rats (Howe and Black, 1963), it may have adversely affected transport in rabbits in the present study.

Since motility, morphology or number of spermatozoa inseminated did not affect transport, some chemical characteristic of the semen or the physiological status of the doe may have been responsible.

Eliasson (1965) postulated that prostaglandin, a substance found in seminal plasma, aided spermatozoan transport by stimulating smooth muscle activity, thus increasing uterine motility. A recent review (von Euler and Eliasson, 1967) indicated that human semen contained very high amounts of prostaglandin, ram semen intermediate amounts, boar semen a low amount and bull and rabbit semen contained no prostaglandin. Although number of spermatozoa inseminated had no apparent affect on transport, its combination with prostaglandin content may be a partial expalanation of the transport of foreign spermatozoa, particularly ram and bull spermatozoa. High numbers of ram and bull spermatozoa were inseminated but high numbers of ram spermatozoa were transported and low numbers of bull spermatozoa.

The physiological status of the does in this experiment may have affected transport of foreign spermatozoa. Braden (1953) recovered extremely high numbers of rabbit spermatozoa from the oviducts of approximately eight percent of mated does. He postulated that the lack of or lowered level of discrimination against spermatozoa by the uterotubal junction in these cases, was due to faulty spermatozoan transport mechanisms in individual does. Another possibility could be that due to random selection of does in Experiment I, approximately 40 percent were in the non-follicular phase of the cycle when selection against spermatozoa may be minimal. Approximately 40 percent of does inseminated with human, boar or bull semen showed transport.

Overall, it appeared that transport of foreign spermatozoa through the uterotubal junction of the rabbit was affected by one, or a combination of three factors, those being; number of spermatozoa inseminated, amount of prostaglandin in the seminal plasma and level of discrimination against spermatozoa exhibited by the uterotubal junction.

Observations of spermatozoan viability in the oviduct were not possible, thus survival was based on motility of uterine spermatozoa. Species that were transported in large numbers also seemed to survive well, but survival was not essential as similar numbers of boar and human spermatozoa were transported but the latter survived significantly better than the former.

Evaluation of transport of foreign spermatozoa to the ampulla was based on the attachment of spermatozoa to rabbit ova. Normal appearing attachment or penetration of rabbit ova by foreign spermatozoa was not observed. Foreign spermatozoa associated with rabbit ova were either stuck in cumulus cells, attached to a mucin layer or lying in a mucin layer. This inability to attach to rabbit ova was particularly noticeable in does inseminated with ram semen as a high proportion of recovered ova were nude and free of mucin. As a result, ram spermatozoa were observed on the ova from four of fourteen oviducts containing spermatozoa. Thus, foreign spermatozoa are transported to the ampulla if they are transported through the uterotubal junction but cannot attach to or penetrate rabbit ova.

Upon establishing that foreign spermatozoa are transported into the oviduct of the rabbit the next phase to test was capacitation of spermatozoa. Assuming that ova play a passive role in the union of gametes, ovulated and follicular ova from pigs, sheep and rabbits were transferred in the oviducts of does inseminated with homologous semen. This technique was used in Experiment II to evaluate transport of foreign ova and capacitation of foreign spermatozoa, based on penetration of homologous ova in the genital tract of the rabbit.

Transport of ova varied both within and between species. The proportion of ovulated ova recovered was greater than that of follicular ova for all three species. Similarly, the proportion of ovulated rabbit and sheep ova recovered in the oviduct was greater than the proportion of follicular rabbit and sheep ova. The majority of ovulated rabbit and pig ova that were transferred, were in a cumulus mass, whereas, follicular ova of the same species were transferred as a group of individuals. The

greater size of a cumulus mass appeared to aid transport and recovery by retaining the ova in the oviduct after transfer and slowing down transport through the oviduct. Similarly, Dickmann (1964) recovered 84 percent of the cumulus-intact ova but only 74 percent of the cumulus-devoid ova that were transferred into the left oviduct of mated does.

The proportion of ovulated rabbit and sheep ova recovered and the proportion of those found in the oviduct were significantly greater than ovulated pig ova from Trial 3 ($P < 0.01$). Only follicular sheep ova were significantly greater than follicular pig ova in the preceeding two categories but the follicular sheep ova were recovered for transfer six to eight hours before ovulation of the donor and as such were more like ovulated ova. Since no sheep ova were transferred in a cumulus mass but recovery rate and retention in the oviduct were very good, it appeared that ova transport mechanisms also involve the type of cumulus surrounding ova.

Ovulation of recipient does increased both recovery rate and more importantly the proportion of pig ova recovered in the oviduct. Non-ligation of the uterine horns did not affect ova transport but did demonstrate that unrecovered ova were being lost out of the ovarian end of the oviduct at the time of transfer or shortly after.

The mechanisms involved in removal of cumulus cells and deposition of mucin are influenced in a manner similar to ova transport mechanisms. The cumulus cells were removed from ovulated ova easier and better than from follicular ova. Perhaps as a result of more rapid cumulus removal or the nature of the surface of the zona pellucida, ovulated ova also were surrounded by more mucin than follicular ova. Again, similar to transport results, rabbit ova, ovulated or follicular were surrounded by more mucin than ovulated or follicular sheep ova which were surrounded by more mucin than pig ova. Ovulation of recipient does resulted in recovery of pig ova with more mucin on them but the native ova so ovulated were surrounded by twice as much mucin as the pig ova.

Transport of ova was influenced by species of ova, type of cumulus cells and amount of cumulus cells.

The mechanisms involved in removal of cumulus cells and deposition of mucin are regulated by species of ova and type of cumulus cells.

Survival of ova was based on amount of segmentation of ovulated ova and nuclear maturation of follicular ova. Less than five percent of the ovulated pig or sheep ova showed any degeneration but more importantly, 74 percent of the follicular pig ova appeared to be undergoing nuclear maturation. A similar portion of the follicular rabbit ova had matured but the low level of fertility was due to the mucin being laid down prior to nuclear maturation. Chang (1955) also observed very low fertility of transferred follicular rabbit ova.

The presence of homologous ova in the oviduct did not affect transport of spermatozoa. The proportion of oviducts containing ova with spermatozoa on them remained approximately the same as in Experiment I. Ligation of uteri did not significantly affect spermatozoan transport, nor did ovulation of recipient does.

Since foreign spermatozoa were only transported in 20 percent of the does, capacitation of spermatozoa and penetration of ova were difficult to assess. At no time were foreign spermatozoa observed either in the zona pellucida or inside ova of any species. All spermatozoa appeared to be either stuck in or on the mucin layer. In Trial 7, 87 percent of pig ova had rabbit spermatozoa stuck in the mucin layer, but rabbit ova had a higher mean number of spermatozoa associated with them. This was not considered to be a chemotoxic effect as rabbit spermatozoa could not attach to, or penetrate, the zona pellucida of pig ova. Similarly, in Experiment I and Trials 5 and 6 of Experiment II, boar

spermatozoa could not penetrate the zona pellucida of rabbit ova. The greater number of rabbit spermatozoa cn rabbit ova was due to the number of spermatozoa in the zona pellucida and perivitelline space. On the basis of lack of ability to penetrate ova of their own species, boar and ram spermatozoa could not be capacitated in the rabbit.

Similarly, after the commencement of this project, the inability of spermatozoa to become capacitated in a foreign tract was reported by other workers. Edwards et al. (1966) found that human spermatozoa incubated in a rabbit uterus for one hour failed to penetrate human follicular oocytes when both were incubated together in vitro for up to 35 hours. Bedford and Shalkovsky (1967) incubated rabbit spermatozoa in the uterus of cats, ferrets, guinea pigs and rats. The spermatozoa did not survive longer than 12 hours in the first three species and did not fertilize any rabbit ova when inseminated into the oviduct of a doe 13 and three-quarters to 14 and a half hours after an ovulating dose of HCG. On the other hand, rabbit spermatozoa survived up to 48 hours in the rat uterus and when inseminated after five to 18 hours of incubation, fertilized three percent of the ova indicating at least partial capacitation.

Despite discouraging capacitation data, 23 percent of the pig ova recovered in Trial 2 appeared to be fertilized. Trial 4 technique was used to increase the fertilization rate but instead, it dropped to four percent indicating that the cleavage was probably segmentation due to age of the ova or some aspect of abattoir processing. Also, none of the cleaved ova in Trial 4 had spermatozoa associated with them.

In summary, it may be said that foreign gametes were transported in the genital tract of the rabbit. However, union and fertilization was prevented by the spermatozoa not being capacitated or if they were capacitated, they were unable to penetrate the ova due to some effect of rabbit oviduct on the zona pellucida.

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APPENDIX

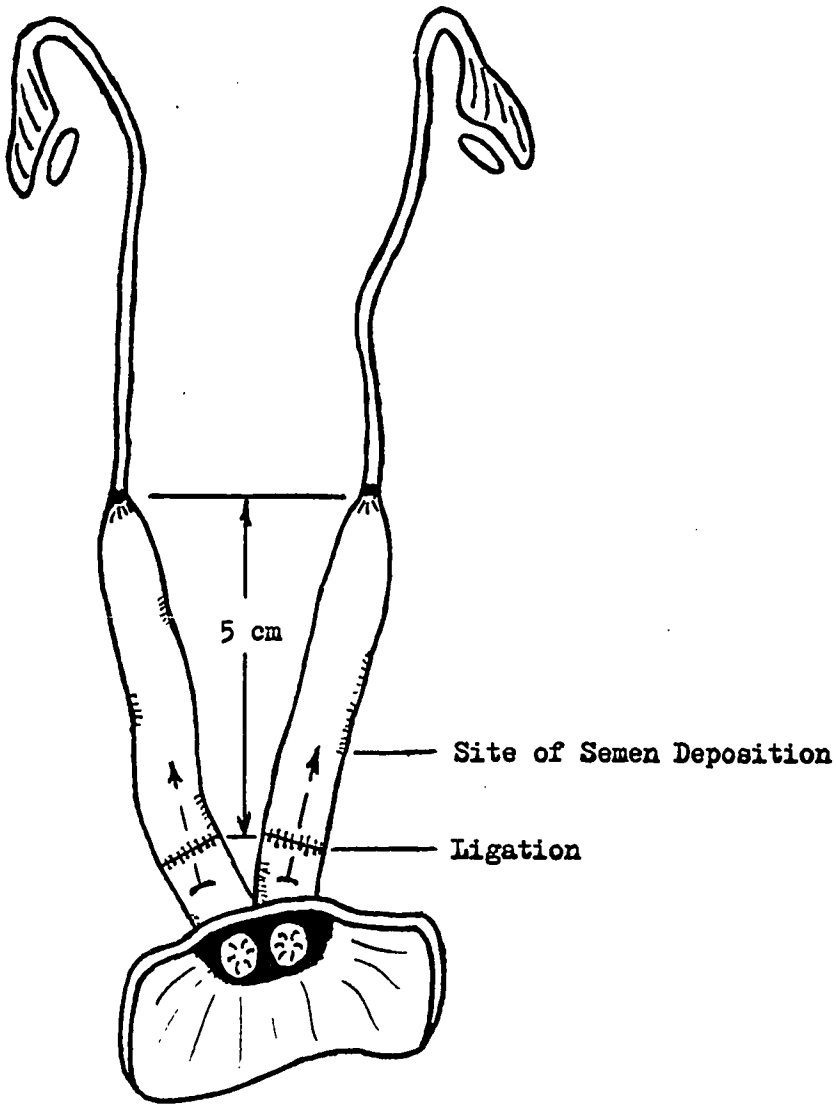


Figure 1. Diagramatic Representation of the Genital Tract of the Female Rabbit.

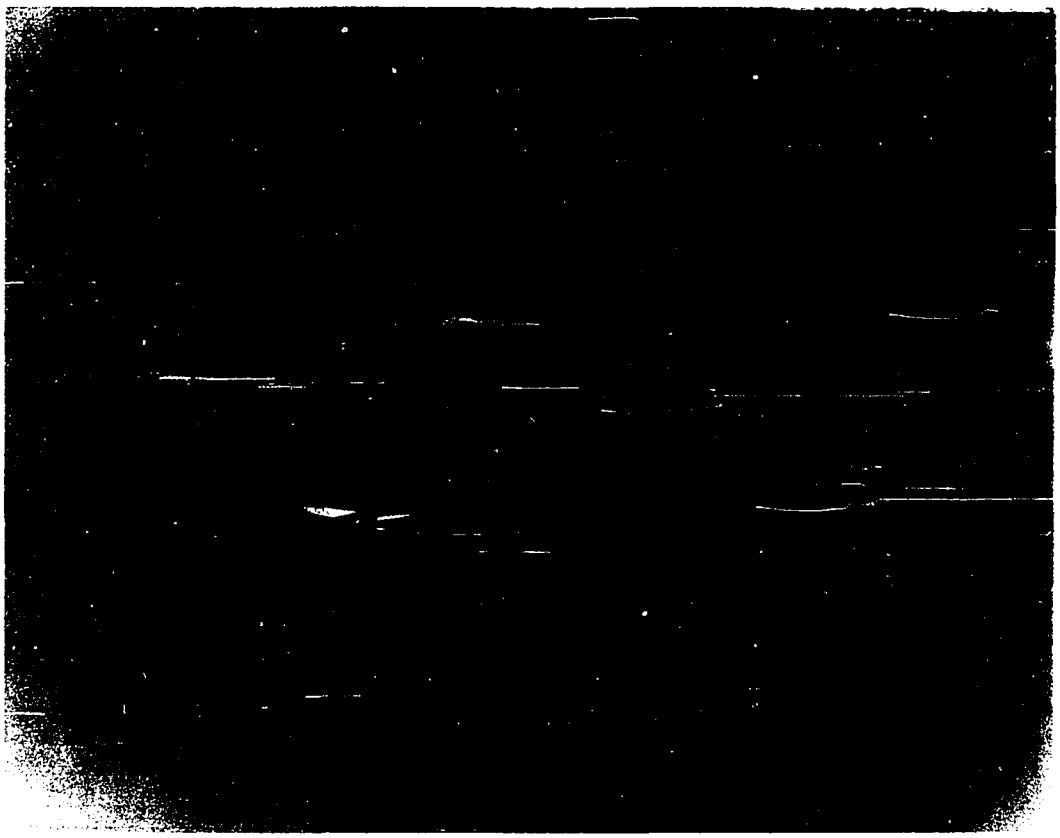


Figure 2. Equipment used for Manipulating Ova.
A - gridded plastic petri dish
B - 20 ml syringe
C & D - flushing needles
E - Iris scissors
F - forceps
G - dissecting needles
H - microsyringe and glass tip

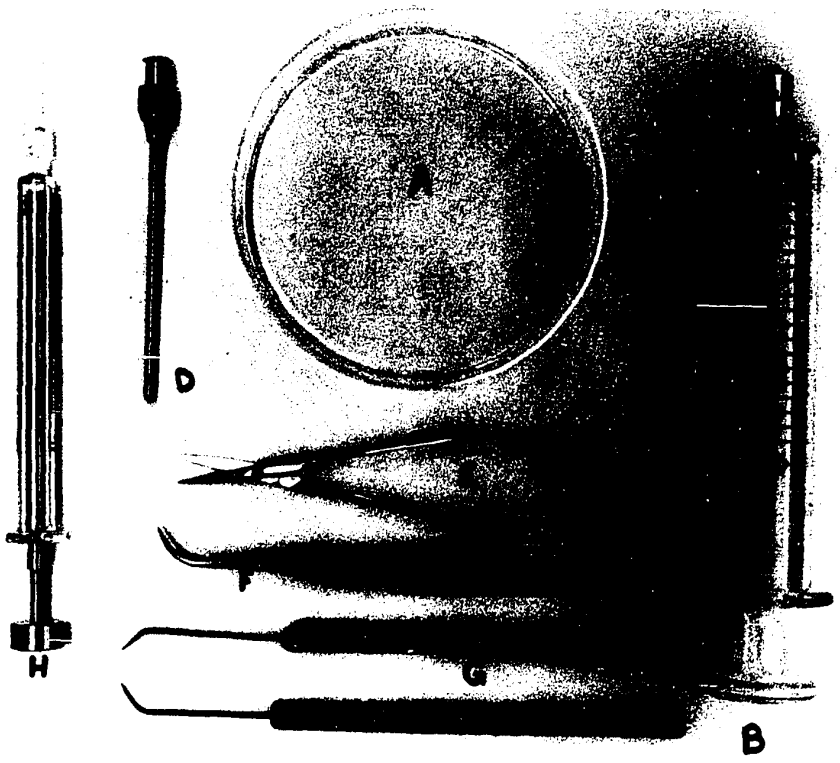


Figure 4. Equipment used for Manipulating Ova.
A - gridded plastic petri dish
B - 20 ml syringe
C & D - flushing needles
E - Iris scissors
F - forceps
G - dissecting needles
H - microsyringe and glass tip

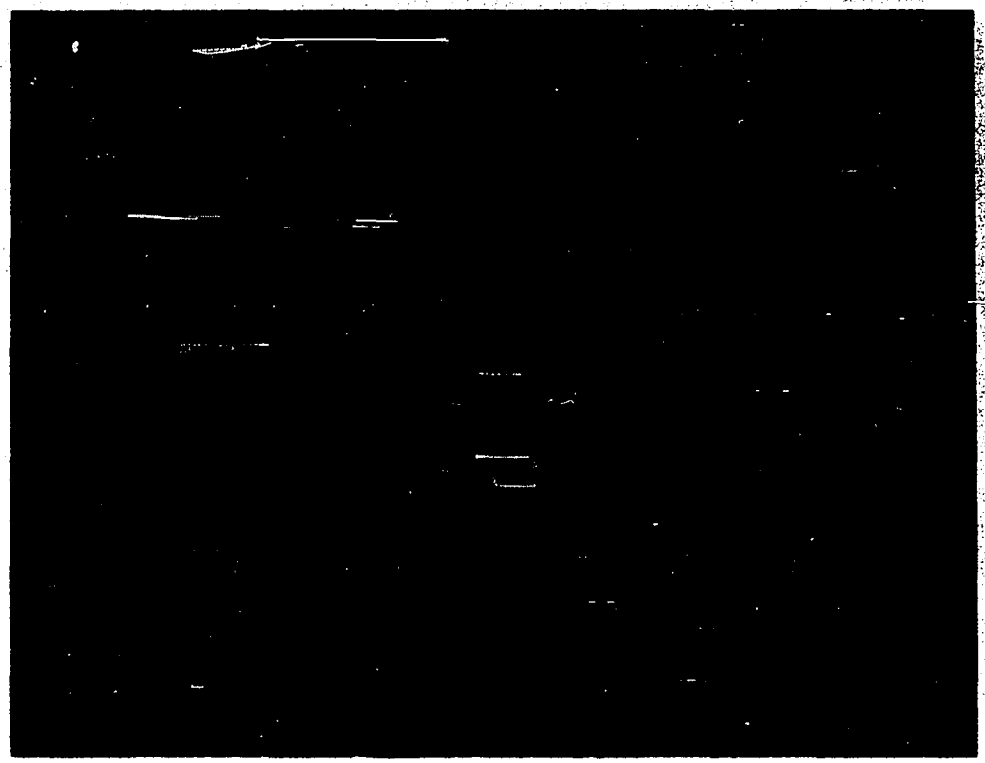


Figure 3. Bright field photomicrograph of four-celled rabbit ovum with rabbit spermatozoa attached to or penetrating the zona pellucida 500x

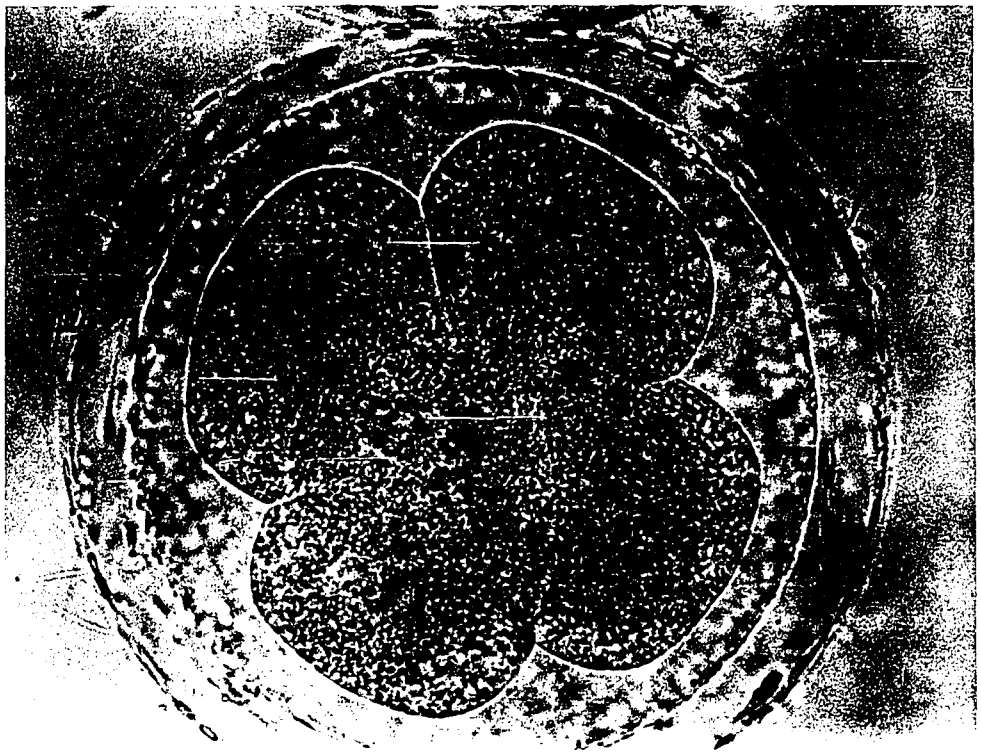


Figure 3 Bright field photomicrograph of four-celled rabbit ovum with rabbit spermatozoa attached to or penetrating the zona pellucida (x)

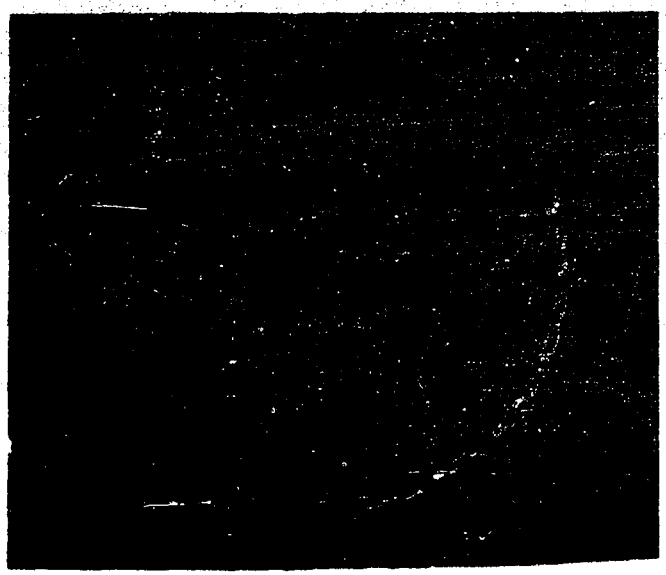


Figure 4. Interference constrast photomicrograph of an unfertilized sheep ovum with ram spermatozoa stuck on or in the mucin layer 300x

Figure 5. Interference contrast photomicrograph of an unfertilized sheep ovum with ram spermatozoa lodged in the cumulus cells 300x

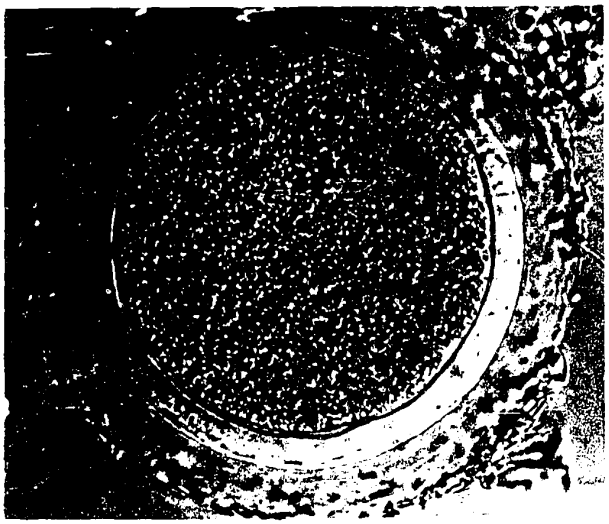


Figure 4. Interference constrast photomicrograph of an unfertilized sheep ovum with ram spermatozoa stuck on or in the mucin layer 300x

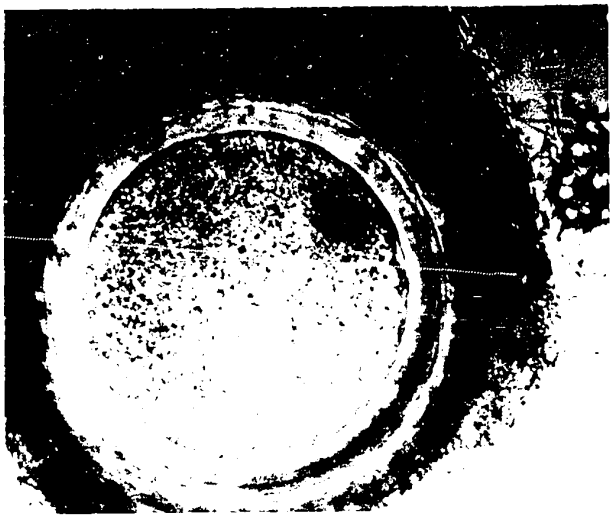


Figure 5. Interference contrast photomicrograph of an unfertilized sheep ovum with ram spermatozoa lodged in the cumulus cells 300x

Figure 6. Interference contrast photomicrograph of two follicular rabbit ova that were recovered from the left uterine horn and oviduct of the same animal.

Single-cell ovum - recovered in the uterus
Two-cell ovum - recovered in the oviduct
400x

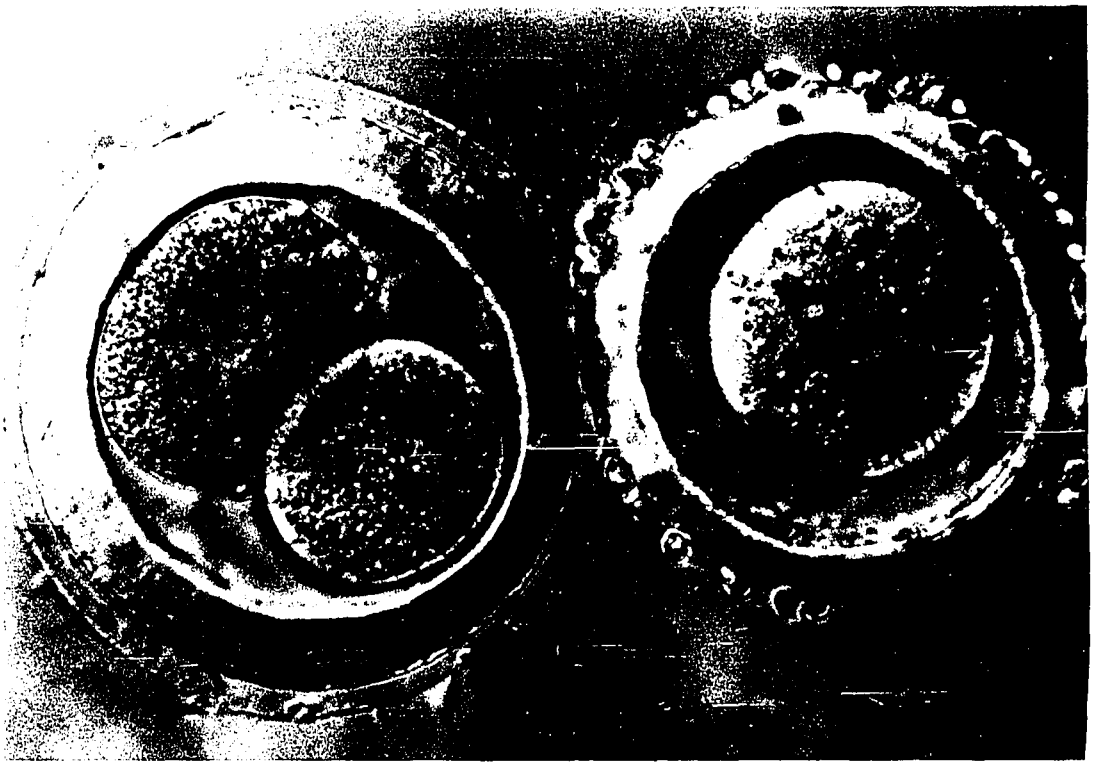


Figure 1. Interference contrast photomicrograph of two follicular cohort ova that were recovered from the left ovaries of two red colobus monkeys.

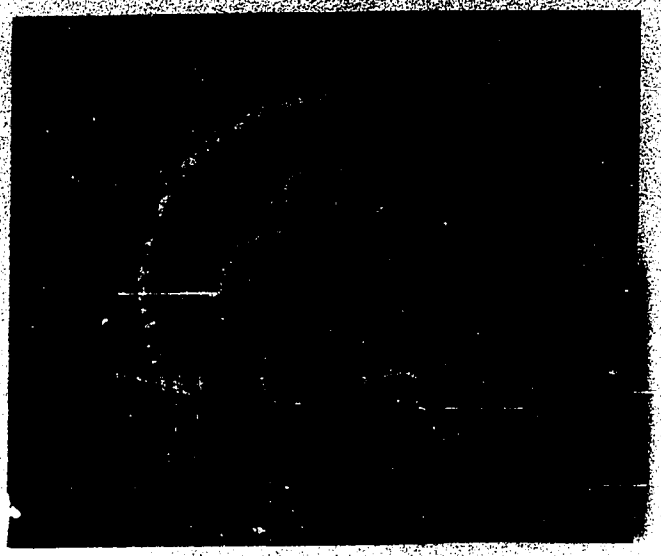


Figure 7. Interference contrast photomicrograph of six-cell ovulated rabbit ovum with mucin coat 300x

Figure 8. Interference contrast photomicrograph of two-cell follicular rabbit ovum with mucin coat 300x

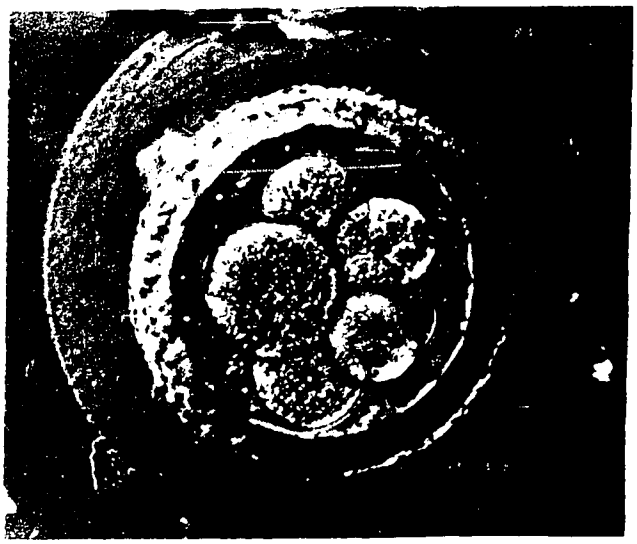


Figure 7. Interference contrast photomicrograph of six-cell ovulated rabbit ovum with mucin coat 300x

Figure 8. Interference contrast photomicrograph of two-cell follicular rabbit ovum with mucin coat 300x

Figure 9. Phase contrast photomicrograph of a two-cell pig ovum - before fixing and staining 450x

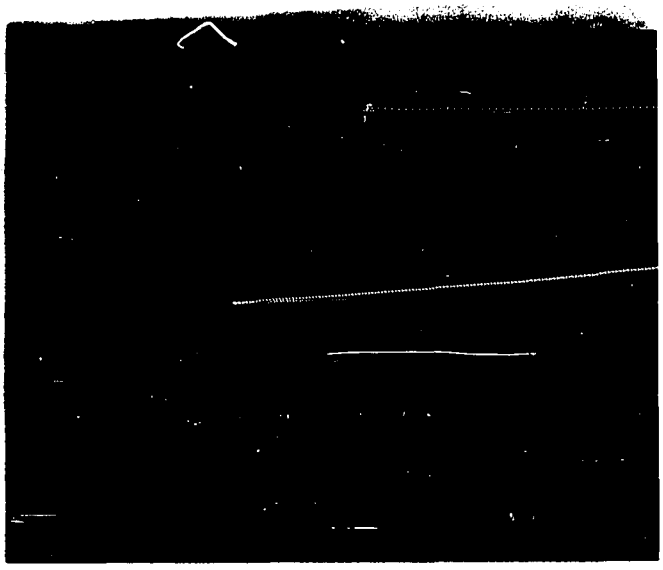


Figure 10. Phase contrast photomicrograph of a two-cell pig ovum - after fixing and staining 450x

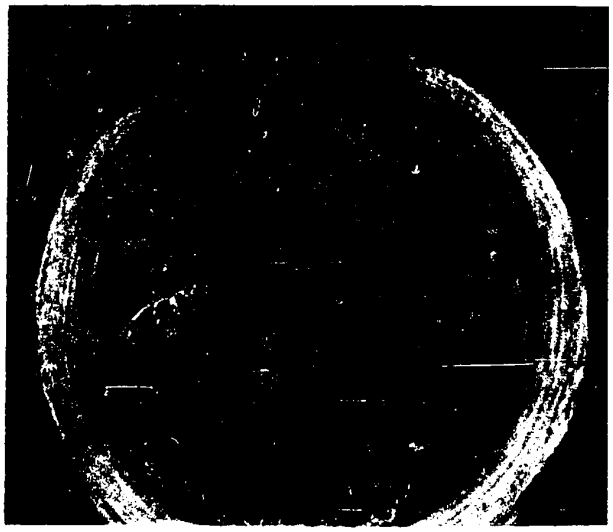


Figure 9. Phase contrast photomicrograph of a two-cell pig ovum - before fixing and staining 450x

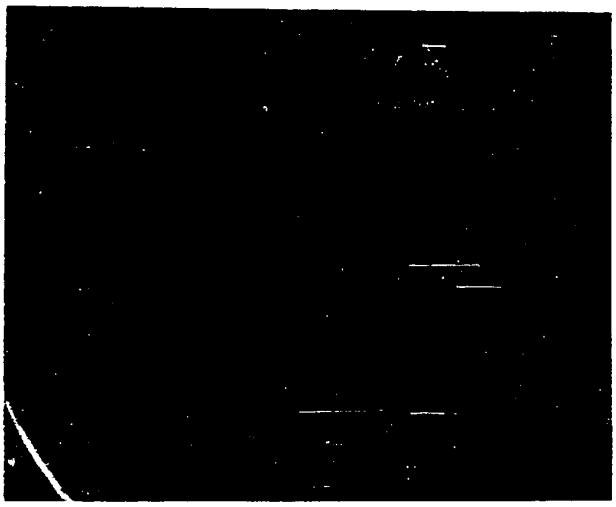


Figure 10. Phase contrast photomicrograph of a two-cell pig ovum - after fixing and staining 450x

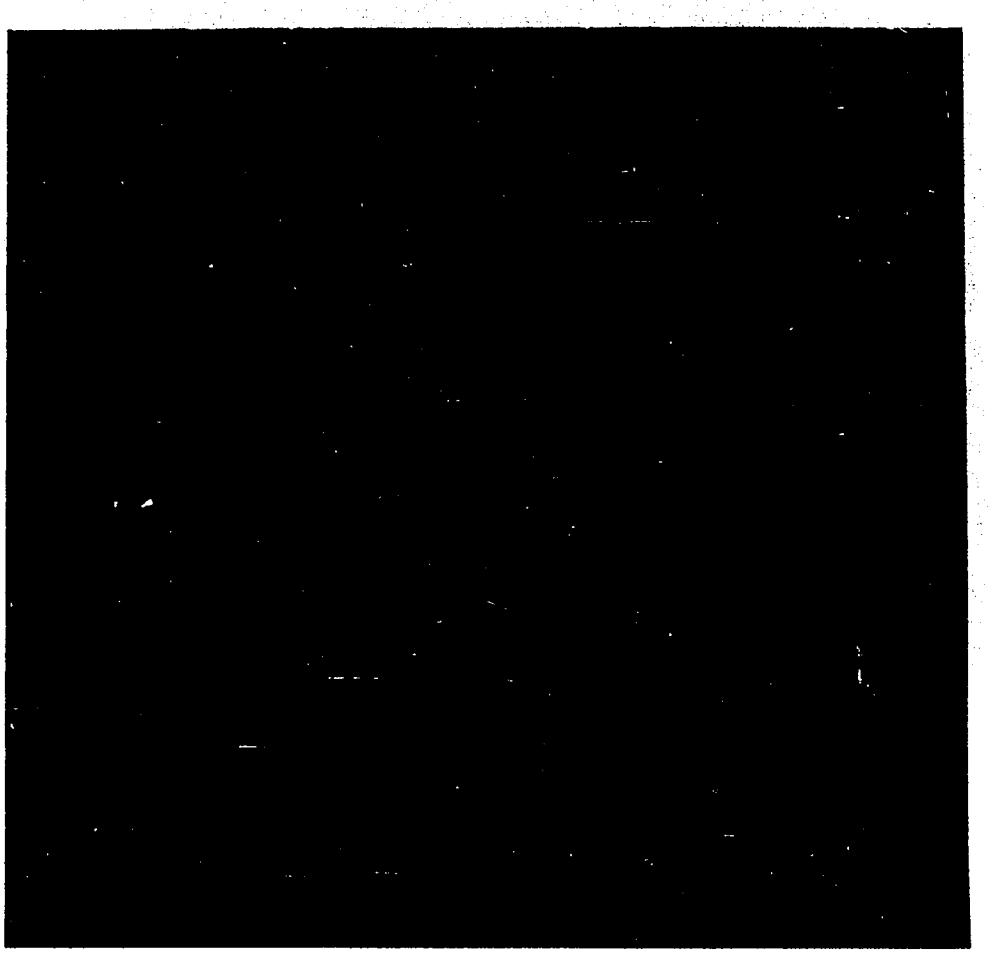
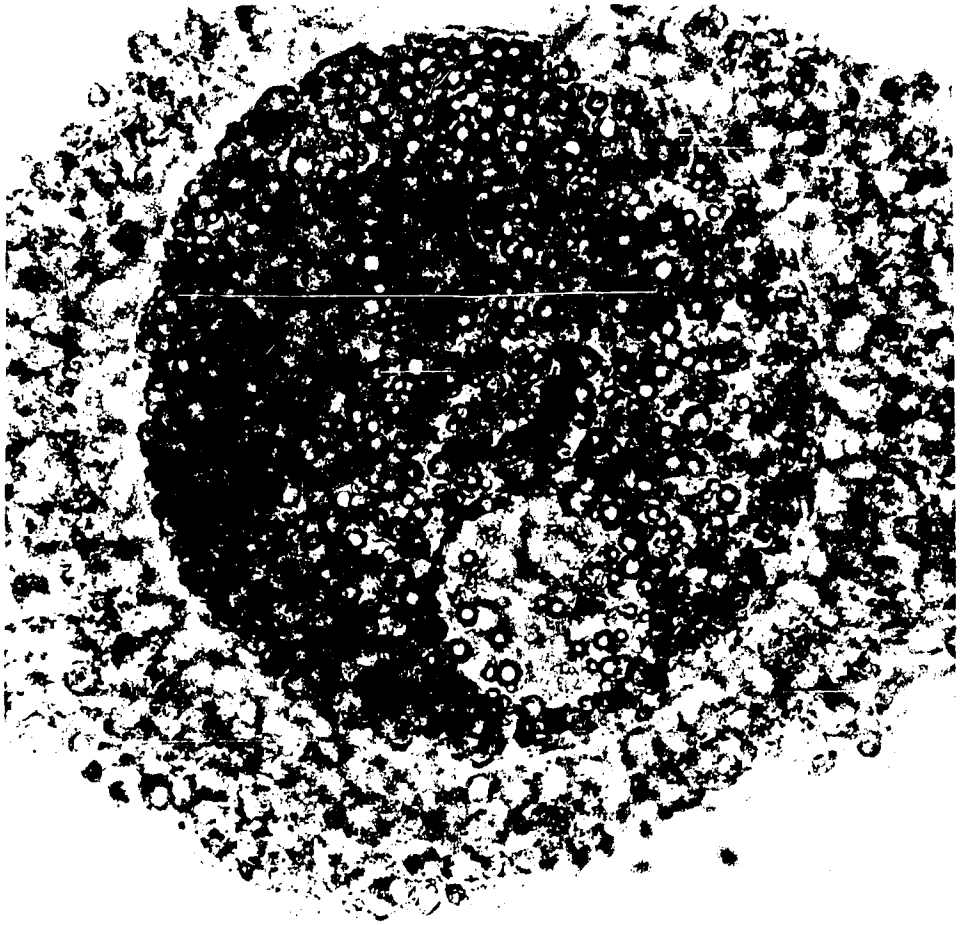


Figure 11. Phase contrast photomicrograph of a control follicular pig ovum with a vesicular nucleus 550x



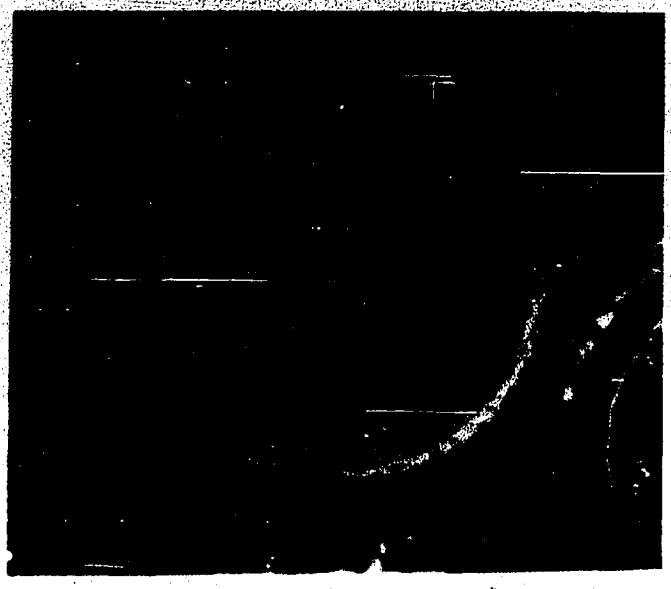


Figure 12. Interference contrast photomicrograph of a pig ovum with slight mucin coat 300x

Figure 13. Interference contrast photomicrograph of a pig ovum with mucin coat 300x

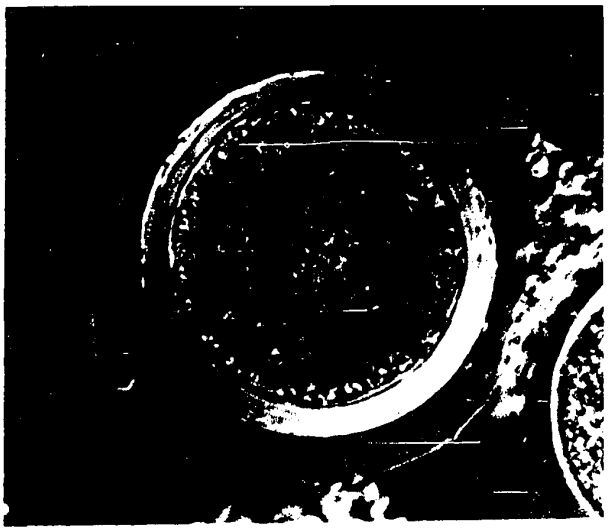


Figure 12. Interference contrast photomicrograph of a pig ovum with slight mucin coat 300x

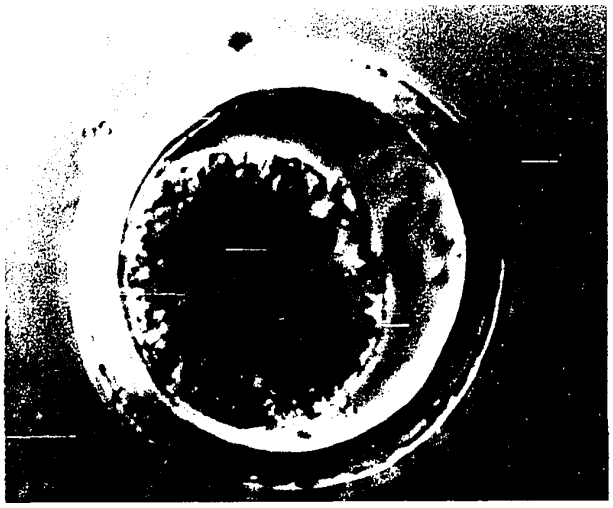


Figure 13. Interference contrast photomicrograph of a pig ovum with mucin coat 300x

Appendix Table 1. VOLUME AND QUANTITY OF SEMEN INSEMINATED IN EXPERIMENT I

Species of Sperm Inseminated	Animals Inseminated	Vol. of Semen Inseminated/Horn	Sperm Inseminated
	No.	ml	$x10^6$
Rabbit	8	0.25	175
Boar	8	0.25	165
Ram	8	0.25	1560
Bull	8	0.25	808
Human	8	0.25	144

Appendix Table 2. TRANSPORT OF RABBIT, BOAR AND RAM SPERMATOZOA IN EXPERIMENT II

Trial Number	Animals Inseminated	No. of Sperm Inseminated/Horn	Animals with Sperm on Ova	Total ova in Oviduct with Sperm Associated	
	No.	$x10^6$	No.	No.	%
1	14	67.3	14	78	95.1
2	5	589.7	3	15	24.6
3	16	83.5	5	23	29.1
4	13	72.2	8	38	56.7
5	4	142.5	1	2	3.2
6	4	67.3	2	6	10.0
7[a]	6	40.2	6	44	100.0
7[b]	6	40.2	4	59	74.2

a - Spermatozoa on rabbit ova

b - Spermatozoa on pig ova

Appendix Table 3. SURVIVAL OF RABBIT, BOAR AND RAM SPERMATOZOA IN EXPERIMENT II

Trial Number	Horns Inseminated	Sperms Motile	Rate of Motility	Horns Containing Motile Sperms
	No.	%		No.
1	28	61.7	1.7	18
2	10	44.0	1.8	0
3	32	72.5	2.4	8
4	26	72.4	2.5	2
5	8	67.5	1.9	0
6	8	67.5	1.9	5
7	12	66.3	1.5	10