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STUDIES RELATING TO OVUM TRANSPLANTATION

in the
DOMESTIC GOAT (Capra hircus)

by
BRUCE PORTWAY

A THESIS SUBMITTED IN PARTIAL
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BIOGRAPHICAL SKETCH

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INTRODUCTION

The possibility of increasing the number of progeny from a genetically desirable dam forms the basis of the work done with the transplantation of ova. From the dairy cow, the number of calves which may be expected during her lifetime is about six. Thus the number of progeny from a high producing dam is limited under conventional methods of husbandry.

A system whereby the number of progeny per year could be increased many times would have enormous economic importance. In the case of artificial insemination, progeny sired by bulls with desirable genetic characters have been increased manyfold.

In view of these facts, research since 1927 has been directed towards the possibility of obtaining living young following the transplantation of ova from donor to recipient. Many workers have interested themselves in the problems involved, and different species have been used as experimental animals.

It is of interest to note that the initial publication on this work is that by Heape (1) in 1890, in which he demonstrated that transferred rabbit ova would develop in the uterus of a foster mother following transplantation. Since Heape published his findings, reports on transplantations were lacking until Warwick et al. (2, 3) in 1934 reported success with sheep and goats. Subsequently, much interest has been shown in the subject.

Workers in different parts of the world have published experimental details of work with various species. Pincus (4, 5)
and Chang (6, 7), using rabbits and mice, investigated the hormonal problems involved in ova transplants. Cattle have been used by American and English investigators to try to obtain a functional system of transplantation which can be commercially applied. Sheep have been used extensively by English and Russian workers. One case of successful transplantation of ova in the pig has been published in Russian literature (8).

Since Warwick et al. (2) reported their early success with the domestic goat, only one other paper has been published giving details of transplantations in this species. This was by Russian investigators. However, American workers have used the goat as a subject for hormonal studies (9, 10).

Superovulation may be defined as an increase in the number of ova released at ovulation over the number which are characteristically released by the species. To increase the progeny from a given dam to an appreciable extent, it is necessary to increase the number of viable ova shed at ovulation. In this respect superovulation is inseparable from a practical system of transplantation. By the judicious use of hormones with gonadotrophic effects, superovulation can be achieved, and has been reported in mice (11), rats (12), rabbits (5), sheep (13) and cattle (14). A review of the literature has uncovered no report concerning superovulation of the domestic goat.

The underlying mechanism involved in the process of superovulation is now understood reasonably well. It involves the
interaction of two hormones with gonadotrophic effects, one of which has follicle stimulating effects and the other luteinising effects. These are known as "Follicle Stimulating Hormone" (F.S.H.), and "Luteinising Hormone" (L.H.) respectively. The former is derived from pituitary and/or chorionic membrane, whereas the latter is only pituitary in origin, in the domestic animals. A balance exists normally between these hormones. Superovulation can be brought about by upsetting this balance.

Transplantations of ova have been carried out successfully in all of the species mentioned previously. Simplicity of procedures involved is a necessary end result of the present and past investigations if the process is to be extensively used on a commercial basis. Complex surgical procedures are still necessary to ensure success, but non-surgical techniques are being investigated. Once the procedures have been simplified, there are good prospects that the livestock industry will benefit considerably.

Only two reports (2, 8) have been published dealing with transplantations in the goat. Much data has been reported on a closely allied species viz., sheep (Ovis aries L.), and this has been used as a basis for the following experimental work with goats (15, 16). In this thesis work has been planned to observe and record all possible details concerning physical factors concerned with the breeding season and oestrous cycles of goats in Southern Ontario. In addition, experiments have been carried out to give details of superovulation procedures, details of ova
and techniques of transplantation.

Published information concerning reproduction in the domestic goat (Capra hircus L.) in Ontario is non-existent. This has necessitated initial observations of physical factors as a pre-requisite for hormonal studies and transplantation of ova. In view of the foregoing, the following studies were carried out to increase the data for the domestic goat specifically, and as a contribution to the general information on superovulation and ovum transplantation in domestic animals.
REVIEW OF THE LITERATURE

The first available information concerning the successful transplantation of ova is that by Heape (1) in 1890, which described the development of transferred rabbit ova in the uteri of foster mothers. Biedl et al. (17) confirmed this work in 1922. Engle (18) in 1927 demonstrated superovulation of mice by the use of a gonadotrophin with follicle stimulating effects. Warwick et al. (3) in 1934 however, published the first report of living young resulting from transplanted ova, in domestic animals. They produced live lambs and kids from auto-transfers, but failed to produce live progeny from cross transfers between these two species. Using cattle, Casida et al. (19) in 1943, first demonstrated that superovulation of this species could be achieved by the injection of pituitary gonadotrophins.

Since the early reports, little interest was shown in superovulation and transplantation of ova, and it was not until about 1947 that this interest was revived.

Willett et al. (20) in 1951, reported the successful transplantation of a fertilized bovine ovum. These workers synchronized the oestrous cycles of donor and recipient animals by the daily injection of progesterone to delay the onset of oestrus in the recipients and to allow donors to undergo normal cycles. Five days after the onset of oestrus in the donor, collections of ova were made, and transfers to recipients were carried out.
Willett et al. (21) in 1933 reported another successful transplantation of bovine ova, when they obtained three calves from five transplanted ova. On the fourth day following a second artificial insemination the donor animals were slaughtered and flushing of the fallopian tubes gave ova of the eight, twelve and fourteen cell stage, for transfer.

Much of the early work dealing with the principles involved in superovulation and transplantation was done with laboratory animals. Beatty (11) in 1951 reported the successful transplantation of mouse ova. Other workers (22, 23, 12) have published work involving recovery and culture of mouse ova. The term "inovulation" to describe the transplantation of fertilized ova from donor to recipient has been suggested by Beatty (11).

The rabbit has also been used by a number of workers in recent years to obtain basic information concerning the processes of superovulation, ovum release, migration and development. Chang (24, 25, 26, 7), Clewe et al. (27) and Fincus (4, 5) have developed collection, storage and transfer techniques for the domestic rabbit. Much of this information has been used by other workers and applied with modifications to domestic animals.

The dairy cow presents the best possibilities for the economic use of the techniques of transplantation, and this species has been used extensively for experimental purposes. Casida et al. (19) in 1943 were the first workers to describe the superovulation
of cattle, and they were followed by a number of other workers in this field (28, 14, 29, 30, 31, 32, 33). All these investigators depended for superovulation upon the follicle-stimulating and luteinising hormones of pituitary or chorionic origin. Willett (34) in 1953 summarised the publications of all workers in the field of superovulation up to that time. He stated that both follicle-stimulating hormone (F.S.H.) and luteinising hormone (L.H.) were necessary to achieve superovulation. The effect of the F.S.H. was to bring about maturation of a number of follicles and L.H. to bring about ovulation. Injected or exogenous F.S.H. can be used to produce follicular maturation and exogenous or endogenous L.H. can be responsible for rupture of the follicles and release of ova.

The maintenance of experimental cows for this work is expensive if slaughter is necessary. Consequently the attention of workers has been directed towards development of a system which allowed ova to be collected from living animals. In 1949, Rowson et al. (36) reported on a procedure for flushing the tips of the uterine cornua with a small volume of fluid (approx. twenty cc's each horn) to obtain the ova. This necessitated the use of a two-way catheter with an inflatable collar which was directed by rectal palpation to near the tip of the uterine horn via the cervix. The collar was inflated to seal off the tip of the uterine horn and this allowed lavage of the sealed tip. Some success with this method was obtained. It had the advantage of using convenient volumes of flushing fluid.
Dracy et al. (35) in 1950 evolved a system of uterine lavage to collect ova, which involved cannulation of the cervix and irrigation of the uterus with several hundred millilitres of fluid. Due to the large volumes of fluid needed, and the poor recovery of ova, the system was impractical.

Transplantation of fertilized ova presented further technical problems. Transfers can be obtained satisfactorily following laparotomy, but to establish the method on a commercial basis such skilled surgical procedures must be eliminated. Attempts by several workers (37, 28, 38, 39, 40) to obtain progeny from the transfer of fertilized ova by non-surgical means, have been unsuccessful so far in all species. These attempts have followed the technique of artificial insemination in that penetration of the cervix has been necessary to deposit the ova in the uterus.

Chang (7) using mice, and Beatty (11) using rabbits, found that for successful development of transferred ova, the interval between ovulation time of donor and recipient should be minimal. Ovulation times in both animals should be made to coincide as closely as possible. Collections of ova from cattle have been made on the fourth or fifth day after oestrus. At this time the donor has passed into the luteal phase of the cycle i.e. metoestrus. Penetration of the cervical canal during metoestrus is likely to result in uterine infection.

Rowson (28) stated that uterine infection seemed to be the
main barrier to the success of ova transfers via the cervix. This is due to the difficulty of maintaining asepsis during the procedure. Penetration of the cervix when endogenous luteinising hormone (L.H.) is high, has been demonstrated by Brock et al. (41), to be a time of minimal resistance to infection. Proliferation of the uterine mucosa in preparation for deciduoma reaction is directly referable to L.H. levels at the time of minimal resistance to infection. Artificial insemination in cattle is undertaken at a stage of the oestrous cycle when uterine resistance to infection is maximal because of the influence of endogenous oestrogens.

Lamming et al. (33) quote their unpublished attempts in cattle at aseptic deposition of ova in the uterus during oestrus, but with delayed release of ova. This work involved the enclosure of ova within a gelatin capsule attached to a small glass dart and embedded in the ovary. After absorption of the gelatin, the ova remained in the vicinity of the fimbria. These trials were unsuccessful.

Hunter et al. (13) in 1955, transferred nineteen ova to eighteen recipient ewes, and a total of eight lambs was born. These workers transferred ova from Border Leicester ewes to Welsh mountain ewes and produced lambs of the opposite breed to the recipient. Pregnancy resulted when oestrus in the recipients began from sixteen hours before to twenty hours after oestrus in the donors. Ova of the four cell, eight cell and sixteen cell stage were transferred. These were exposed to room temperature
for thirty to forty-five minutes before deposition within the donors. One ovum developed after exposure for seventy minutes to room temperature.

Rowson et al. (42) in 1957 transferred fifteen ova following two superovulations of a single donor ewe with subsequent birth of ten live lambs from ten recipients. This donor was mated naturally following a normal oestrus after the second P.M.S. treatment and produced a single lamb from the uninterrupted pregnancy. Thus a total of eleven live lambs were produced from a single ewe in one breeding season. Averill et al. (16) in 1958 transferred one hundred and twenty-seven fertilized sheep ova to ninety-one recipient ewes. Fifty-two ewes received ova of the six to sixteen cell stage, and forty-one of these gave birth to one or more lambs. Holding periods between collection and transfer reached a maximum of one hundred and fifteen minutes.

Development of ova in the recipient seems to be related to the site of placement in the genital tract, and according to the number of blastomeres present in the ovum at collection. All workers with sheep have found it necessary to employ surgical procedures for collection from donors and transfer to recipients.

Russian investigators (43, 44, 45, 46) working with sheep have undertaken transfers using Merino, Karakul, Chuntuk and Tsigai breeds. Lambs resulted from transferred fertilized ova and transferred unfertilized ova, followed by mating of the recipient to
obtain conceptions. Unilateral flushing of the oviducts of donors and recipients was accomplished and the ova transferred to the contralateral oviduct of the recipient. The flushed oviduct of the donor then became the recipient oviduct for ova from another donor. The effect of these procedures was to have each ewe acting as a donor and as a recipient simultaneously.

Lopyrin et al. (45) 1952, reported inter-species transfers of ova between sheep and goats, in which thirteen females of each species were used for transfers. Only one animal, a doe, gave birth to young, producing a viable kid and a stillborn lamb which was presumed to have died shortly before parturition, and which although small (1.2 Kg) had a fully developed coat. This case of inter-species transfer from sheep to goat, represents an advance on the work of Warwick et al. (3) in 1934, in which was reported small resorbing embryos of thirty, thirty and forty-five days respectively in three recipient ewes. They reported on five goat recipients of sheep ova, in which two older embryos were resorbing between thirty and fifty days after transfer, but three live embryos were present.

Since 1955 Hunter et al. (13) and Averill et al. (16) have investigated the possibilities of storage of sheep ova. This follows the early work by Chang in 1947, (24) and later in 1952 (25), with rabbits. Chang (24) produced evidence to show that temperature shock resulted in rabbit ova cooled rapidly to 0°C. or 5°C.,
but this effect was considerably lessened by slow cooling to the same temperatures. The same author in 1949 (25) published on the fertilizability of rabbit ova following storage.

In 1955, Averill et al. (47) published experiments in which sheep ova were transplanted to the uterus of a recipient rabbit for at least five days, after which time the developing ova were re-transplanted to the uterus of a recipient ewe, with apparently normal development from the two blastomeres to the eight day blastocyst stage. These workers also mention that species differences in rates of cleavage are inherent in the ova themselves, following simultaneous transfer of sheep and rabbit ova to a recipient doe. Marden et al. (48) in 1952, reported the aerial transport of rabbit ova from U.S.A. to England in vials of blood serum at 2 - 5°C., with subsequent development in a recipient doe.

Various storage media have been used including physiological saline and hen-egg extract at pH 7.2 - 7.4 (22), blood serum (8), Locke's solution (49), 0.8% sodium chloride buffered to pH 7.4 (50), Tyrode's solution (4) and Ringer's solution (42). These media have been developed and used to minimize damage to ova and to simulate conditions within the body as closely as possible. Blood serum has been used extensively and is regarded as satisfactory. Chang in 1949 (26) showed the presence of an ovicidal factor in the blood serum of some animals but not in others. Cow's blood contains this factor, but it is inactivated by heating at 55°C.,
for thirty-nine minutes or storing at ordinary refrigerator temperatures (3°C. approx.) for forty-eight hours.

Dracy (51) has pointed out that for any solution to be desirable as a flushing agent, it must be nearly transparent, and he suggests the use of a fluid having the same physiological properties as blood serum, and yet is transparent. Willett (34) in 1953 reviewed the main work on this question of media and concluded that the solutions in current use are fairly satisfactory for flushing and holding ova up to a few hours. Hunter et al. (13) and Rowson et al. (42) have restricted the media used to Ringer's solution and homologous serum, with good results in both cases. These workers made no attempt to maintain constant temperatures of the media holding the ova, providing transfer from donor to recipient was completed within fifty minutes. For transfers requiring longer holding time Averill et al. (16) made use of the dialysis chamber as described by Dott et al. (52) in 1953, but slightly modified. This chamber was maintained at 37°C. in a water bath.

Dracy (51) in 1953 published observations on the future of ova transfers in the light of problems current at that time, and listed the following factors which must be considered if ova transfers are to become a practical reality: economic aspects of the technique, simplicity of the procedures involved, and assurance that the correct offspring is born. Certain fundamentals pertaining
to these factors must be thoroughly investigated and understood. These include superovulation of the donor, synchronization of oestrus in donor and recipient, simple non-surgical methods for recovery of ova, suitable media for maintaining ova for long periods of time and simple methods for introduction of ova into recipients.

From the literature reviewed it is evident that investigations are slowly proceeding in several aspects of transplantation work and new light is being shed on various problems from time to time. The most recent of investigations is that of McLaren et al. (23) in 1958 concerning the in vitro culture of eight to sixteen cell embryos of mice. These embryos developed to term following transplantation to recipients. Two hundred and sixteen out of two hundred and forty-nine (87%) embryos developed to the blastocyst stage in vitro.

From the foregoing review of the literature there is a noticeable lack of information concerning the domestic goat. Only two references to transplantation of ova in the goat have been noted (3, 8). Furthermore, only two references to hormonal studies in the goat have been found (9, 10).
MATERIALS AND METHODS

In this study twenty does were used, during two breeding seasons. The fourteen Saanen, three Toggenburg and three Alpine does were maintained at the Ontario Veterinary College, Guelph, Ontario. Seven of the does bred naturally during the 1957-58 breeding season gave three female kids for experimental use during the 1958-59 breeding season. The does were of different ages, and originated in the herds of local goat breeders in Ontario.

In addition to the above does, three bucks were used. A Toggenburg and a Saanen were used for natural service. A Nubian buck had been vasectomized before reaching maturity and was allowed to run with the does in the early part of the 1958-59 breeding season. This Nubian buck was used from September to January to detect oestrus.

Mating was carried out by a fertile buck within twelve hours of the onset of oestrus and repeated when possible within the next twelve hours.

Each doe was identified by a coloured, numbered tag on a neck chain. Does were easily recognized in pens or in the field by this system. Tag colours varied with the breeding seasons. During the 1957-58 breeding season does were identified by yellow tags. Kids born during 1958 from the previous season's matings were tagged blue. All other does used in the 1958-59 experiments were tagged red.
Oestrus was determined in all cases by the behavioural response of the does to the vasectomized buck, namely bleating, tail wagging, following the buck and allowing other does or the buck to mount.

In one case (RED 13) artificial insemination was performed in addition to natural service by the Saanen buck. Semen was collected by means of an artificial vagina, and deposited in the vaginal fundus of the doe. This insemination was done to try to ensure fertilization of the ova, because the doe was a shy breeder and natural service was unsatisfactory.

Pregnant Mares' Serum (P.M.S.) (Antex brand) was used to effect superovulation of the does. This was supplied in vials containing 5,000 International Units (I.U.) of dry equine chorionic gonadotrophin, to be made up with diluent as required. This product was used during both breeding seasons. Initial attempts at superovulation using P.M.S. involved intravenous and subcutaneous injections. Only four does received injections via this route.

All does during the 1958-59 breeding season received intramuscular injections of 1,000 I.U.'s of P.M.S. This was administered on the thirteenth day following the onset of oestrus. No variation of dosage or time of injection was made due to the small number of experimental animals available. The criterion of superovulation was the presence of three or more corpora haemorrhagica

1 Ayerst, McKenna and Harrison Ltd., Montreal, Canada.
present on the ovaries.

Anaesthesia was induced and maintained by the use of intravenous Thiopental sodium (Pentothal)\(^2\) during 1957-58. In the experiments carried out in the 1958-59 season, induction of anaesthesia was also achieved by intravenous injection of "Pentothal"\(^2\), but maintenance was by ether (di-ethyl-ether) administered via an endotracheal tube attached to an open circuit anaesthetic machine.

Laparotomies in four cases during the 1957-58 season (Table A) were performed in the flank region. The flank approach gave reasonable access to the near-side genitalia, but manipulations of the far-side genitalia were extremely difficult. This site was therefore abandoned for the 1958-59 season, in favour of the pre-pubic midline site. All does during 1958-59 underwent surgery at this site. Laparotomies were carried out not sooner than seventy-two hours after the onset of oestrus, on the assumption that the ova had reached the uterus by this time.

After opening the peritoneum, a Balfour retractor was inserted and the internal genitalia were exteriorized and examined. Care had to be taken to avoid incising mammary tissue. Separation of the two mammary glands was possible, and this allowed the abdominal opening to extend from an immediate pre-pubic point to a point some three inches anterior.

Examination of each ovary and the fallopian tubes was carried out to detect adhesions or other abnormalities. Corpora

\(^2\) Abbott Laboratories Ltd., Montreal, Canada.
haemorrhagica were counted and recorded for each ovary. The procedure for flushing the tip of the uterine horn and the fallopian tubes was then carried out, to collect the released ova.

Ringer's solution was used to flush the fallopian tubes and for the holding of ova for examination or transplantation. This solution was prepared from the formula of Hunter et al. (13) which was given as:

- Sodium chloride 0.50 grams
- Potassium chloride 0.15 grams
- Sodium bicarbonate 0.20 grams
- Calcium chloride 0.12 grams
- Sodium dihydrogenphosphate 0.01 grams
- Water 1,000 millilitres

This was autoclaved and stored in rubber-stoppered one hundred millilitre bottles. This solution was also used to cleanse the polyethylene tubing of antiseptic solution (65% alcohol) prior to collecting ova. The polyethylene tubing had an internal diameter 0.062 inches and external diameter 0.082 inches, and was cut into approximately ten inch lengths. One end of this tubing was inserted through the fimbriated end of the fallopian tube for a distance of approximately one-half inch. The tubing was manually held in position between the thumb and index finger with the free end directed into a watch glass.

A 10 cc. syringe containing Ringer's solution at 37°C.
was attached to a sixteen gauge needle, one inch in length. This needle was passed through the wall of the uterus approximately one inch posterior to the tubo-uterine junction. With the tip of the needle in the lumen of the uterine horn, and the lumen of the uterine horn occluded posteriorly by digital pressure, flushing was commenced. Gentle pressure of fluid into the uterine horn forced the Ringer's solution into the watch glass via the polyethylene tubing, carrying with it the ova and cellular debris. This fluid in the watch glass was then examined under the dissecting microscope (45X) for the presence of ova.

If surgery was performed at a time when the ova were assumed to be in the fallopian tubes, puncture of the wall of the uterus was carried out close to the uterine ostium. Conversely, ova which were assumed to be in the uterine horn were collected by flushing a variable length of the horn. The above procedures were repeated for each uterine horn and fallopian tube. The volume of Ringer's solution used to flush each side varied from two to six millilitres.

Examination of the ova by means of the dissecting microscope revealed the numbers of ova and the stages of development. Cellular debris present in the fluid tended to cover up some of the ova, due to aggregation at the bottom of the watch glass. By rocking the watch glass, movement of the ova and debris could be achieved. Actual manipulation of the ova was done by careful use
of micro-dissecting needles attached to balsa handles. With these instruments debris was removed from the solution, and the ova were rolled together.

Ova which were collected but not transferred to recipient animals were held in the Ringer's solution and were measured (Table C) and/or photomicrographed (Plates 2-8) as soon as possible after collection.

The criteria of fertilization used in these experiments were the presence of polar bodies and blastomere development. Since surgery was performed approximately seventy-two hours after the onset of oestrus, development of the ova to the four blastomere stage was expected. Unicellular ova found at this time were deemed to be unfertilized. Rolling the ova with micro-dissecting needles made blastomere and polar body examinations easier. In addition, fractures or abnormalities of the zona pellucida were detected more readily when the ova were rolled.

Transfer of ova from a donor to a recipient required the synchronizing of the onset of oestrus in both animals. A difference in time of twenty-four hours between the two animals' cycles was the maximum time allowed in this study. No attempt was made to synchronize cycles by the use of hormones, but naturally coincident oestrus occurred in the experimental animals.

Transfers of ova between such animals were performed. The laparotomy incision was made at the same site in the recipient as
the donor i.e. pre-pubic midline, and the internal genitalia exteriorized in the same manner. The ipsilateral horn to the ovary showing one or more corpora haemorrhagica was selected for deposition of ova. Ova with less than seven blastomeres were selected for transfer to the fallopian tubes of the recipient. Ova with seven or more blastomeres were chosen for deposition in the tip of the uterine horn.

Transfer of ova from the watch glass to the recipient was achieved by picking up the ova in the tip of a Pasteur pipette. The pipette was attached to a two millilitre syringe by means of a rubber artificial insemination adaptor. A minimum of solution was taken up with the ova. The tip of the Pasteur pipette was passed through the wall of the uterine horn at or near the tubo-uterine junction, or alternatively into the fallopian tube via the fimbriated end. Deposition of ova and holding fluid was accomplished in the lumen of the uterus or the fallopian tube.

Trauma of the internal genitalia was kept to a minimum during all procedures. This was necessary to maintain maximum chance of deciduoma, continued development of the transplanted ova and to minimize the development of adhesions.

Routine closure of laparotomies was carried out using Chromic catgut No. 0 or No. 1, for deep sutures. All skin sutures were braided silk. No post-surgical treatment was given, and the does were allowed to recover in their stalls. Recovery was slow.
and obviously painful in does that underwent surgery in the flank region. All does that were opened on the midline showed uneventful recovery and minimal discomfort.
Physical Factors

Observations on the experimental does indicate that the breeding season begins in Southern Ontario during the latter part of September. Does, during the 1957-58 breeding season, showed first signs of oestrus on the 20th September, 1957. All does in the flock had shown one or more oestrous periods by the end of October, 1957. These findings were consistent with those noticed during the 1958-59 season. The first doe to show oestrus was noticed on September 24th, 1958. Again, all does except two had shown one or more cycles by the end of October, 1958.

Of the two does that did not show oestrus by the end of October 1958, one (RED 1) remained anoestrus during the whole of the breeding season. The other (RED 14) following treatment with hormones, showed two oestrous periods and was used in the studies. Both oestrous periods occurred late in the breeding season.

Regular oestrous cycles were evident in the flocks during both seasons, until late December, after which time oestrus showed sporadically. Oestrus did not occur regularly after the last week in December during both seasons.

The duration of oestrus showed a mean of 31 hours with a spread from 17 hours to 41 hours approximately. Young does showed irregularities of cycle length and duration of oestrus in comparison with older does. The duration of oestrus was shorter at the beginning and end of the breeding season.
Physical Response to P.M.S. Injections

Intravenous and subcutaneous injections of P.M.S. given to seven does during the 1957-58 breeding season were erratic in bringing about a visible response. Two of the does (YELLOW 4, YELLOW 2) received two injections of P.M.S. and showed oestrus on the first and second, and fourth and seventh days respectively, after injection. Four other does failed to respond visibly to the hormone, i.e. no evidence of oestrus was manifested within fourteen days of the injections. These effects are summarized in Table A.

During the 1958-59 season, nine does which received 1,000 I.U.'s of P.M.S. by intramuscular injection, showed a better response. Six of these does showed oestrus at a mean time of eight days approximately after the injection. In these six does the spread from the mean was from six to eleven days. However, three does failed to respond to the injections, i.e. they did not show signs of oestrus within fourteen days of the hormone injection.

Five of these does received a second injection of 1,000 I.U.'s of P.M.S. each. The time of injection varied from twenty to fifty-five days after the first injection. Again, a third injection of 1,000 I.U.'s of P.M.S. was administered to two of these five does. The times of these injections are set out in Table B.
Ovarian Response to P.M.S. Injections

Results during the 1957-58 breeding season were unsatisfactory. Two of the seven does showed a maximum of two ovulations each. No ova were collected from any does during this season.

The 1958-59 breeding season gave better results. Nine does received the primary P.M.S. injection, and five of these showed superovulation. The criterion used was the presence of more than three ovulations per doe. The five does varied in response, from four to twenty-six ovulations. Two other does showed no response to injected P.M.S. given as the primary injection. One doe (BLUE 20) received the primary P.M.S. injection, but no surgery was performed. This doe was held in readiness as recipient of ova from another doe, (RED 13).

Second injections of the same dosage of P.M.S. again given thirteen days after a later oestrus produced superovulation in two out of three does. Third injections of P.M.S. again at the same dose rate, failed to produce superovulation of the two does treated.

The response by the ovaries of nine does to P.M.S. injections, is set out in Table C together with the number of ova collected.

Embryological Data

During the 1957-58 breeding season, no collections of ova were made.

The 1958-59 season provided forty-eight ova for study.
These were collected from six different does during eight surgical operations, as set out in Table C.

Ova were recovered in various stages of development from unicellular (Plate 2) i.e. uncleaved, to the morula stage (Plate 3). Three ova at the morula stage were collected seven days after the onset of oestrus. This was the maximum time post-oestrus that collections were made. Table D gives the relevant details of ova development.

Measurements of the overall diameter of those ova that were available for microscopic examination, varied from 168 microns to 193 microns. This gave a mean overall diameter of 177.3 microns for the ova measured.

Measurements of the zona pellucida of the ova showed a spread of 9.8 microns to 14.9 microns from a mean diameter of 12.3 microns. One ovum collected from RED 12, in the two blastomere stage, showed a fracture of the zona pellucida.

The polar bodies of six of the ova were measured, but difficulty was experienced identifying these structures in the remainder. Details of measurements therefore, are not complete. Six ova showed polar bodies which were measured, to give a mean value of 13.2 microns, with a spread of 9.9 microns to 16.5 microns. In two ova, two polar bodies were seen (RED 12, RED 45).

Variations in the size of blastomeres were evident in ova up to the eight cell stage. One or more blastomeres in developing
ova showed enlargement to twice the size of the other blastomeres. The technique of collection of ova from the genital tract varied slightly with the time which had elapsed between onset of oestrus and time of surgery. Flushing the fallopian tubes only, yielded ova which had developed to the five blastomere stage, or less. Flushing of the tip of the uterine horn and fallopian tubes yielded ova developed to the fourteen to sixteen blastomere stage. Three morulae were collected (RED 14) by flushing the full length of the uterine horns.

Ova in small amounts of Ringer's solution showed rapid and marked plasmolysis after approximately an hour at room temperature. This occurred also in ova held for times up to two hours in Ringer's solution. Plate 6 demonstrates this effect clearly.

Two transplantations of ova were carried out during the 1958-59 season. Due to the small number of does with naturally synchronous cycles, the number of transplantations was restricted. Two other recipient does during this season showed synchronous oestrus with two donor does. However, collections of ova from both these donors indicated that fertilization had not occurred. No cleavage had taken place in the ova. Transfers were not carried out for this reason.

In the above two transplantations, the ova were at the eight blastomere stage and the fourteen to sixteen blastomere stage respectively. Six other ova collected concurrently from
this donor (RED 45) were uncleaved and hence were discarded as unfertilized. Each of the fertilized ova was transferred to the lumen of a uterine horn of RED 5. Neither of the two transplanted ova developed in the recipient.

Histopathological Data

The outstanding pathological changes found during both breeding seasons were referrable to surgical procedures per se. All animals which underwent one or more surgical interferences with the genitalia developed adhesions. Manipulations of the genitalia caused inflammatory reactions which resulted in adhesion formation involving the internal genitalia, small and large intestines, omentum and bladder. In three cases (RED 5, RED 45, RED 6) adhesions were so extensive that flushing procedures were extremely difficult or impossible. Excision of the internal genitalia was carried out in two cases (RED 45, RED 6) to achieve ova collections.

Adhesions of the fimbriae to the ovary were common following flushing techniques.

One doe (RED 6) had an occluded left fallopian tube which interfered with flushing during the first collection. The same doe, at a second ova collection, one week later, showed a marked oedema of the left mesosalpinx, fallopian tube, tip of the uterine horn and mesometrium. A third flushing on the same doe forty-nine days later, indicated occlusion of the lumina of both fallopian
tubes. There was extensive adhesion formation by this time.

Post-mortem examination of one sacrificed doe (RED 45) showed extensive abscessation in the region of the surgical incision. Pockets of pus were found in the subcutis, muscular layers and peritoneal surfaces. The viscera involved were small intestine, large intestine, omentum and uterus.

Histopathological examination of the uterus of RED 45 revealed local suppurative peritonitis. Abscesses were evident along the wall of the uterine horn. These suppurative changes were restricted to the serous and subserous areas. The endometrium showed no pathological changes.

Histopathological examinations were made of the internal genitalia of five does (RED 5, RED 6, RED 12, RED 45, RED 13) at post-mortem. Tissues examined included the uterus, fallopian tubes and ovaries. All post-mortem specimens showed a mild degree of serositis, but no inflammatory changes were seen in the endometrium or the epithelium of the fallopian tubes.

Sections of the ovaries of these does were examined, but no pathological abnormalities were noticed, except in the case of RED 45. This doe showed inflammatory changes on the serous surface of the ovary which was consistent with the fibrosis and abscessation seen grossly at post-mortem.

At the time of surgery, two does (RED 45, RED 14) showed cystic development of the ovaries. RED 45 had one cyst approximately
one-half inch in diameter present on the right ovary when the second surgical operation was performed. RED 14, at the first ova collection, had a cyst on each ovary. Both cysts were of three-quarter inch diameter approximately and ruptured easily during manipulations. In addition, both ovaries in this doe showed approximately eight to ten small atretic follicles on each.
DISCUSSIONS AND CONCLUSIONS

Physical Factors

The start of the breeding season in the domestic goat in Southern Ontario is late September. This is based on observations in the experimental herd of does at the Ontario Veterinary College from August, 1957 to February 1959, and confirms local opinions. The earliest date of onset of the breeding season was September 20th, 1957. The following year, the first doe to show oestrus was noticed on September 24th, 1958.

Oestrus occurred regularly until late December in the experimental does. The peak of the breeding season occurred in October, as evidenced by all does showing one oestrous period by the end of October and the grouping of oestrous periods. RED 1 and RED 14 were exceptions to this. By December, a noticeable decline in breeding activity had occurred. Only one doe showed oestrus in January, and this was following injection of P.M.S. to achieve superovulation.

Asdell (53) quotes American goat statistics as indicating that the breeding season starts about September in U.S.A., reaches its greatest intensity in October and gradually tapers off by the end of the year. He indicates that few goats will come in oestrus in the New Year. This is in accord with the findings in this experimental herd.

Variations in the onset of the breeding season of the domestic
goat are probably due to light intensity, acting via the optic nerve, on the pituitary or hypothalamus. Temperature would seem to be of lesser importance. Yeates (54) in 1949 demonstrated this effect with sheep, by experimental procedures. Temperature changes may bring about minor seasonal breeding variations.

Variations may be expected in the breeding season of the domestic goat at different latitudes. Southern Ontario presents a slightly different breeding season to areas in Southern U.S.A., and a reversed breeding season to goats in the southern hemisphere. The breeding season may be expected to begin in September and continue until the end of December, under normal conditions in Southern Ontario.

Variations in the breeding season in the British Isles are given by Mackenzie (55). This reference indicates that Southern England has a comparable time of onset of the breeding season to Southern Ontario. However, the latitude of Southern England is some degrees north of Southern Ontario.

The length of the oestrus cycle is quoted by Asdell (53) as a mean time of 19.4 days with a spread from 12 to 24 days. These figures are given for does in U.S.A.

In the work carried out at the Ontario Veterinary College, no reliable assessment of cycle length was made. This was due to the use of P.M.S. injections to achieve superovulation. P.M.S. was given on the thirteenth day following the first oestrus of the
breeding season during 1958. As a result of this interference with
normal cycles, attempts to calculate cycle length were not made.
A cycle length of twenty days was assumed for all experimental
animals and is justified by experimental results.

Duration of oestrus in the does varied from seventeen hours
to forty-one hours approximately. The onset of oestrus was noted
by the response of the doe to the presence of the vasectomized
buck. The latter was taken to the does daily approximately every
eight to ten hours, and the reaction of the does served as the
basis of the above calculations. These procedures obviated over-
looking oestrus.

The signs of approaching oestrus included tail movements,
seeking of company of the buck, but refusal to allow the buck to
mount.

Onset of oestrus was characterized by continued bleating
and tail wagging, seeking the company of the male, and standing
to allow mounting and service. Rarely a thin vaginal discharge
was seen, but visible changes in the external genitalia were
minimal or absent. Only one doe (RED 13) showed shyness at breeding.

From the observed physical factors, it may be concluded
that:

1. The breeding season of the domestic goat commences
late in September, reaches a peak in October, and finishes by
the end of December.
2. Cycle length is approximately an average of twenty days.
3. Oestrus lasts for an average of thirty-one hours, with variations from seventeen to forty-one hours.
4. Behavioural signs are indications of approaching oestrus and oestrus itself.
5. Visible changes in the external genitalia at oestrus, are minimal or absent.

**Physical Response to P.M.S. Injections**

Administration of P.M.S. by intravenous and subcutaneous routes during the 1957-58 season did not produce a satisfactory physical response. The onset of oestrus following P.M.S. injections was erratic. It occurred from one to seven days after injection at a mean time of three days.

Because four does out of seven failed to show any physical response during this season, as a result of intravenous or subcutaneous injections of P.M.S., it may be concluded that these routes of administration are unsatisfactory. The three does showing oestrus following treatment did not show superovulation.

In the 1958-59 season, P.M.S. injections given intramuscularly brought about a physical response within six to eleven days. The average time of onset of oestrus was eight days.

Each of nine does received 1,000 units of P.M.S. by the intramuscular route on the thirteenth day following oestrus. Only two of these does failed to respond to the injection. Repeat
injections on five of these does were given also by intramuscular injection thirteen days after the onset of a later oestrus. All five repeat injections brought about the desired physical response.

Conclusions that may be drawn from the foregoing results are:

1. The intramuscular route of administration of P.M.S. is likely to give a better physical response than administration via the subcutaneous or intravenous routes.

2. Oestrus may be expected to occur at an average time of eight days following injection of 1,000 I.U.'s of P.M.S. intramuscularly on the thirteenth day of the oestrous cycle.

3. Second and third injections of P.M.S. at the same dose rate, route of administration and day of the cycle, will elicit a response similar to the primary injection.

Ovarian Response to P.M.S. Injections

The does used in the 1957-58 season did not give satisfactory responses to injected P.M.S. Surgery was performed on four does, sixty hours after service by the buck. The maximum number of ovulations seen in an individual animal was two.

It was concluded that the dose rate and the route of administration of the P.M.S. used for these animals were unsatisfactory as a means of superovulation in does. This conclusion was reached because of the complete failure to achieve superovulation in any of the does during this season.
By using the system previously described, of injection of a standard dose on a fixed day of the cycle, by the intramuscular route of administration, superovulation can be achieved. The criterion of superovulation was taken to be the presence of more than three corpora haemorrhagica in a single doe (Plate 1). Asdell (53) states that twins and triplets are common in the domestic goat, but four and five kids extremely rare. Five of the does were superovulated on seven separate occasions. All five does showed a minimum of four ovulations. The average number of ovulations was 10.8 with a spread about the mean from four to twenty-six. This average is for the superovulations resulting from the initial injection of P.M.S.

Second injections were given to three does, only one of which (RED 6) had been superovulated by the initial P.M.S. injection. The other two does (RED 45, BLUE 20) had not been superovulated or had not been investigated. RED 45 had responded physically to the primary P.M.S. injection, but had shown only two ovulations. BLUE 20 had responded physically, but had not undergone surgery to ascertain the ovarian response. This doe was held as a recipient for ova from RED 13, but no transplant was carried out. Subsequently a second injection of P.M.S. did not bring about superovulation of BLUE 20, only one ovulation point was observed.

Third injections were given to two does (RED 45, RED 6),
neither of which was superovulated. Each doe showed evidence of two ovulations. The limited number of does available restricted further studies of ovarian response.

From the data collected concerning the effects of P.M.S. on the ovaries of nine does in this breeding season, it can be claimed that a successful system of superovulation has been evolved. Injections of P.M.S. were administered at a stage of the oestrous cycle which was thought would achieve optimum results. This time was believed to be the start of the pro-oestrus stage of the cycle, and was the thirteenth day after the onset of oestrus. Five of the does gave a good response to the hormone injections, as previously noted. The outstanding superovulation was doe RED 12 that showed thirteen ovulations on each ovary.

In all hormonal studies, variations are to be expected. This is due largely to the inadequate state of knowledge concerning hormone effects. Gonadotrophins are no exception to this broad statement, and thus the variation in response shown by the experimental does, was satisfactory. Further studies on these aspects of superovulation are necessary however. Plate 1 shows the ovaries of doe RED 13 with ten corpora haemorrhagica.

Two does (RED 45, RED 14) showed cystic conditions of the ovaries. After the second injection of P.M.S., doe RED 45 had a cyst present on the right ovary. This cyst was approximately one-half inch in diameter. There were three corpora haemorrhagica
on this ovary in addition to the cyst. In the case of RED 14, one cyst was present on each ovary. The cysts were three-quarter inch in diameter, and there were two corpora haemorrhagica on each ovary in addition to the cysts.

The presence of follicular cysts in these two animals had not interfered with ovulation or corpus luteum formation. Normal fertilization and ovum cleavage had also occurred. There were no signs of abnormal oestrus or nymphomania in the does with cystic ovaries.

Subsequent treatment of RED 45 with a third injection of P.M.S. produced no further cyst formation. Rupture of the cyst occurred during the second surgical operation.

It would appear that there is a refractoriness to repeated injections of P.M.S. Ovarian response seems to be reduced by second and/or third injections. In one case only (RED 45) was a second or third injection successful in bringing about more ovulations than a primary injection. All other does showed a maximum super-ovulation following the primary injection of P.M.S.

This observation supports Willett's work (56) in which he reported a refractoriness of dairy cows to repeated gonadotrophin injections.

The conclusions to be drawn from these aspects of the work are as follows:
1. Intramuscular injections of P.M.S. brought about better ovarian response than either intravenous or subcutaneous injections.

2. Superovulation results if the injection of 1,000 I.U.'s of P.M.S. is given on the thirteenth day following the onset of oestrus.

3. Wide variation in ovarian response by individual does can be expected.

4. Based on the presence of cystic follicles on the ovaries of two does, superfolliculation without ovulation may occur under the above conditions.

5. Repeated injections of P.M.S. appear to result in a refractoriness by the ovaries.

**Embryological Data**

All does during 1957-58 underwent surgery in the flank region. This gave reasonable access to the near side ovary, fallopian tube and uterine horn, but very difficult access to the far side genitalia. In addition, to difficult access, adhesions involving the internal genitalia were prone to occur. This made collections of ova difficult or impossible.

Six does in the 1958-59 breeding season provided forty-eight ova for observation. The sources of the ova and their development are shown in Tables C and D. Excellent exposure of the internal genitalia was largely responsible for collection of these ova.
Surgery was performed at times varying from seventy-two hours to seven days after the onset of oestrus. Since service was performed twelve to twenty-four hours after the onset of oestrus, surgery may be calculated as occurring forty-eight to sixty hours after service. In effect, no surgery was performed sooner than sixty hours after service.

Variation in the time of ova collection was made to try to assess the rate of cleavage of ova by the time of entry into the uterine horns. From the data in Table C, it can be seen that ova varying from the uncleaved to the morula stage, were collected on eight occasions (Plates 2, 8). Three does (RED 12, RED 45, RED 6) had collections of ova made from them between sixty-eight and seventy hours after service. Ova collected from all three had reached maximum development of five, eight and six blastomeres respectively (Plates 5, 6). All three collections were made by flushing the tips of the uterine horns and the fallopian tubes. In the case of RED 12, the left oviduct and tip of the uterine horn were flushed, and four and five blastomere ova were recovered. In the same animal, the right oviduct only was flushed and the maximum development of the recovered ova was the three blastomere stage.

It appears that ova reach the uterine ostium of the oviduct when they have developed to about the five or six blastomere stage. This is approximately eighty hours after the onset of oestrus, and
conforms to the classical idea that the ova enter the uterus in domestic animals seventy-two to ninety hours after ovulation. Pomeroy (57) claims that this period is only twenty-four to forty-eight hours after ovulation in the pig. Amoroso et al. (58) indicate that the ova of the goat do not reach the uterus until approximately the eight blastomere stage, which they claim is four to five days after mating. The studies reported here indicate that ova enter the uterus before this time, and at an earlier stage of development.

In all collections of ova, the first two millilitres of Ringer's solution was found to contain the ova plus cellular debris. The uneven development of blastomeres was presumably due to an increase in size immediately before division. This difference in blastomere size was noticed in ova which had developed up to the eight cell stage. Ova with more than eight blastomeres did not show this size discrepancy. Because the overall size of the ovum does not appreciably alter until the late morula or blastocyst stage, variations in blastomere size will be less obvious as blastomere numbers increase. Plate 7 depicts this inequality of blastomeres.

Plasmolysis of blastomeres is evident in ova held in Ringer's solution for up to two hours at room temperature. Concentration of the Ringer's solution due to evaporation of the water, led to an osmotic effect on the blastomeres, with decrease in size, and
shrinkage away from the inner margin of the zona pellucida. Plates 3, 6 show this effect clearly. In any transplantation of ova, this effect must be prevented. Rapid transplantations from donor to recipient must be carried out to avoid this plasmolysis.

Some ova were collected and found to be uncleaved after seventy-two hours had elapsed since service. In one case at least (RED 6), this was probably due to adhesions involving the internal genitalia. Such adhesions can prevent normal sperm migration to the oviduct, and hence prevent fertilization. Two other does (RED 13, BLUE 20) yielded uncleaved ova after a similar time, but without any adhesions evident during surgery. BLUE 20 was nine months old, and had shown only one ovulation following a second injection of P.M.S. The youth of this doe could have been responsible for the non-conception. No other explanation is offered in view of normal anatomy and absence of obvious pathology.

Conclusions that may be drawn from the embryological data obtained are as follows:

1. Ova in various stages of development can be collected from the oviducts and uterine cornua of the doe.
2. Pre-pubic midline is the operative site of choice for ova collections.
3. Genital adhesions restrict collection procedures and may prevent conception.
4. Ova collections can be made from seventy-two hours
to seven days after the onset of oestrus. Development of the ova to the morula stage can be expected by the seventh day.

5. Ova reach the uterine ostium of the oviduct at approximately the five to six blastomere stage.

6. The time taken for ova to reach the uterine horn after ovulation is sixty to seventy-two hours.

7. Ova and loose cellular debris are flushed out with the first one or two millilitres of flushing medium.

8. The average overall diameter of ova of the goat, up to the morula stage, is approximately 177.3 microns.

9. The average thickness of the zona pellucida of ova of the goat, up to the morula stage, is 12.3 microns.

10. Plasmolysis of blastomeres is liable to result from evaporation of water from the holding medium.

11. Ova should be examined for development prior to transplantation to prevent transplantation of uncleaved ova.

Pathology

Adhesions of the uterus, oviducts and ovaries were the main post-operative pathological conditions seen in the does. Second and third operations which were performed to collect ova revealed the presence of adhesions. These adhesions complicated or rendered impossible the collection of ova.

Two does (YELLOW 2, YELLOW 7) used during 1957-58 had
abdominal adhesions involving the ovaries, uterus and oviducts. Neither of these does had a history of previous surgery.

Manipulations of the internal genitalia caused local peritonitis. Instruments and the techniques used to insert the polyethylene tube into the oviduct were in some cases responsible for adhesion of the fimbria to the ovary.

Abscessation occurred in one doe only (RED 45) and involved the peritoneal surface of the ovary and abdominal wall. Contamination of the surgical incision was apparently the source of the peritoneal infection. A local suppurative peritonitis was evident on examination of sections of the uterus.

Histopathological examination of the endometrium and oviducts of five does was carried out. All five sets of genitalia showed normal epithelia. This would indicate that the procedures for the collection of tubal and/or uterine ova have no detrimental effect on the epithelia of the organs concerned. (Plates 9, 9A).

Ovarian changes were restricted to the presence of adhesions and mild inflammation of the serosal surface of the ovary. Although cysts of the ovaries were seen in two does (RED 14, RED 45), no evidence of cystic follicles was seen in the sections of ovaries of the five does that were examined at post-mortem.

Normal corpus luteum formation was seen in all five specimens.
It may be concluded that:

1. The common pathological change in the genitalia after collections of ova, is local peritonitis with adhesions.

2. Flushing procedures to collect ova can be expected to cause no pathological changes in the epithelium of the uterus or oviducts when aseptic techniques are maintained.

General Conclusions

The experiments during the two breeding seasons were undertaken after close study of the publications of similar projects with sheep. It is realized that sheep and goats are closely allied species and have the same chromosome numbers.

It was assumed that data concerning sheep would be the best basis on which to carry out investigations with domestic goats. Publications by Averill et al. (16), Hunter et al. (13) and Rowson et al. (42) provided sufficient information to evolve a comparative method of superovulation, ova collection and ova transplantation in the goat. A functional system of superovulation and ova collection has been tried and found to be satisfactory.

The hormonal aspects of this study have been most important because superovulation is a necessary pre-requisite for commercial transplantations of ova.

Surgical techniques of ova collection and transplantation
can be carried out with a minimum of difficulties.

On the basis of the work in this thesis, it can be stated that further work in the field of ovum transplantation and related studies can follow directly along the above lines with good prospects of successful pregnancies resulting.

Synchronization of oestrous cycles of donor and recipient does warrants further study. Provided that sufficient does are available to act as recipients, it can be expected that results with does will be as favourable as those published with ewes.
SUMMARY

1. Seventeen does provided data concerned directly or indirectly with studies related to ovum transplantation in this thesis.

2. Observations concerning the onset and conclusion of the breeding season in the goat in Southern Ontario were made. It was observed that the onset of the breeding season is late September, the season reaches a peak in October, and is finished by the end of December.

3. Data concerning the duration of oestrus was recorded. A mean duration of oestrus was found to be thirty-one hours with a spread about the mean from seventeen to forty-one hours approximately. Young does tended to show irregularities in duration of oestrus when compared with older does.

4. Observations on the physical responses to injections of P.M.S. were made. Erratic responses resulted during the 1957-58 breeding season. Does used in the 1958-59 season showed a mean time of physical response to P.M.S. injections of eight days. The spread about this mean was from six to eleven days.

5. Intravenous and subcutaneous injections of P.M.S. were administered during the 1957-58 season with generally unsatisfactory physical and ovarian responses. Intramuscular injections of P.M.S. during the 1958-59 season produced reliable physical and ovarian responses.
6. Laparatomies were performed in the flank region during 1957-58 and on the pre-pubic midline in 1958-59. The flank approach gave poor access, and maximum post-operative discomfort. The pre-pubic midline approach gave optimum exposure with minimum post-operative discomfort.

7. Ovarian responses to P.M.S. injections by intravenous, subcutaneous and intramuscular routes, were observed, recorded and assessed for the two breeding seasons.

8. Collections of forty-eight ova were made during the two breeding seasons. Development of the ova varied from the uncleaved single blastomere stage to the morula stage.

9. The ova were collected by a technique of flushing the uterine horns and/or the oviducts with Ringer's solution.

10. Following the collection the ova were microscopically examined, measured and photographed. Ova which were to be transplanted were not measured or photographed, but were transferred with a minimum of delay between donor and recipient.

11. Two ova were transferred. A recipient doe that had shown naturally synchronous oestrus with the donor doe was used. One of the transplanted ova was at the eight blastomere stage of development. The other had developed to the fourteen - sixteen blastomere stage. No pregnancy resulted.

12. Post-mortem and histopathological examinations were performed on the genitalia of five does. Adhesion formation was the only
consistent finding. In one case, abscessation had occurred following contamination of the surgical wound. Histopathological examinations revealed no pathological changes of the endometrium, tubal epithelium or ovary as a result of flushing techniques.

13. A reliable method of superovulation and ovum collection from does has been evolved as follows:

On the thirteenth day post-coitus, 1,000 I.U.'s of P.M.S. are given intramuscularly, the internal genitalia are exposed sixty hours to seven days later and ova are recovered by flushing the uterine horn and/or fallopian tube.
REFERENCES


25. Chang, M.C. Fertilizability of rabbit ova and the effects


TABLES

AND

PHOTOGRAPHS
<table>
<thead>
<tr>
<th>DOE</th>
<th>DOSE RATE OF P.M.S. (I.U.)</th>
<th>ROUTE OF ADMINISTRATION</th>
<th>OESTRUS (DAYS AFTER INJ'N)</th>
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<td>I.V.</td>
<td>not observed within 14 days</td>
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<tr>
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<td>1. 4 days</td>
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<td>2. 750</td>
<td>subcut.</td>
<td>2. 7 days</td>
</tr>
<tr>
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<td>I.V.</td>
<td>not observed within 14 days</td>
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<tr>
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<td>1. 500</td>
<td>I.V.</td>
<td>1. 2 days</td>
</tr>
<tr>
<td></td>
<td>2. 500</td>
<td>I.V.</td>
<td>2. 1 day</td>
</tr>
<tr>
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</tr>
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<td>4 days</td>
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<td>YELLOW 7</td>
<td>1,000</td>
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**TABLE A.** P.M.S. injections and their effects 1957-58 season.
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<th>Doe</th>
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<td>Red 15</td>
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<td>no response within 14 days</td>
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<td>5</td>
</tr>
<tr>
<td>Blue 20</td>
<td>1</td>
<td>11</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Blue 21</td>
<td>1</td>
<td>no response within 14 days</td>
<td></td>
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</table>

**Table B.** Times of P.M.S. injections, and the physical responses during 1958-59 breeding season.

* All P.M.S. injections given intramuscularly 13 days after onset of oestrus.
<table>
<thead>
<tr>
<th>DCE</th>
<th>1st P.M.S. Injection 1,000 I.U. Intramuscularly</th>
<th>2nd P.M.S. Injection 1,000 I.U. Intramuscularly</th>
<th>3rd P.M.S. Injection 1,000 I.U. Intramuscularly</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Ovulations</td>
<td>No. of Ova Collected</td>
<td>No. of Ovulations</td>
</tr>
<tr>
<td>RED 5</td>
<td>6</td>
<td>nil</td>
<td></td>
</tr>
<tr>
<td>RED 6</td>
<td>8</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>RED 12</td>
<td>26</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>RED 13</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>RED 14</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>RED 15</td>
<td>No physical response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RED 45</td>
<td>2</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>BLUE 20</td>
<td>Not investigated</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>BLUE 21</td>
<td>No physical response</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE C.** Ovarian response to P.M.S. injections, and number of ova collected during 1958-59 breeding season.
<table>
<thead>
<tr>
<th>DOE</th>
<th>DATE OF OPERATION</th>
<th>NO. OF OVA COLLECTED</th>
<th>DEVELOPMENT</th>
<th>OVERALL OR AV. DIAM. OF OVA (u)</th>
<th>DIAM. OR AV. DIAM. OF ZONA PELLUCIDA (u)</th>
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</thead>
<tbody>
<tr>
<td>RED 6</td>
<td>23/10/58</td>
<td>3</td>
<td>5 blastomeres</td>
<td>175</td>
<td>14.9</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>7 blastomeres</td>
<td>180</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7 blastomeres</td>
<td>182</td>
<td>10.0</td>
</tr>
<tr>
<td>RED 6</td>
<td>19/12/58</td>
<td>2</td>
<td>1 blastomere</td>
<td>183</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 blastomere</td>
<td>182</td>
<td>14.0</td>
</tr>
<tr>
<td>RED 12</td>
<td>22/10/58</td>
<td>20</td>
<td>7 with 1 blastomere</td>
<td>193 (av)</td>
<td>14.9 (av)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9 with 2 blastomeres</td>
<td>172 (av)</td>
<td>12.1 (av)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>1 with 3 blastomeres</td>
<td>179</td>
<td>11.6</td>
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<td></td>
<td></td>
<td></td>
<td>1 with 4 blastomeres</td>
<td>183</td>
<td>9.8</td>
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<tr>
<td>RED 13</td>
<td>21/11/58</td>
<td>9</td>
<td>9 with 1 blastomere</td>
<td>178 (av)</td>
<td>13.4 (av)</td>
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<tr>
<td>RED 14</td>
<td>12/1/59</td>
<td>3</td>
<td>morula</td>
<td>168</td>
<td>13.2</td>
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<td></td>
<td></td>
<td></td>
<td>morula</td>
<td>175</td>
<td>10.2</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>morula</td>
<td>178</td>
<td>10.2</td>
</tr>
<tr>
<td>RED 45</td>
<td>11/11/58</td>
<td>2</td>
<td>4 blastomeres</td>
<td>168</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8 blastomeres</td>
<td>168</td>
<td>13.0</td>
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<tr>
<td>RED 45</td>
<td>30/11/58</td>
<td>8</td>
<td>5 with 1 blastomere</td>
<td>Not</td>
<td>Not</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1 with 8 blastomeres</td>
<td>Measured</td>
<td>Measured</td>
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<td></td>
<td></td>
<td>2 with 14-16 blastomeres</td>
<td>Measured</td>
<td>Measured</td>
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<td>BLUE 20</td>
<td>12/12/58</td>
<td>1</td>
<td>7 blastomeres</td>
<td>173</td>
<td>12.5</td>
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</table>

**TABLE D.** Development and dimensions of ova collected at laparotomy.
Plate 1. Superovulation in doe RED 13
Plate 2. Uncleaved ovum from doe RED 6 (X 85 and enlarged).
Plate 3. Uncleaved ovum from doe RED 14, showing plasmolysis (X 85 and enlarged).
Plate 4. Two blastomere stage of ovum development from doe RED 12 (x 30 and enlarged).
Plate 5. Four blastomere stage of ovum development from doe RED 12 (X 30 and enlarged).
Plate 6. Eight blastomere stage of ovum development with plasmolysis from doe RED 12 (X 85 and enlarged).
Plate 7. Six blastomere stage ovum with uneven blastomere development from doe RED 12 (X 85 and enlarged).
Plate 8. Morula from doe RED 14 (X 350).
Plate 9. Low power magnification of section of fallopian tube two hours after flushing RED 6 (X 30).
Plate 9A. High power magnification of section of fallopian tube two hours after flushing RED 6 (X 350).
<table>
<thead>
<tr>
<th>Date</th>
<th>Due Date</th>
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<td>SEP 22 1959</td>
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<td>OCT 6 1967</td>
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<td>MAY 1 3 1970</td>
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<td>DEC 22 1970</td>
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SM-12-55
THESIS:

SF 756.T  Portway, Bruce
P 838  Studies relating to ovum transplantation in domestic goat.
1959