AN ABSTRACT OF THE THESIS OF

WILLIAM LYNN HUNT for the Ph. D. in Animal Science
(Name) (Degree) (Major)

Date thesis is presented January 28, 1964

Title OVA TRANSFERS IN SHEEP AND RABBITS: STUDIES ON MATERNAL INFLUENCES AND IRRADIATION DAMAGE

Redacted for Privacy

Abstract approved _____
(Major professor)

The first study utilized ova transfer in sheep and involved hormonal treatments for synchronization of estrus and superovulation in an investigation of crossbred maternal influences on inbred and linecross lambs.

Synchronization of estrus in ewes was achieved effectively with either oral progestogen, 6α-methyl-17α-hydroxy-progesterone acetate (60 mg./ewe/day), or intramuscular injections of progesterone (10 mg./ewe/day). Satisfactory superovulation was not obtained with pregnant mare serum preparations and alterations in oviduct morphology were noted following oral progestogen therapy. After progesterone injections, superovulation with a mean of 13.4 ovulations per ewe was obtained using pituitary extracts. Successful treatments began day after final progesterone injection with primary injection of 25 mg. followed in two days with 15 mg. of pituitary extract. An

intravenous injection of 25 mg. pituitary leutinizing hormone followed at onset of estrus.

Twelve Suffolk ewes of three inbred lines were bred to produce fertilized ova from each of three lines and from each possible linecross. Surgical transfers of ova from Suffolk donors were made to nine recipients which were similar in size and consisted genetically of Columbia, Dorset and Cheviot crosses.

Based on corpora lutea numbers, in vivo ova recovery rates increased from 39 percent for the first year to 53 percent for the second year. Cleavage rates were 54 and 52 percent for the two years.

The inbred line II lamb which developed in a crossbred maternal environment weighed 12.3 percent more at birth than its non-transfer line II counterpart. The transferred linecross III x II lamb weighed 30.6 percent more at birth than its non-transfer counterpart. The linecross took most advantage of prenatal nutrition. Adjusted 120-day weights, condition and conformation scores were similar for transfer and non-transfer lambs at weaning. Under similar postnatal environment, genotype for size is expressed in lambs at weaning.

In the second study effects of in vitro x-irradiation of fertilized mammalian ova on their subsequent in vivo development were investigated by means of rabbit ova transfer. Non-irradiated and irradiated

two-cell ova were transferred to non-irradiated and irradiated uteri of recipients to discriminate between embryonic and uterine injury. Irradiation was applied to two-cell ova in vitro at levels of 0, 15.4, 61.2, 91.8, and 122.5 rads using a 100 kVp x-ray machine (1 ma., HVL 1 mm. Al., distance 37.4 cm., dose 14.5 r./min.). Ova were transferred into oviducts of prepared recipients.

Uteri of recipients were exposed to the same radiation levels as the ova and in addition to 250.2, 265.3, and 530.5 rads.

Combination of ova/uterus irradiation showed additive effects of x-ray damage. One step increases of either ova or uterus above 61.2/250.3 rads caused 100 percent embryo mortality.

Two-cell ova which were given 122.5 rads of irradiation failed to develop into fetuses and uteri which were given 530.5 rads failed to contain implantations. Irradiation with 91.8 rads killed all but the most hardy ova and produced an all or none effect, while 61.2 rads caused abnormal, dead, and resorbed fetuses as well as living offspring. Two such newborn developed latent sequelae in the form of spreading limbs. Deformities became obvious at one month and progressed until death at four months.

Histological examinations of eight-day embryos which received 61.2 rads or no irradiation as two-cell ova revealed delayed development in irradiated embryos.

Mean increase in weight for the first 50 days of surviving offspring from irradiated ova was 6 gms. /day more than that of controls.

OVA TRANSFERS IN SHEEP AND RABBITS: STUDIES ON MATERNAL INFLUENCES AND IRRADIATION DAMAGE

by

WILLIAM LYNN HUNT

A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1964

APPROVED:

Redacted for Privacy

Professor of Animal Science

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Date thesis is presented January 28, 1964

Typed by Nancy Kerley

ACKNOWLEDGEMENTS

The research which is reported in this thesis was accomplished within the Animal Science Department under the competent guidance of Dr. Ralph Bogart, Professor of Animal Genetics. The author wishes to sincerely thank Dr. Bogart for his understanding and many faceted intellectualism.

Sincere appreciation is extended to Dr. J. C. Miller, Head of the Department of Animal Science, Dr. Jim Oldfield, Professor of Animal Nutrition, and Dr. Rod Frakes, Assistant Professor of Farm Crops, for their encouragement and counsel.

The sheep research problem could not have been accomplished without the able assistance of Mr. Duane Addleman and Mr. Joe Riker.

Appreciation is also expressed to Dr. T. Dale Trout for his advice concerning irradiation problems.

Special acknowledgement is made of Oregon State University as an institution where the possibilities are unlimited for those who desire to learn.

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INTRODUCTION

A standing goal of the livestock industry is to produce efficiently young livestock of desirable quality which exhibit greater size and a higher growth rate than the average of their predecessors. Many of the early investigations along these lines gave evidence that led to the conclusion that body size and growth were determined in a quantitative manner by a large number of genes, and that, after appropriate analyses of sufficient data, a reasonable theory of quantitative inheritance would explain these factors. Evidence favoring this concept can be noted in the genetic variation between breeds of different weights and the fact that these differences can be observed as early as the time of the first cell division (26, p. 284). However, omitted from this conclusion is one of the major influences--that of the maternal side in providing the growing organism with its many necessities for development. Several methods of inbreeding and crossbreeding animals of large and small sizes have proven that this maternal influence does exert a marked effect on the offspring, at least for some portion of its life. A classic demonstration was the reciprocal crosses between the Shire horse and Shetland pony which were carried out by Walton and Hammond in 1938 (77). From recent studies certain breeds of sheep are believed to offer greater area in

the uterus for the embryo to develop its placenta to maximum size than others, thereby effecting increased nourishment. This prenatal nourishment would seem to be especially important during the period of most rapid growth of the fetus, or after the 91st day of gestation in sheep. The postnatal maternal influence also has been shown to exert a measurable effect on the young animals and in addition, interactions between the prenatal and postnatal performance of the mother and the genotype of the young exist (9, p. 55). Although the previously mentioned environmental size effects have been demonstrated, there has been little experimental discrimination in livestock between maternal environments as affected by their own genetic make-up, i. e., inbred maternal environments versus crossbred maternal environments, and the offspring's phenotype as affected by its own genetic make-up, i. e., inbred offspring versus crossbred offspring, and the accompanying interaction between the two. If size and growth limitations which are observed in the offspring of a given line of livestock were controlled largely by the offspring's genetic make-up, then the maternal environment should not change these given characteristics to any great extent. That the genotype of the maternal animal effects control over her ability to reproduce is obvious, but the extent to which this condition affects certain economically important characteristics in the offspring has not been

established. The interaction that is continuously present between the maternal animal and the offspring must always be considered.

If accurate evaluations can be established for the separate average effects of genetic, environmental, and the interaction contributions to the performance of the animal which we observe, tremendous progress could be realized in determining heritability and in properly constructing and properly weighing a selection index. Selection methods presently in use have generally overlooked maternal influences for many years. It was recently recognized, however, that maternal effects appear to be masking the genetic change which was thought to be occurring in individual and family selection methods (4, p. 252). Obtaining large animals after selection for several generations may not mean as much actual genetic change as earlier believed, since the accumulative influence of each generation's heritable maternal effects hides the genetic progress. Breeding systems might well be modified in lieu of information regarding the inbred lamb's and the inbred ewe's actual potentials and limitations. The approach to this problem should be such that inbred and linecross lambs which developed in an inbred maternal environment may be compared with similar inbred and linecross lambs which developed in a crossbred environment.

Among the many new experimental procedures now being

employed to focus attention on various problems in genetics, and also in cytology, immunology, and reproductive physiology, is ova transfer. The ova transfer technique provides a method of locating, to some extent, the factors which influence the characteristics exhibited by the offspring. Several apparent advantages of ova transfer are manifest in its possibilities of multiplying the genetic contribution of a given mating to the next generation, the utilizing of ova from younger animals for shortening the generation intervals, as in progeny testing, and, most recently tested, the transporting of ova long distances for subsequent development in foster mothers (37).

The application of the ova transfer technique to this genetic-environmental study in sheep incurs the combination of two preparatory procedures. These are first, the synchronization of estrus for donor and recipient animals, and second, superovulatory treatment of the donor ewes. Although it has not proven difficult in sheep to synchronize estrus (51; 20; 32; 1), nor to cause multiovulations (48, p. 336; 28, p. 299), the successful combination of these hormonal treatments has not been reported in the literature. Hormonal therapy has become a major tool of workers in reproductive physiology and genetics, thus to incorporate the results of estrous synchronization and superovulation of these experimental sheep is considered paramount.

In attempting to elucidate these problems as summarized, this study presents the results of (a) hormonal therapy for synchronization of estrous cycles and superovulation of sheep, as well as certain other requirements of ova transfer, and (b) the attempt to measure maternal influences of inbred and crossbred ewes on inbred and non-inbred lambs.

REVIEW OF LITERATURE

The realization that maternal influences were important in livestock probably originated with the very earliest breeders, especially in regard to the postnatal effects, i. e., milk supply and maternal care. However, that the fetus has been completely dependent on its mother prior to birth, and that its constitution at birth may be related partially to the maternal environment has been acknowledged with concern in humans as well as in livestock only in recent times (72, p. 5; 79, p. 22). Different aggregates of factors have been used to describe maternal effects. According to a recent description (16, p. 40), they include the non-chromosomal direct influence of the dam upon the development of her progeny by means of nutrients, pathogens, or antibodies provided in the uterus, in the egg, or in the milk. A more general description, which is acceptable for this study, is "... the sum of the effects of those maternal factors which influence the growth of the young after fertilization of the egg" (35, p. 39).

Early studies of the maternal environmental effects were carried out by crossing breeds which differed considerably in size. Walton and Hammond's reciprocal crosses of the Shire horse and Shetland pony established the presence of this environmental influence

and its long term effect (77, p. 334). Genetic differences in rates of growth appeared only after weaning in these equine crosses. With similar methods cattle breeds of different sizes were crossed (39), as were sheep (35) and rabbit breeds (72). In these crossing experiments it was necessary to assume that few or no sex-linked factors were concerned with body size or growth, since the crossbred offspring resulting from the male of the large breed and the female of the small breed was assumed to be approximately the same genotype as the offspring resulting from the reciprocal cross. Following such as assumption, marked differences were shown between the crossbred individuals resulting from the two breeds which differed in size. In such an experiment with large South Devon and small Dexter cattle the crossbred calves which were born from the South Devon dams averaged 14. 5 pounds more at birth than the crossbred calves which were produced by the Dexter dams (39, p. 647). Maternal influences could be obscured by the lengthened gestation periods or by variations in litter size among multiparous animals (39, p. 647).

Birth weights of lambs have been shown to be directly related to the breed differences (41; 40) and even among the same breed, heavier ewes give birth to larger lambs (75, p. 101-109). Investigators have narrowed the portion of uncontrolled variance in their studies of body size and maternal effects through use of ova transfers.

Inheritance studies utilizing this procedure have been reported in mice (25; 47), rabbits (72; 73) and sheep (35; 15). In a study involving sheep ova transfer, it was concluded that maternal effects are important only when the genotype for size of the fetus is markedly different from its mother (35, p. 55). Although there are other data supporting this conclusion (15, p. 76), the accurate evaluation of maternal effects, genotypes, and interactions in regards to their relative importance will hardly hold true for any comparison except the particular one being studied. It has been reported that in a normal maternal environment with reasonable nutrition, the average birth weight of a single lamb is probably near its upper genetic limit (15, p. 76). Evidence for this was found in reciprocal ova transfers between the small Welsh and large Lincoln breeds of sheep. The genotypically large embryos developing in a large environment were much larger than genetically similar embryos which developed in a restricted environment (Welsh ewes), while genotypically small embryos exhibited very minor size increases when they developed inside the larger maternal environment (Lincoln ewes).

Inbreeding of sheep has shown evidence of depressing the birth weights (17; 7; 83) and weaning weights (56) of lambs. Also the ability of the inbred lamb to survive is considered as diminished (18; 50). The deterioration in many traits due to inbreeding, especially

birth weights, weaning weights, and liveability, are sometimes offset by the selection procedures which are practiced (14, p. 80). Under natural selection, birth weights and gains after birth in guinea pigs were severely depressed in the extreme inbreeding experiment of Sewall Wright (85). Also with only natural selection, the growth rate of inbred Merino sheep averaged considerably lower than that of the non-inbred Merinos (17). It is noted that in these studies inbreeding effects are always represented by conditions which are measured on the inbred offspring which were born from inbred mothers.

Synchronization of Estrual Cycles, Superovulation and Ova Transfer

Effective synchronization of estrous cycles with no important detrimental effects on the animal's fertility was first reported in sheep by Dutt and Casida (20). The optimum dose for preventing estrus and ovulation in the ewe appeared to be a daily injection of 10 mg. progesterone. This method of repressing estrus and ovulation until such time that discontinuance of the hormone brings on the breeding cycle in all treated females within a few days has proven to be quite successful in sheep (51; 34; 43). After the oral progestational compounds became available, considerable experimentation produced good evidence of their effectiveness for synchronizing estrual cycles in sheep (32; 71; 1). No adverse effects on the ewe's

future fertility or productivity were ascertained after causing synchronization of the estrous cycle by feeding 50 mg. of 6 α-methyl-17α-hydroxyprogesterone acetate per ewe per day (22, p. 807), but at this same level, indications of disturbed hormonal balance were observed (1, p. XLVI-6). However, a satisfactory ovulation rate occurred in a high percentage of the ewes after cessation of the hormone treatment.

Injections of gonadotrophins to evoke multiovulations in the ovine have been used both during the normal breeding season (58; 76; 24) and during the anestrous period (13; 52; 57). Gonadotrophins have also been used to induce ovulations in ewes after progestational synchronization of the estrual cycle (19; 36; 59; 55), but no trend in the ovulation rates could be established following progesterone priming with varying dosages of pregnant mare serum (PMS) and human chorionic gonadotrophin (HCG) (42, p. 711). In one such experiment ovulations in 25 ewes which received 800, 1,500, and 2,000 international units of PMS ranged from 0 to 12, 1 to 10, and 0 to 9, respectively (36, p. 145). Among these ewes, some were superovulated for either the first, second, or third estrus following the progesterone priming with the PMS being injected either the last day with the progesterone, or the 12th day following the previous estrus.

Quite divergent results with very low fertility have

characterized progesterone-PMS or progesterone-PMS-HCG treatments (48). The dose, method of injection, and time in relation to follicular growth appear as important factors in HCG administration.

Use of anterior pituitary extract has shown promise in effecting superovulation. A total of 75 mg. for maiden ewes and 100 mg. for mature ewes of horse anterior pituitary extract was administered subcutaneously in six equal injections 12 hours apart beginning on the 12th day following estrus (50, p. 719). Ovulations ranged from 1 to 21 with an average of 11.3 for 74 treated ewes. However, only 79 percent of the ova shed were recovered and, of these only 72 percent were fertilized (49, p. 721).

The successful utilization of ova transfer in laboratory animals has led to refined and rigorous studies within the fields of reproductive physiology, genetics, cytology and immunology. The technique has been successful in most farm animals under experimental conditions and these include goats and sheep (78; 36), cattle (80) and swine (29). It has not been accepted for any large scale breeding program, although an attempt with sheep in Australia showed that numerous successful transfers could be made (49, p. 723). Threefold benefits are apparent in its increasing the frequencies of certain genetic characters in the population, rapid identification of a ewe's genotype, and the fact that more rigid selection can be

practiced on fewer replacement females. Low fertilization rates and the apparent vulnerability of ova to changes in their environment are the major limiting factors (49, p. 723).

MATERIALS AND METHODS

Three inbred lines of registered Suffolk sheep were available in 1962 and 1963 for this study. These Suffolk sheep have been developed through selective breeding from one-sire lines at Oregon State University over the last eight years. With good records of the inbreeding coefficients of these sheep, they served very appropriately as the experimental animals for (1) hormonal treatments to induce superovulation, (2) donors of the ova for transfer into crossbred foster-mothers, and (3) production of inbred and linecross lambs to complete the desired comparisons.

The original crossbred material which was used in the development of the Willamette sheep served as recipient animals. The Willamette breed is being developed from crosses between the Dorset Horn, the Columbia, and the Border Cheviot breeds. For convenience in this presentation, these early crossbred animals will be referred to as Willamette. Normal management practices were carried out for each of the two breeds at the Oregon State University hill pasture sheep farm. Mature ewes of both the Suffolk and Willamette breeds do not tend to vary greatly in size and weight (Figures 1 and 2).

Fertile rams from each of the three inbred lines of Suffolk

Figure 1. Suffolk ewe, weight, 145 pounds, which was the type used as donor animals.

Figure 2. Willamette ewe, weight, 148 pounds, which was the type used as recipients.

sheep were maintained. Five vasectomized rams were available for detecting estrus in the recipient ewes. The total number of experimental animals, breeds, and their utilization for 1962 are listed in Table 1.

In order to produce ova in the transfer group which were linebred and crossbred from each of the three lines involved, the Suffolk donors were marked and assigned to a given ram as shown in Table 2. It is seen from Table 2 that the maximum linebred ova are thus produced while still allowing for a linecross with each of the other two lines.

An additional 21 Suffolk ewes and five Willamette ewes were available in 1963 for further testing of estrous synchronization and superovulatory treatment.

Synchronization of Estrual Cycles

In 1962 an oral progestogen, 6α-methyl-17α-acetoxy-progesterone ("Repromix," Upjohn Company) was used to synchronize the estrous cycles of donors and recipients. A pretreatment feeding period of seven days was carried out before adding the hormone to the feed. The ewes were divided into small groups of approximately 14 to insure adequate distribution of the feed, the amount of which was equivalent to one-half pound per ewe. The ration containing the

Table 1. Experimental animals, their breeds and utilization in 1962.

No. of Animals	Breed	Line	Utilization
		EWES - NON-TRANSFER	
6	Suffolk	I	Produce inbred Line I lambs
6	Suffolk	II	Produce inbred Line II lambs
6	Suffolk	III	Produce inbred Line III lambs
6	Suffolk	I	Linecross (I x II) lambs
6	Suffolk	II	Linecross (I x III) lambs
6	Suffolk	III	Linecross (II x III) lambs
		EWES - TRANSFER	
20	Willamette	-	Recipients of Linecross ova
20	Willamette	-	Recipients of Inbred ova
		EWES - DONORS	
4	Suffolk	I	Donors of fertilized ova
4	Suffolk	II	Donors of fertilized ova
4	Suffolk	III	Donors of fertilized ova
		RAMS	
6	Suffolk	I, II, III	Fertile breeding
3	Vasectomized		Indicate estrus

Table 2. Breeding plan for the donor ewes (Suffolk) in 1962.

Ram Line I		Ram Line II		Ram Line III	
Ewe No.	Line	Ewe No.	Line	Ewe No.	Line
1	I	5	II	9	III
2	I	6	II	10	III
3	II	7	III	11	I
4	III	8	I	12	II

progestogen hormone at the rate of 60 mg. per half pound of grain was fed for 14 consecutive days following the pretreatment period. Due to the results of the superovulation following the oral progestogen treatment in 1962, the synchronization procedure was changed for 1963. In accordance with the method that has proven successful (20; 51), daily injections of 10 mg. progesterone in one cc. of corn oil were used. The injections were given intramuscularly from 1 to 33 days in the rear legs of both donor and recipient ewes. Length of the injection period depended on the date of the previous estrus and the day during which surgical recovery could be performed.

Superovulation of Donors

Injections of various materials containing gonadotrophins, follicle stimulating hormone (FSH) and leutinizing hormone (LH),

were used in an attempt to induce multiovulations in the donor ewes. The hormonal preparations utilized for their follicle stimulating activity included raw PMS ("Colorado Pregnant Mare Serum," Colorado Serum Company), two different purified preparations of PMS ("Equinex," Ayerst Company; "Gonadogen," Upjohn Company), and anterior pituitary extract from domestic animals ("FSH-P," Armour Company). Hormones utilized for their leutinizing activity included human chorionic gonadotrophin ("HCG," Upjohn Company) and pituitary leutinizing hormone ("P-LH," Armour Company).

In 1962 following the oral progestogen feeding, four, five and three Suffolk donors were scheduled to be superovulated for the first, second and third estrus, respectively. Due to an interruption during the experiment, ova recovery was performed on only one ewe prepared for the third estrus. This same animal and one other donor were treated for the fourth estrus. Those four ewes which underwent treatment for the first cycle received their PMS injection, subcutaneously, on the 14th and last day of the oral progestogen. Day 14 from the first, second, and third estrus became PMS injection day for those subsequent donors of ova. Purified PMS and HCG were the hormones used primarily in 1962, however two test animals were given the raw PMS on the fourth cycle. The doses, frequencies of hormonal injections of the four general treatments

concerned are shown for 1962 in Table 3.

Table 3. Hormonal treatment of donor ewes with PMS and HCG following oral progestogen in 1962.

Treatment	PMS Dose (Given on Day 14)			HCG (Given at Estrus)		Interval Between PMS and HCG Injections (days)
Group	Purified PMS (i.u.)	Raw PMS (i.u.)	Frequency of Injection	Dose (i.u.)	Frequency of Injection	
1	1000	-	X 1	800	X 1	1
	500	-	X 2	800	X 1	1
	1000	-	X 2	800	X 1	2
2	800	-	X 2	800	X 1	1
	800	-	X 3	800	X 1	1
3	800	-	X 2	800[1]	X 2	
				1000	X 1	1
4	-	1125	X 2	1000	X 1	2

[1]Two injections of HCG were mixed with the PMS injection.

In 1963 the superovulatory treatment was changed as well as the synchronization procedure in an attempt to produce more fertilized sheep ova at desired intervals. Five of the 21 Suffolk ewes were given superovulatory treatment during two different cycles and each treatment is tabulated separately. For a comparison of the responses of inbred and crossbred animals, five crossbred Willamette ewes were also given superovulatory treatment. In five instances 17β-estradiol was administered to bring on estrus following the gonadotrophin treatment. The hormonal therapy methods were partitioned into four treatment groups depending on the type or

mixture of gonadotrophins used. These treatment groups, the progesterone injection period, the doses and injection frequencies of the gonadotrophins are shown in Table 4.

Table 4. Hormonal treatment with gonadotrophins to induce superovulation in ewes during 1963.

Treatment Group	10 mg. Progesterone Injections	FSH[1]		HCG[2]
		Total Dose	No. of Injections	Dose
	(days)	(i.u.)		(i.u.)
		Purified PMS		
I	9-14	1000	1 & 2	800
	11-15	1500	1 & 2	1000
	10	2000	1	1000
		Purified + Raw PMS		
II	15	500 + 2500	1 & 2	800
	13	2000 + 2500	2 & 1	1000
		Raw PMS		
III	13-16	2500	1	800
	15-17	4000	3	1000
		Anterior Pituitary Extract[3]		
		(mg.)		(mg.)
IV	5-22	25	1	5
	15-23	25	1	25
	0	25	1	30
	0	25	1	50
	0	30	2	0
	0	40	2	0
	9-19	40	2	25
	14	50	2	25

[1]Injected on day following end of progesterone therapy.

[2]Injected at beginning of estrus.

[3]Variation in assays prevents expression in i.u.. but on clinical basis in cattle 50 mg. FSH-P = 5000 i.u. PMS and 25 mg. LH-P = 1500 i.u. HCG.

Ova Transfer Techniques

After synchronization of donors and recipients, and superovulation and breeding of the donors, the animals were fed sparsely in preparation for the required laparotomy. The major parts of the surgical techniques employed were common to both the donors and recipients. Laparotomies were usually performed between 48 and 72 hours following the appearance of estrus, at which time the ova should be fertilized and located in the Fallopian tubes, a location which facilitates flushing.

Anesthesia consisted of sodium pentobarbital (1 gr./ml.) which was injected into the jugular vein. Epinephrine (heart stimulant) and picrotoxin (anti-barbiturate) were kept in readiness for possible emergencies. The anesthetized ewe was fastened to a surgical table which was tilted forward. A midline incision just anterior to the mammary gland and about nine cm. long was made through which the ovaries and uterus were slowly drawn. Ovulations and follicular development were recorded. For ova recovery a polyethylene tube of two mm. outside diameter was guided two cm. down the Fallopian tube via the fimbria. The plastic tube was held in place by gently squeezing the portion of the oviduct which was cannulated between the fingers. The other end of the 30 cm. long tube was held over a watchglass. Using a blunted number 16

hypodermic needle the uterus was punctured two cm. from the utero-tubal junction and the needle point guided into the lumen to within a few mm. of the junction. Pressure was placed on the uterus and needle in a manner which allowed the flushing solution from the syringe to enter the oviduct and prevented its backflow into the uterus. Flushing solution for both 1962 and 1963 consisted of autologous serum which had been prepared several days earlier. Penicillen G potassium (100 units/ml.) was added to the newly prepared serum after which it was stored at -19° C. ± 2° C. until needed. Just prior to use, it was thawed and warmed to 38.6° C. ± 0.3° C. Flushing of the ova directly into a watchglass with eight ml. of serum facilitated rapid location and storage of the ova until a recipient was prepared (Figure 3). The ova were examined under a binocular, the cleavage recorded, then stored in small 8.0 ml. test tubes. The test tubes were filled to within a few mm. of a non-pressure type cap, and placed into the waterbath at 38.6° C. ± 0.3° C.

After exposing the reproductive tract of the recipient ewe ova were placed either approximately 2.5 cm. down the Fallopian tube or into the lumen of a uterine horn. The former method involved the expulsion of the ovum or ova with a small amount of serum into the oviduct through use of a glass pipette fitted with a rubber bulb.

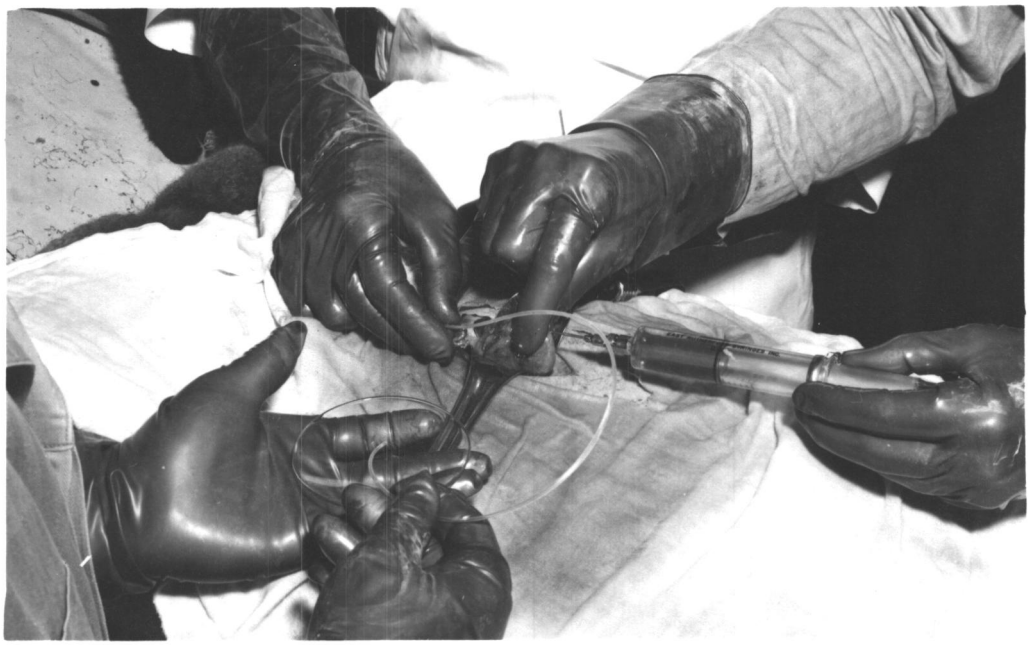

Figure 3. In vivo recovery of ova from Suffolk donor by flushing oviduct from utero-tubal junction.

Figure 4. Inserting pipette through cannula into uterine lumen for expulsion of ova.

The latter method involved puncture of the uterine horn approximately three cm. from the utero-tubal junction. A large, shortened bleeding needle served as a cannula which stayed in the uterine puncture as the smaller, but longer, bleeding needle, which had served as a trocar, was withdrawn. Through this cannula the glass pipette containing the ova in a small amount of serum was carefully guided into the lumen of the uterine horn for expulsion (Figure 4). The glass pipette and the cannula were withdrawn together. After suturing, the animals were given an antibiotic ("Combiotic," Pfizer Company) to prevent infection. Aseptic conditions were maintained to the highest degree possible. With small modifications most of the ova transfer techniques employed are similar to those employed or described by previous investigators (36; 48; 28; 49).

Subsequent Data

Following laparotomy the donor ewes were checked for estrus and bred again to their designated ram if they returned in estrus. Records were maintained on donors and recipients as to their condition following ova transfer. A complete record was maintained on the lamb when it was born, which included birth weight, genetic background, type of birth, sex, and gestation length. Bi-monthly or monthly weights were recorded during the preweaning

period and the average gain and gain per day of age was calculated for each period and for the total period. The lambs were weaned at 85 pounds. At weaning, days of age were recorded and a 120-day adjusted weight was calculated. Conformation and condition scores from four evaluators were averaged and recorded for each lamb at weaning.

RESULTS

The methods of synchronizing estrous cycles in the sheep will be outlined first, followed by the findings in regards to superovulatory treatment, ova recovery, and transfer, and lastly, the comparisons obtained for evaluation of maternal influences.

Synchronization of Estrous Cycles

The 60 mg. of oral progestogen in one-half pound of grain which was fed daily to each ewe in 1962 proved its effectiveness in that 11 of the 12 donors and 41 of the 42 recipients exhibited estrus within 72 hours following the last feeding. The two ewes that failed to exhibit estrus continued in an anestrus state throughout an observation period of 45 days. Following the progestogen feeding, checks were made among all donors and recipients with vasectomized rams to detect the first estrus at 24, 39, 48, 63, and 72 hours and the findings are shown in Table 5. Estrus also appeared in each of the 11 donors, including those which had undergone the first cycle transfer laparotomy, and in all remaining recipient ewes within 14 to 18 days following their first estrus. Enough recipients were still synchronized for the third and fourth cycles to satisfy the requirements of the ova transfer.

Table 5. Synchronization of estrus in Suffolk donors and Willamette recipients after feeding of oral progestogen for 14 days.

Observation Periods Following Last Oral Progestogen Feeding (hours)	Suffolk Donors Exhibiting Estrus		Willamette Recipients Exhibiting Estrus
24	(1)	-	-
39	(2)	1	7
48	-	2	9
63	(1)	4	12
72	-	-	13
Total		11	41

Numbers in parentheses indicate those donor ewes which received PMS treatment on last day of progestogen feeding.

A total of 257 injections of one ml. of corn oil containing ten mg. of crystalline progesterone was administered to the 21 Suffolk ewes over a 51-day period in 1963. None of the ewes which received the daily injection showed estrus during the period of therapy. Following cessation of the injections, estrus appeared in the ewes covering the range between one to five days, however 16 of the 21 ewes exhibited estrus between 64 and 120 hours.

Superovulatory Responses

Results from the treatments to stimulate multiovulations were

classified as "successful" if the number of ovulations were four or more, and "unsuccessful" if the ovulations were less than four. The patterns of treatments which resulted in the higher ovulatory rates are presented in Table 6. A varied number of hormonal therapies which resulted in "unsuccessful" superovulation are presented in Table 7. These sequences of hormonal treatments are given individually in these tables due to the apparent sensitivity of response of the ovary to previous hormonal environments and the time relationships. It is evident from the tables that among those animals receiving purified PMS in 1962, any ewe receiving less than 2000 i.u. failed to superovulate. Lack of superovulatory response was the basis for changing to ten mg. progesterone injections for estrous synchronization in 1963. It is noted, however, that among "unsuccessful" ewes of 1962 which received more than 2000 i.u. of purified PMS, considerable follicular development was exhibited. Following the progesterone injections of 1963, three ewes superovulated which had received 1000, 1500, and 2000 i.u. of purified PMS. No superovulations were realized through the use of raw PMS or a combination of raw PMS and purified PMS. The highest number of ovulations were evident in the ewes receiving a primary injection of 25 mg. of the pituitary extract (FSH-P), followed by a secondary dose of 15 mg. two or three days later (Figure 5).

Table 6. Progesterone-FSH-LH hormonal therapy for "successful" superovulation and follicular development of Suffolk donors in 1962 and 1963.

Progesterone Therapy		Ewe	Follicle Stimulating Hormone		Leutinizing Hormone		Follicles Present	
Type	Days[1]	No.	Injection Day(s)	Amt. of Injection	Injection Day	Amt. of Injection	(unruptured)	Ovulations
1962			(purified PMS in i. u.)		(HCG in i. u.)			
Oral	1-14	5	14, 15	1000	16	800	6	7
Oral	1-14	3	14, 15, 16	800	16	800	7	4
Oral	1-14	6	14, 15, 16	800	17	800	9	5
1963								
Injection	9	1P	9	1000	-	0	8	4
Injection	11	2P	11	1500	12	1000	1	7
Injection	12	3P	10, 12	1000	16	1000	5	5
			(anterior pituitary extract in mg.)		(P-LH in mg.)			
Injection	33	11	34	25	36	25	2	10
None[2]		64	12	25	14, 15	25	3	13
Injection	4	R1	5	25				
			9	15	-	0	20	4
None[2]		R5	14	25				
			16	15	-	0	12	10
None[2]		R9	15	25				
			17	15	-	0	8	19
Injection	10	3	11	25				
			13	15	14	25	19	12
Injection	19	10	20	25				
			22	15	23	25	1	25
Injection	9	13	10	25				
			13	15	14	25	0	25

[1]Beginning of progestogen or progesterone is day 1.

[2]Superovulatory injection day is the number of days from previous estrus.

Table 7. Progesterone-FSH-LH hormonal therapy resulting in "unsuccessful" superovulation in sheep during 1962 and 1963.

Progesterone Therapy			Follicle Stimulating Hormone		Leutinizing Hormone		Follicles Present	
Type	Days[1]	Ewe No. & Cycle	Injection Day(s)	Amt. of Injection	Injection Day(s)	Amt. of Injection	(unruptured)	Ovulations
1962			(purified PMS in i.u.)		(HCG in i.u.)			
Oral	14	9-1	14am, 14am	500	15	800	5	1
Oral	14	1-1	14	1000	15	800	16	2
Oral	14	10-2	14, 15	800	15	800	14	3
Oral	14	8-3	13, 14	800	14, 15	800	7	2
Oral	14	12-2	14, 15, 16	800	16	800	15	2
Oral	14	4-2	14, 15, 16	800	16	800	28	1
Oral	14	7-2	14, 15, 16	800	16	1000	26	3
			(raw PMS in i.u.)					
Oral	14	7-4	13, 14	1125	15	1000	6	2
Oral	14	8-4	14, 15	1125	15	1000	0	1
1963			(purified PMS in i.u.)		(HCG in i.u.)			
Injection	17	13-1	15	500				
			17	1000	20	800	0	1
Injection	17	4-1	14	500				
			17	1000	21	800	0	2
Injection	14	8-1	14	1500	16	1000	4	1
Injection	14	7-1	14	1500	17	1000	6	2
Injection	15	1-1	15	1500	17	1000	7	2
			(purified-raw PMS in i.u.)		(HCG in i.u.)			
Injection	17	14-1	15	500p				
			18	2500r	21	800	0	0
Injection	14	2-1	13	500p				
			14	1500p				
			19	2500r	19	1000	0	2
			(raw PMS in i.u.)		(HCG in i.u.)			
None[2]		3-1	13	2500	14	800	0	1
Injection	16	R6-1	17	2500	--	0	12	2
			(anterior pituitary extract in mg.)		(P-LH in mg.)			
Injection	22	5-1	23	25	25	5	20	1
Injection	4	14-2	5	25	7	5	25	0
Injection	12	15-2	13	25	15	25	14	2
None[2]		4-2	16	25	17	5		
					18	25	8	0
None[2]		1-2	21	25	22	5		
					23	25	12	3

(continued on page 31)

Table 7 (continued)

Progesterone Therapy Type	Days[1]	Ewe No. & Cycle	Follicle Stimulating Hormone: Injection Day(s)	Follicle Stimulating Hormone: Amt. of Injection	Leutinizing Hormone: Injection Day(s)	Leutinizing Hormone: Amt. of Injection	Follicles Present (unruptured)	Ovulations
Injection	8	R2-2	9	25				
			16	15	--	0	9	3
Injection	14	E1-2	15	25				
			16	25	17	25	4	2

[1]Beginning of progestogen or progesterone is day 1.

[2]Superovulatory injection day is number of days from previous estrus.

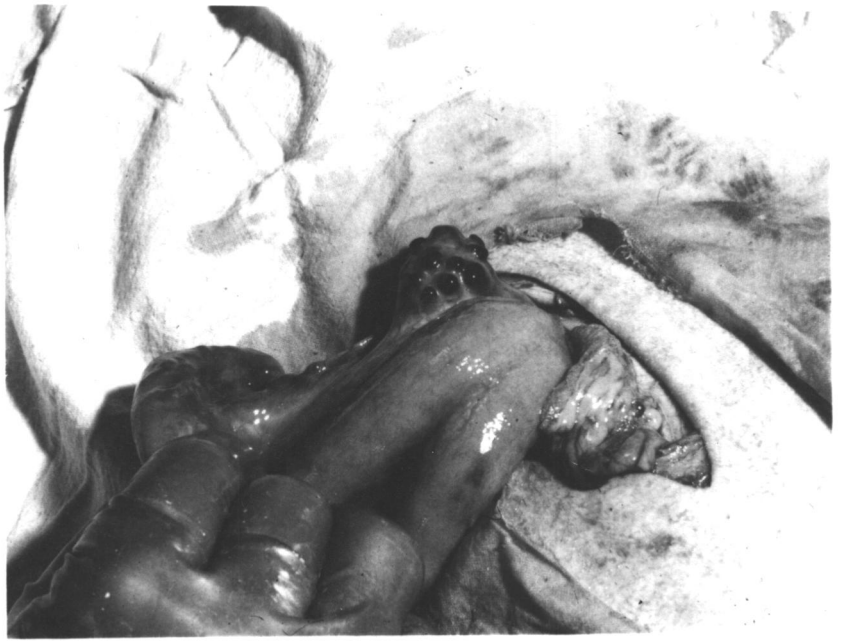

Figure 5. Exposure of uterus and superovulated ovary with 13 corpora lutea.

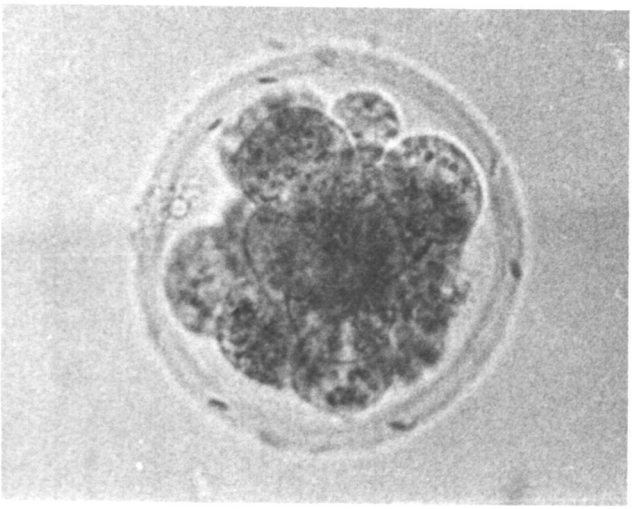

Figure 6. Sixteen-cell sheep ovum typical of ova transferred to uteri of recipients (X463).

No clear effect on the ewe's ability to be superovulated was apparent between the ones that received progesterone injections and those that did not. If the additional injection of 25 mg. of P-LH is considered as the only variable, it significantly increased the ovulations of three ewes, numbers (3), (10), (13) over three ewes in which P-LH was omitted, numbers (R1), (R5), (R9). Follicular development was not lacking in the non-P-LH ewes, however.

Administration of four mg. of 17β-estradiol intramuscularly to five ewes failing to exhibit estrus following the FSH therapy caused the ewes to mate with the ram, however, four of the five did not ovulate and the remaining ewe ovulated twice.

Ova Recovery and Transfer

Thirteen of the twenty-two laparotomies performed the first year were attempts to recover ova. In a similar manner, 30 such recoveries were repeated in 1963. For each ewe the number and cell stage of the recovered ova are shown for both years in Table 8. Only 13 ova from 33 ovulations or 39 percent were recovered the first year, but during the second year this increased to 65 ova from 122 ovulations or 53 percent. Fifty-four and fifty-two percent of the recovered ova exhibited cleavage in 1962 and 1963, respectively (Figure 6). Ten of the thirteen ova from the first group of donors

Table 8. Recovery of ova and their cleavage stage from superovulated sheep in 1962 and 1963.

Ewe No. & Cycle	Ovulations	Number of Ova Recovered — Stage of Cleavage (Blastomeres) 1	2	4	8	16	Total
1962							
1	2	-	-	-	-	-	0
2[1]	-	-	-	-	-	-	-
3	4	1	-	1	-	-	2
4	1	-	-	-	-	-	0
5	7	1	-	-	-	-	1
6	5	-	-	-	1	-	1
7-2	3	-	1	1	-	-	2
7-4	2	-	2	-	-	-	2
8-3	2	2	-	-	-	-	2
8-4	1	1	-	-	-	-	1
9	1	-	-	-	-	-	0
10	3	1	-	-	-	-	1
12	2	-	1	-	-	-	1
1963							
1P	4	-	-	-	2	-	2
2P	7	-	-	1	1	-	2
3P	5	-	-	-	-	-	0
1-1	2	1	-	-	-	-	1
1-2	3	-	-	-	-	-	0
2	2	-	-	-	-	-	0
3-1	1	-	-	-	-	-	0
3-2	12	-	-	-	-	6	6
4-1	0	-	-	-	-	-	0
4-2	0	-	-	-	-	-	0
5	1	1	-	-	-	-	1
6	0	-	-	-	-	-	0
7	2	-	-	2	-	-	2
8	1	-	-	1	-	-	1
9[1]	-	-	-	-	-	-	-
10	25	3	3	2	2	2	12
11	10	5	3	-	-	-	8

(continued on page 35)

Table 8 (continued)

Ewe No. & Cycle	Ovulations	Number of Ova Recovered: Stage of Cleavage (Blastomeres) 1	2	4	8	16	Total
E1	2	-	1	1	-	-	2
12	1	-	-	-	-	-	0
13-1	1	1	-	-	-	-	1
13-2	25	17	-	-	-	-	17
14-1	0	-	-	-	-	-	0
14-2	0	-	-	-	-	-	0
15	2	-	-	-	-	-	2
64	13	-	-	3	8	-	7
R2	3	3	-	-	-	-	3
Others[2]	38	4	-	-	-	2	6

[1] Ewes with adhesions.

[2] Four ewes in which laparotomies to inspect ovaries were made and maximum ova recovery was not attempted.

were transferred into nine synchronized recipients. In eight of these receipients the ova were transferred into the oviducts and the one exception received an eight-cell egg in the left uterine horn. Post-surgical investigations of donors and recipients revealed only one minor infected incision which was cleaned and successfully treated. In 1963 all 65 ova were transferred to 25 synchronized recipients. No estrus was observed in these recipients after the transfer operation.

Maternal Influences

Lambing in 1963 was under close surveillance and assistance was given the ewes when necessary. Two of the nine Willamette ewes which had received transferred Suffolk embryos gave birth to single female Suffolk lambs. One lamb, a line II, originated from the eight-cell egg which had been inserted into the recipient's uterus. The other lamb originated from a line III x II cross. This lamb was transferred as a two-cell egg which was placed six mm. into the recipient's left Fallopian tube. Both lambs were healthy and received no special handling. Single female lambs from the non-transfer group included two line II lambs, one line III x II lamb, and three line III lambs.

The birth weights, adjusted 120-day weights, conformation

and condition scores of the transferred lambs and the non-transfer lambs are shown in Table 9. The average birth weight of the line II non-transfer lambs was considerably below that of the transfer lamb of the same lineage. However, at weaning, no marked differences were apparent in the adjusted 120-day weight, the conformation score or the condition score. In comparison the transfer III x II linecross again exhibits a superior birth weight over the non-transfer III x II lambs. Average birth weights of line III lambs are noted to be decidedly lower than the non-transfer III x II linecrosses. The values obtained at weaning time exhibited similar results as the previous groups, i.e., the non-transfer lambs were actually leading in adjusted 120-day weights, conformation and condition scores. Non-transfer line III lambs exhibited the lowest values of all the animals in this comparison.

Gestation lengths, as calculated from the day that the donor was bred, were 148 days for the line II lamb which was transferred as a eight-cell egg, and 149 days for the III x II lamb, which was transferred as a two-cell egg. Average gestation lengths in days for the other non-transfer lambs used for comparison were 146 for the line II, 148 for the III x II, and 146 for the line III.

Table 9. Average birth weights, adjusted 120-day weights, conformation and condition scoring of lambs for comparison of maternal influences.

Type	Line	Birth Weight	Adjusted 120-Day Weight	Conformation Score	Condition Score
Transfer	II x II	12.6	70	86	86
Non-transfer	II x II	11.3	77	90	88
Transfer	III x II	15.8	90	83	84
Non-transfer	III x II	12.1	94.4	93	95
Non-transfer	III x III	9.8	74.2	78.7	79.3

DISCUSSION

No single factor can explain a phenomenon such as successful synchronization or superovulation, however, the range of response within a species to a changed hormonal titer can be utilized in research if it produces a consistent pattern. Use of the oral progestogen, 6 α-methyl-17 α-hydroxyprogesterone acetate, was effective at the rate of 60 mg. per ewe per day, and this result agrees with other reports (71; 1). However, in many of the sheep that received this treatment the Fallopian tubes were noted to be approximately five mm. in diameter and extremely flacid. It was unhandy to hold the two mm. cannula in place for flushing and this difficulty caused loss of fluid, and perhaps ova, in several instances.

Although evidence is meagre, a possibility also exists that the low superovulatory responses (see Tables 6 and 7), which followed the oral progestogen, were due to the interrelated side effects of the progestogen therapy. Results do show that less than 2000 i. u. of purified PMS failed to superovulate any ewe which received oral progestogen, while there were "successful" multiovulations among ewes which received a lesser dose of PMS following progesterone injections. Without synchronization, a standard dose of 1500 i. u. of PMS given on the 12th or 13th day of the cycle caused

ovulations in 40 ewes ranging from 2 to 38, with a mean of 9.0 ova (48, p. 335).

Obtaining fertilized ova was also important to the investigation. The interval between breeding and laparotomy of the donor was scheduled to allow ample time for divisions to occur in the ovulated egg cells. Using cleavage of the ova as the criterion for fertilization, only 54 percent of the recovered ova were fertilized during the first year. These adverse considerations were important in realizing the goals of this experiment, and therefore, even though synchronization of the estrous cycle was effectively brought about by oral progestogen, the treatment the following year was changed to daily injections of 10 mg. progesterone per ewe. This level of progesterone has been shown to be consistent in its effect of inhibiting ovulation in sheep (20). The decreased hormonal titer was expected to be less damaging to flushing technique, superovulation and fertilization rate, if in truth, these last two items were diminished. Laparotomies subsequent to the injection method revealed Fallopian tubes which were approximately two mm. in diameter. Thus, a snug fitting between the oviduct and cannula apparently allowed the increase in ova recovery rates from 39 percent for the first year to 53 percent for the second year. Other reasons for low recovery rates should be noted. If the interval between ovulation and the recovery attempt

is too long, the oviduct flushings may yield few ova since many may have already reached the uterus. This explanation might suffice for the few ova recoveries in which 16-cell and 8-cell ova were found. In certain instances, such as donor 13-2 (see Table 8), the ovaries of which had 25 corpora lutea and only 17 one-cell ova were recovered, a possibility exists that the rapidly forming corpora lutea entrapped many ova.

The various hormones and intervals of administration which were tested obscure the picture as to which synchronization method was more conducive, or less depressing, to superovulation. Cleaved ova represented 52 percent of the ova recovered during the second year; therefore, the suspected association of flacid oviducts and lowered fertilization rates were false. Other investigators have also found the fertilization rate following progesterone and PMS administration to be in the neighborhood of 50 percent (28, p. 299; 84, p. 206).

The general application of gonadotrophic hormones in sheep to stimulate multiovulations has produced evidence of wide inter-individual variability. Data from Tables 6 and 7 on animals receiving only purified PMS show ovulations ranging from 1-4, 1-7, 5-7, and 1-5 for 1000, 1500, 2000, and 2400 i.u., respectively. In a similar investigation after synchronization with progesterone, ovulations in

sheep receiving 800, 1500, and 2000 i.u. of PMS ranged from 0-12, 1-11, and 0-9, respectively (36, p. 145). Little definitive evidence exists as to whether this variation can be limited through a more optimum chronology of the PMS hormone administration.

After converting to different gonadotrophins, pituitary extract from domestic animals and pituitary leutinizing hormone, superovulation in sheep following progesterone synchronization was attained. Under the pituitary extract group, an inspection of Table 6 discloses 108 ovulations from eight ewes for an average of 13.5 ovulations per ewe, a value which compares favorably with other mean ovulation rates of five (36, p. 146), nine (48, p. 335), and 11.4 (49, p. 721). Ewes receiving these hormone preparations which did not superovulate (Table 7) were part of an exploratory test pattern. Among six of these "unsuccessful" animals it appeared that either not enough P-LH or an improper chronology of P-LH injections and amounts had been administered. Interpretations of the responses of the two remaining "unsuccessful" ewes are less clear. A disturbed endocrinological balance was suspected in one of them, E1-2, because of her continued state of anestrous. Follicular development was induced in the other ewe, 15-2, but for unknown reasons, she failed to ovulate.

The first part of the successful superovulatory treatment began

with a large 25 mg. dose of pituitary extract (FSH-P), which was given intramuscularly, and was followed in two days by a second dose of 15 mg. In 1944 it was reported that injections of sheep anterior pituitary powder produced multiovulations in ewes (9, p. 23), however, gonadotrophic potency was determined by the investigator's assay for each batch of pituitary and comparative values are probably not relevant. Horse anterior pituitary extract has also been reported as an effective gonadotrophin for ovine superovulation (49), however, most of the experimentation which tends to explain the action of exogenous gonadotrophins has been carried out with laboratory animals. After the original stimulation of follicular growth with PMS in the hamster ovary, it was recently shown that additional PMS is needed by the reserve follicles to continue their development (2, p. 440). In these experiments an anti-PMS serum was obtained from rabbits and injected in the hamster to cut off the exogenous PMS titer at various stages of the estrous cycle. The anti-PMS serum did not react with the endogeneous gonadotrophins in control animals, yet its injection into PMS-treated hamsters late in the cycle inhibited all ovulations. This and other information from different stages of the hamster's estrous cycle support the postulate that ". . . the presence of a greater number of Graafian follicles might increase ovarian steroid production, which in turn would decrease the release of

endogenous gonadotrophins" (2, p. 440).

In sheep, the fact that two injections of FSH-P produced such favorable reactions agrees with the earlier report that additional exogenous gonadotrophin is needed after the initial stimulation. Such chemically purified follicle stimulating hormone is said to be destroyed quickly by the body, and according to this same reference, injections should be made every 12 hours (27, p. 169). In opposition to the postulate on increased ovarian steriods and pituitary feedback is the report that pituitaries of sheep treated with progesterone and PMS have less gonadotrophic potency (2, p. 220). This report interprets the finding as an indication that progesterone-PMS treatment causes the release of endogenous gonadotrophin in the ewe. The low ovulation rates of 19 ewes which possessed numerous follicles (Table 7) apparently do not support this conclusion. On the other hand, the successful superovulation of three ewes receiving only FSH-P must be explained by either extra endogenous gonadotrophin or LH contamination of the FSH-P. An investigation of these phenomena could be carried out with assays on blood-serum samples obtained from the ewe's cavernous sinus according to a recently developed technique (46; 21). The study should include examination of pituitaries of individual ewes at the various stages for the histological indications.

The second step in the successful superovulation of ewes was the intravenous injection of 25 mg. of P-LH. How important this rapid increase of blood LH is to superovulation was indicated by comparing the ewes receiving LH with the ewes not receiving LH (Table 6). Desirable effects were produced when one injection of 25 mg. of P-LH was given at the beginning of estrus. Assuming a 30-hour estrus with ovulation occurring during its final 12 hours, it seems probable that only one injection early in the breeding period would induce ovulations at approximately the correct time. This depends, of course, on an optimum LH dose for the number of ready follicles.

Follicle stimulation and ovulation have been effected to different degrees in this experiment by PMS and pituitary FSH. Active portions of the two materials containing the hormone have not been purified so that a given assay can measure the activity in both preparations. Perhaps additional information concerning the contrasting stimulatory actions of these two well-known gonadotrophic materials could be obtained through producing anti-PMS and anti-pituitary FSH, and then testing these anti-serums against each other's antigens. Development of the ovaries under such treatments might well expose different stimulatory actions of the anterior pituitary extract and PMS.

The transfer of fertilized ova, the genetic composition of which is either an inbred line or a linecross, into crossbred maternal environments was successful, but limited in number by other problems already discussed. Seven out of 13 ova recovered showed cleavage, and ten ova were transferred to nine recipient ewes. The pregnancy rate was two out of nine ewes or 22.2 percent. Other investigators have performed similar transfers with successful lambing in four out of 13 or 28.6 percent (84, p. 206) and in eight out of 18 recipients or 44.4 percent (36). Using large numbers of donors and recipients and no synchronization, it was possible to produce a transfer pregnancy rate of 28 percent, where only one egg was transferred and up to 71 percent where two or three eggs were transferred (49, p. 723).

The average weights for the three types of ewes involved in these results were 146.7 pounds for the line II Suffolk, 144.5 pounds for the line III Suffolk, and 148.5 pounds for the Willamette crossbred.

A direct comparison of the single, female, transfer lambs with single, female, non-transfer lambs of the same lineage appeared most appropriate. Lambs of like sex and type of birth from mature ewes were used in the comparison. The birth weight of the line II transfer lamb was 12.6 pounds or a 12.3 percent increase over that

of its non-transfer genetic counterparts. Further, the line III x II transfer lamb weighed 15.8 pounds at birth which is a 30.6 percent increase over that of its non-transfer genetic counterpart. Birth weights are taken as the only available measure of prenatal nutrition and maternal hormonal control. The transfer lamb, line II, indicates to some degree that fetal growth differences can be expressed between the inbred lamb within its inbred mother and a similar inbred lamb within a crossbred mother.

Assuming equal capabilities of the two crossbred recipients, it appears that the linecross embryo with its seemingly small advantage of hybridity can, if given the maternal environment, take advantage of prenatal nutrition to a much larger extent than the more inbred or line II embryo.

At weaning the adjusted 120-day weight values give no clue that the lambs had undergone such different types of prenatal environment. Conformation and condition scores of the non-transfer lambs show higher values than the transfer lambs, which may be only normal variation. These results indicate that the genotype for size of the lamb is expressed at weaning. This finding was also true with the reciprocal crosses between large and small breeds of sheep (35) and likewise, in mice (8). The rapid expression of the genetic control in these lambs may be representative of the postnatal

similarity of the maternal environments. Suffolk ewes provide ample milk and are good mothers, and no wide margin between these factors of maternal environment has been observed between the crossbred Willamettes and the Suffolks.

The non-transfer line III x II lambs were on an average 2.3 pounds heavier at birth than the line III lambs, and again, 22.2 pounds heavier at weaning. Since birth weight of non-transfer lambs should be correlated to rate of gain (6, p. 315), this linecross gives promise of rapid growth. Its prenatal growth potential would appear to be capable of a much higher expression as well.

SUMMARY AND CONCLUSIONS

This study has utilized several recently developed methodologies to bring about a fuller understanding of the hormonal treatments for synchronization of estrous cycles and superovulation in the ewe, and to produce information on inbred and linecross lambs for evaluation of maternal influences. Hormonal therapy for synchronization of estrous cycles involved 35 Suffolk ewes of three different inbred lines and 45 Willamette ewes of crossbred genetic constitution. Hormonal therapy for superovulation of ewes following synchronization of estrus and surgical recovery of ovulated ova were carried out using 35 Suffolk and three Willamette ewes. Suffolk ewes of three inbred lines were bred in order to obtain ova from each of the three lines and ova from each possible linecross. Surgical transfers of ova obtained from the Suffolk donors were made to nine crossbred Willamette Recipients the first year. Sixty-five ova were transferred to 25 recipients the second year and none of these recipients exhibited estrus after the transfer. Observations and conclusions which appear as answers to the problems which were set forth are:

1. Synchronization of the estrous cycles of ewes was achieved effectively with either oral progestogen, 6α-methyl-17α-hydroxyprogesterone acetate, at the rate of 60 mg. per ewe per day,

or daily injections of 10 mg. progesterone.

2. Satisfactory superovulation of the ewes was not obtained with pregnant mare serum preparations and alterations in oviduct morphology were noticed following synchronization of estrus with oral progestogen.

3. Following synchronization of estrus with injections of 10 mg. progesterone, successful superovulation with a mean of 13.4 ovulations per ewe was obtained with pituitary extract and pituitary leutinizing hormone. The sequence of follicle stimulating injections began one day after the last progesterone treatment with a primary injection of 25 mg. of pituitary extract, followed in two days with 15 mg. of the same preparation. The final injection was 25 mg. of pituitary leutinizing hormone which was administered intravenously at the onset of estrus.

4. Recovery rates calculated from the number of ova flushed from the oviducts and the number of corpora lutea observed were 39 percent for the first year and 53 percent for the second year. Cleavage rates for the recovered ova were 54 percent and 52 percent for the first and second years, respectively.

5. Two of the nine Willamette ewes which had received transferred Suffolk embryos gave birth to single, female, Suffolk lambs. A comparison of these lambs, a line II and a linecross

III x II, with single, female, non-transfer lambs of line II, linecross III x II, and line III, was presented. The inbred line II lamb which had been transferred weighed 1.3 pounds, or 12.3 percent, more at birth than its non-transfer, line II counterpart. The linecross III x II lamb which had been transferred weighed 3.7 pounds, or 30.6 percent, more at birth than its non-transfer, line III x II counterpart.

6. Assuming equal capabilities of the two crossbred recipients, it appeared that the linecross embryo with its seemingly small advantage of hybridity can, if given the maternal environment, take advantage of prenatal nutrition to a much larger extent than the more inbred embryo.

7. At weaning the adjusted 120-day weight values as well as the condition and conformation scores were similar for both the transfer and non-transfer lambs. The limited interpretation is that under similar postnatal environment, the genotype for size is expressed in lambs at weaning.

OVA TRANSFERS IN SHEEP AND RABBITS
II. STUDIES ON IRRADIATION DAMAGE

INTRODUCTION

The role of radiation in animal science has become prominent through its use as either an analytical agent or as a treatment variable. Many contributions have been realized with tracers, activation analyses and other quantitative measurements while studies on treatment effects have been limited by lack of information. Experimental studies involving radiation of mammals usually encompass a large number of undesired influences, interactions, and side effects. Irradiation of embryos is of particular interest since the newly fertilized ovum, although consisting of one or a few cells if cleaved, represents the entire organism and alterations in certain mechanisms within these cells can be distinguished many times by obvious changes in later differentiating characteristics. The extreme radiosensitivity of the mammalian ovum has been shown (64; 64; 66). Typical embryo radiation research has usually been carried out by irradiating the embryos in utero at certain stages and then observing the changes leading to mortality of the embryo, or to a deviation from the normal ontogenetic pathways. Evaluations of these radiation effects have been concerned with only the embryo and have usually overlooked

any damage to the uterine environment which may have been caused. That the ionizing ray conveys its energy on the individual cells of the uterus as well as the embryonic cells is obvious.

It is recognized that the number of cells inactivated by a given radiation dose depends on the actual number of cells exposed. This constant for numbers of cells damaged over numbers of cells exposed per unit dose is a phenomenon which is also applicable to the number of cells exhibiting mutations, i. e., the mutation rate is a simple function of the number of living cells exposed (33).

Results which followed irradiation of fertilized mammalian ova during their early cleavage stages has led some investigators to the all-or-none conclusion that radiation either produces a lethal change or does not affect the mammalian ovum (69, p. 126; 60, p. 51; 11, p. 519). Free radicals produced by ionizing rays are thought to react with the molecules of cellular constituents and to produce all sorts of new end products. It appears improbable that all alterations within the ovum would prove lethal. There is limited evidence supporting this thesis in that unequal effects were shown in mouse ova which received equal irradiation (64, p. 308). A rigorous experimental design which would increase the probability of more equally absorbed dose by the ova and allow their development without the influences of an irradiated maternal environment can be found in the

use of ova transfer technique. In addition, most present day embryo radiation research has not been concerned with the development of the embryo beyond its fetal stages. Animal scientists, on the other hand, closely observe post-partum development and are particularly interested in the causes for any changes that might appear much later during the animal's growth to maturity.

With cognizance of some of the errors encountered in evaluating embryo radiation, this study was designed to differentiate the effects of low level x-irradiation on the early cleavage stages of mammalian ova with respect to uterine or embryonic damage. The investigation of non-lethal alterations in the mammalian ovum by various levels of low level x-irradiation is a secondary objective. Joined to these studies is the goal of investigating possibilities of late sequelae due to ova irradiation which might occur in individuals long after birth.

REVIEW OF LITERATURE

The effect of exposure to ionizing radiations has become a subject of monumental proportions during the last decade and a half. Embryonic radiation is one part of this subject in which important contributions through research have appeared slowly due to the nature of the treatment variable and the variety of experimental conditions. However, numerous studies with mammals have been concerned with irradiation of ovarian oöcytes (68; 45; 62), unfertilized ova (12; 44), fertilized ova (11; 12; 68; 62), and developing embryos at later stages (65; 82; 81; 66; 63; 60). Considerable data exist on irradiation of the embryo while it is differentiating and it is generally recognized that numerous malformations are produced in embryos by irradiation during this critical period (38; 82; 81). This period refers only to the stage at which an initial disturbance sets off a series of transformations leading to a subsequent change. This does not assume that the immediate primordium of the character which is malformed must be damaged, since the anomaly may be the result of a complex chain of processes which involve irradiation-damaged cells interacting with normal cells (69, p. 132).

The initial effect of radiation on preimplantation stages of mammalian ova was found in early studies to be a high incidence of

early death but the surviving embryos appeared normal (54; 38). This led to the conclusion that irradiation at early cleavage stages produced either death or unaffected survivors (69, p. 126; 60, p. 51; 11, p. 519). Later research has produced evidence that abnormalities were produced in one and two-cell mouse ova which were observed at 6 and 24 hours following irradiation; these results tended to suggest that there is no lower limit to x-ray effects on the early embryo (64, p. 309). Early irradiation of the mammalian ova has shown that if the mouse embryo is exposed to 50 roentgen x-ray at any time from fertilization of the egg through the ninth day of gestation, its probability of being killed or developing gross abnormalities averages about 13 percent (63, p. 478). Prior to the first cleavage, fertilized mouse ova are highly susceptible to irradiation insult, with 42 percent being resorbed after 50 roentgen and 64 percent after 200 roentgen (63, p. 478). Even lower levels have proven deleterious to the 0.5 day embryo in terms of increased embryonic death. An exposure of five roentgen caused embryonic death at a rate 11 percent higher than the controls, and 25 roentgen caused a 38 percent increase in mortality rates (63, p. 477). Survival of the ova which were irradiated at the one or two-cell stage left the question open as to what had been altered by the radiation. Following such treatment, latent changes which appeared in the embryos have

been attributed to genetic or chromosomal effects (68), however, this concept was not accepted as the whole answer (60, p. 63). Evidence is lacking that these alterations, which were produced by irradiation of early cleavage stages, can be transmitted to progeny. Cytoplasmic changes have been ruled out as being the area of alteration because doses of much greater magnitude are needed to effect a cytoplasmic change that is detectable (69, p. 129). The site of general radiation damage which is hazardous to humans has most of the evidence placing it in the genetic apparatus and the damage appears to be primarily with the cellular reproductive ability and chromosomal changes (54).

Few reports are found in the literature regarding irradiation effects on embryos in regards to sex ratios, birth weights and growth rates. Alteration of the sex ratio to 62 percent male among rats was reported quite early in embryonic radiation history (38), but this claim has not been substantiated by later studies (69, p. 113). Birth weights have been found to be significantly lowered in mice litters which were irradiated on days $8\frac{1}{2}$ through $13\frac{1}{2}$ (69, p. 114). These were postimplantation embryos.

The chain of events in irradiated cells has been postulated by Russell and Russell (69, p. 137) to be: ionizations (generally distributed throughout the cells)$\rightarrow$chromosome breaks = primary

intracellular damage→ chromosome aberrations→ failure at mitoses or aneuploidy after mitosis→ death of cells = initial cellular effect (probably unevenly distributed)→ (if regulatory power of process inadequate for given amount of damage) initial developmental effect→ (i) observed damage related by cellular descent to initial developmental effect; (ii) observed damage caused indirectly by initial developmental effect or (iii) interaction of (i) and (ii).

MATERIALS AND METHODS

Procedures of ova transfer between rabbits have been described in detail (53; 72). Modifications which were performed specifically for this experiment will be explained. All rabbits utilized in the experiment were purchased locally and were fed ad libitum throughout the experiment. Mature female rabbits were allocated to either the donor or recipient category as shown in the schedule in Table 10. Three breeds of males, previously proven fertile, and vasectomized males were used for fertile and sterile matings, respectively (Table 10).

Collection of Ova from Donors

Sixty-four donor animals which included New Zealand White, California, and Checker Giant breeds were available. The donor rabbits were mildly superovulated with subcutaneous injections of purified PMS ("Equinix," Ayerst Company). One injection of 50 i.u. PMS per day for two days was followed on the third day by breeding and an intravenous injection of 25 i.u. of HCG ("Chorionic Gonadotrophin," Upjohn Company). Fertilized rabbit ova were collected at 24 to 28 hours post coitum from the oviducts of the slaughtered donors by flushing. Flushing solutions consisted of

Table 10. Number, breed, and utilization of rabbits for the study on irradiation damage.

Number	Breed	Utilization
Recipients		
26	New Zealand White	Transfer of irradiated ova into a non-irradiated uterus.
26	New Zealand White	Transfer of non-irradiated ova into an irradiated uterus.
12	New Zealand White	Transfer of irradiated ova into an irradiated uterus.
Donors		
20	New Zealand White	To supply fertilized ova at proper time for recipients.
20	California and Checker Giant	To supply genetically marked fertilized ova at proper time for recipients.
Males		
2	New Zealand White	For fertile breeding of donors.
1	California	For fertile breeding of donors.
1	Checker Giant	For fertile breeding of donors.
2	Non-descript	Vasectomized for sterile breeding of recipients.

either physiological saline or a mixture of half saline and half autologus serum. Two-cell ova were selected for the transfer in all but a few instances where four-cell ova were used. A total of 576 ova were transferred during the experiment.

Irradiation of Ova

For irradiation of ova a 100 kilovolt potential (kVp) x-ray machine was set at 1 milliampere (mA), with a half-value layer (HVL) of 1 mm. aluminum and a distance of 37.4 cm. The dose rate was measured with a Victoreen meter and retained at 14.5 roentgen per minute. The selected ova were irradiated in the open watchglass containing 3 ml. of the flushing medium for intervals which allowed total doses of either 0, 20, 80, 120, or 160 roentgen. After allowing for air incident radiation and the absorbing action of two mm. of fluid through which the x-ray must pass to strike the ova, the roentgen absorbed dose (rad) was calculated for these aforementioned values to be 0, 15.4, 61.2, 91.8, and 122.5 rads.

Recipients of Ova

Immediately following the irradiation the ova were placed about one-half inch into the right and left oviducts of the prepared recipient. Either four or five ova to a side were transferred to

approximate the natural number of embryos. The control of non-irradiated ova were handled the same as treated ova except for the irradiation. Control ova were transferred to the left or the right oviduct of a recipient that had received irradiated ova in the other oviduct. Thus controls grew within the same maternal environment as the treated ova at each of the different irradiation levels.

For transfer of non-irradiated ova into an irradiated uterus, the recipient underwent a midline laparotomy at 24 hours after mating with a vasectomized buck. Both uteri were drawn through a 2.8 cm. incision. A gauze dressing dampened with physiological saline kept the exposed uteri from drying during irradiation. After shielding all other portions of the rabbit with a lead sheet (3 mm. thick), the uteri of the recipient were irradiated so that they received either 0, 20, 80, 120, 160, 300, 500, or 1000 roentgen. In order to shorten the time for these higher levels, the conditions of the x-ray machine were changed to 8 mA., and a distance of 15 cm., after which the dose rate was 233 roentgen per minute. Expressed in roentgen absorbed dose the uteri received either 0, 15.4, 61.2, 91.8, 122.5, 250.2, 265.3, or 530.5 rads. These doses were calculated assuming a backscatter factor of 110, and average target tissue thickness of 2 cm., and using the conversion factor, one roentgen equals 0.91 rad.

Results from the first two categories of recipients, i. e., the transfer of irradiated ova into a non-irradiated uterus and non-irradiated ova into an irradiated uterus, were utilized in selecting levels of radiation for the third category, or that of irradiated ova into irradiated uteri. Four combinations of irradiated ova and irradiated uteri levels were tested. Expressed as ova/uteri first in roentgen with the calculated rad dose in parentheses, these combinations were 80/300 (61. 2/250. 2), 80/500 (61. 2/265. 3), 120/300 (91. 8/250. 2), and 120/500 (91. 8/265. 3).

A midline laparotomy was performed on each recipient eight days following the transfer and implantation sites were recorded. At parturition each offspring was recorded as to type of birth, color, weight, sex, crown-to-rump length, width of head, and presence of abnormalities. All offspring were weighed each week until they reached 56 days of age, at which time they were weaned.

RESULTS

It is important to evaluate the efficacy of the ova transfer technique since all pregnancies which occurred in this experiment arose from transferred ova. For controls 43 ova were placed into 12 uteri of 10 recipients. Of these 10 recipients, eight received ova from the irradiated treatment groups as well as control ova. Thus, in most instances the control embryos developed under the same maternal environment as the treated embryos. A second set of controls consisted of four recipients which were subjected to a midline laparotomy, exposure of their uteri for a period equal to that required for irradiation of uteri, and the transfer of 38 ova. Overall rates of transferred control ova which implanted and survived to term were 74.6 and 64.7 percent, respectively.

Irradiation Effects on Pregnancy Rates

Each uterus of the recipient was considered the individual unit when comparing the effects of irradiation on pregnancy because in most recipients of the irradiated ova group, one uterus contained differently treated ova than the other. Irradiation of ova with 122.5 rads prevented any pregnancy from developing to term (Table 11). It was obvious that all implantations at this level were undersized

Table 11. Effects of ova and uterine irradiation treatments on incidence of uteri exhibiting pregnancy at nine days and parturition as a result of transferred ova.

	AMOUNT OF IRRADIATION (RADS)															
	0		15.4		61.2		91.8		122.5		250.2		265.3		530.5	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent
I. Irradiated ova in non-irradiated uteri																
Pregnancy at 9 days	12	(100)	10	(83)	8	(50)	7	(78)	4	(57)	-	-	-	-	-	-
Parturition	8[1]	(73)	8[1]	(67)	6[1]	(43)	4	(44)	0	(0)	-	-	-	-	-	-
II. Non-irradiated ova in irradiated uteri																
Pregnancy at 9 days	6	(100)	4	(100)	3	(100)	2	(100)	7	(58)	3	(38)	3	(50)	0	(0)
Paturition	6	(100)	4	(100)	3	(100)	2	(100)	7[1]	(58)	3[1]	(38)	2[2]	(33)	0	(0)

	AMOUNT OF IRRADIATION GIVEN FOR OVA/UTERUS							
	61.2/250.2		61.2/265.3		91.8/250.2		91.8/265.3	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent
III. Irradiated ova in irradiated uteri								
Pregnancy at 9 days	6	(100)	2	(33)	2	(33)	0	(0)
Parturition	6[1]	(100)	0	(0)	2[2]	(33)	0	(0)

[1]Litters contained some stillborn fetuses.

[2]All fetuses were stillborn.

except one. In general, one observes from Table 11 that with increased x-irradiation of ova, pregnancy rates declined rapidly. However, there was an increase in the pregnancy rate as revealed by observations at nine days in uteri receiving ova which were given 91.8 rads of irradiation over uteri receiving ova which were given 61.2 rads of x-irradiation.

The decreasing incidence of pregnancy at nine days and parturition in recipients with irradiated uteri occurred at radiation levels greater than 91.8 rads. All pregnancies were prevented in the recipients which underwent uteri irradiation of 530.5 rads (1000 roentgen).

In recipients where both ova and uteri were irradiated separately and then considered as a combination, rates of pregnant uteri varied from 100 percent for both implantation and parturition when ova/uteri dose was 61.2/250.2 rads to zero percent for both implantation and parturition at the 91.8/265.3 level (Table 11).

Irradiation Effects on Rates of Implantation and Parturition

Numbers and percentages of ova which implanted and developed to term for the various levels of ova and uterine irradiation are shown in Table 12. In recipients with non-irradiated uteri, the decrease of implantation numbers which was due to the increase in

Table 12. Effects of ova and uterine irradiation treatments on the incidence of implantations and development to term of transferred ova.

	AMOUNT OF IRRADIATION (RADS)															
	0		15.4		61.2		91.8		122.5		250.2		265.3		530.5	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent
I. Irradiated ova in non-irradiated uteri																
Pregnancy at 9 days	29	(67)	23	(58)	18	(30)	10	(28)	8[1]	(29)	-	-	-	-	-	-
Parturition	22[2]	(58)	18[2]	(45)	8[2 3]	(17)	9	(25)	0	(0)	-	-	-	-	-	-
II. Non-irradiated ova in irradiated uteri																
Pregnancy at 9 days	24	(86)	15	(94)	8	(57)	7	(70)	24	(51)	9	(26)	6[1]	(25)	0	(0)
Parturition	20	(71)	10	(63)	8	(57)	6	(60)	15[2]	(32)	7[2]	(20)	2[4]	(8)	0	(0)

	AMOUNT OF IRRADIATION GIVEN FOR OVA/UTERUS							
	61.2/250.2		61.2/265.3		91.8/250.2		91.8/265.3	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent
III. Irradiated ova in irradiated uteri								
Pregnancy at 9 days	12	(43)	4	(17)	7[1]	(23)	0	(0)
Parturition	6[2]	(21)	0	(0)	2[4]	(7)	0	(0)

[1]Included are undersized and probably degenerating implantations.

[2]Litters contained some stillborn fetuses.

[3]Litters contained some malformed fetuses.

[4]All fetuses were stillborn.

ova irradiation from zero to 122.5 rads was significant ($p < .05$). The true difference was expressed between the radiation treatment means of 15.4 rads or less and 61.2 rads or more. Also in these recipients the decrease in numbers of young that were born with increased levels of x-irradiation was highly significant ($p < .01$). Again the means of the treatments producing this highly significant difference partitioned their effects between the levels of 15.4 and 61.2 rads.

Irradiation of the uteri did not appear to affect the development of the non-irradiated ova until the level was 122.5 rads or higher. No viable fetuses were obtained with the uterine radiation level of either 265.3 or 530.5 rads. Fetuses were considered stillborn if, after autopsy, their lungs failed to float in water.

The implantation and parturition responses of irradiated ova which were placed in an irradiated uteri showed additive effects at the levels tested (Table 12). At the lower doses in rads for the ova/uterus (61.2/250.2), the implantation response was high and the parturition response compared well with group I, ova irradiation at 61.2 rads. However, one step increases in either ova or uterine irradiation above this combined dose, 61.2/250.2 rads, caused 100 percent mortality among the transferred embryos.

Malformations Among the Offspring

Viable, stillborn, malformed and resorbed fetuses resulted from two-cell ova which were irradiated 61.2 rads. This was the only level of irradiation which produced malformed fetuses. Developing to term among these embryos was a stillborn fetus which exhibited cranial and thoracic abnormalities (Figure 7). This fetus exhibited cranium bifidum which appeared as a diamond shaped cleft in the frontal bones of its large skull.

Delayed sequalae were observed in two male offspring which had been recorded as normal at birth (Figures 8 and 9). At 30 days of age a decided weakening of their right rear legs became apparent. The spreading condition which resulted was limited to this leg at 52 days of age (Figure 8). The colored male died at 57 days of age, the day after weaning, from rupture of the stomach wall. The condition progressed in the other male with the spreading of the left rear leg, which was followed by spreading of both front legs. At 72 days of age, locomotion was difficult for this animal (Figure 10). With further aging, the forelimbs of this rabbit arched up on its back and extreme skeletal deformities were apparent at 127 days of age. At this time radiographs of a normal and the deformed rabbit were produced for comparison (Figure 11). Although the normal was 14

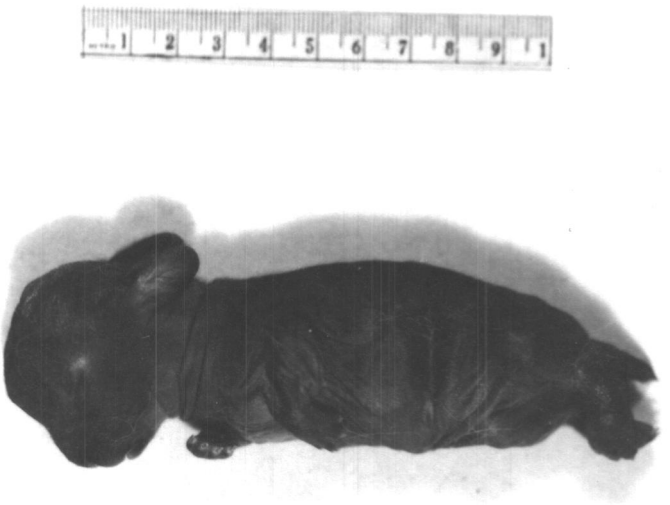

Figure 7. Abnormal stillborn fetus resulting from ovum irradiated 61.2 rads.

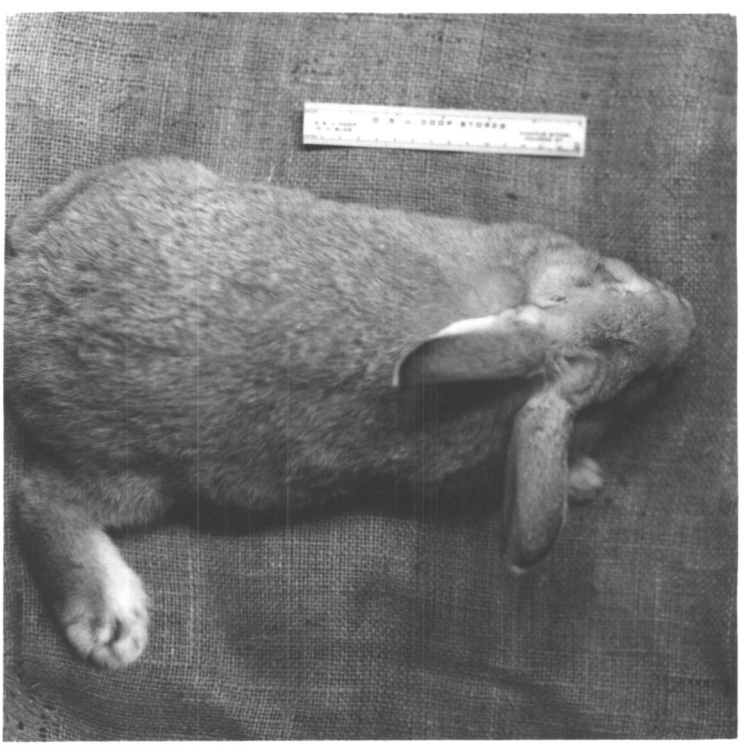

Figure 8. Delayed effect, spreading leg, in male. Ovum irradiated 61.2 rads.

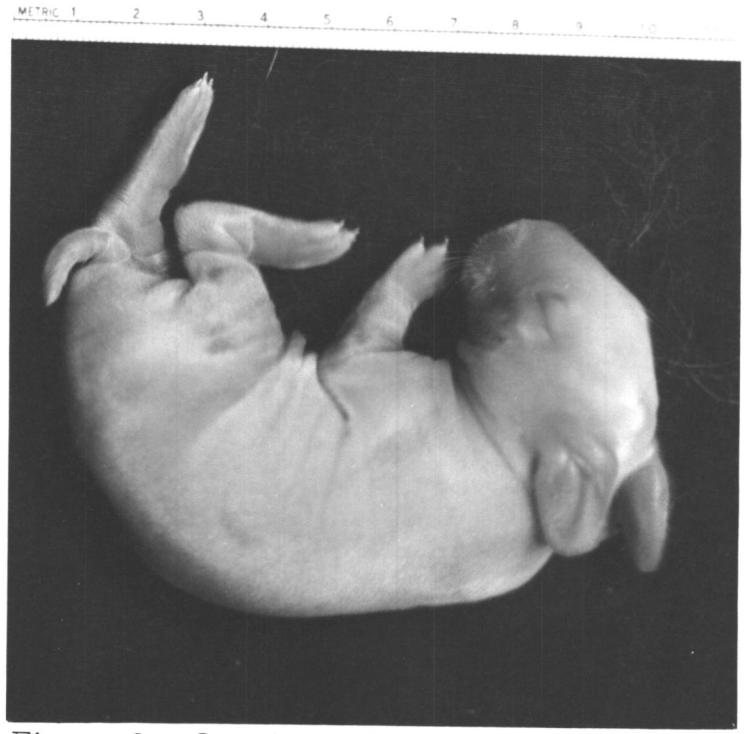

Figure 9. One day old normal appearing newborn. Deformity was delayed.

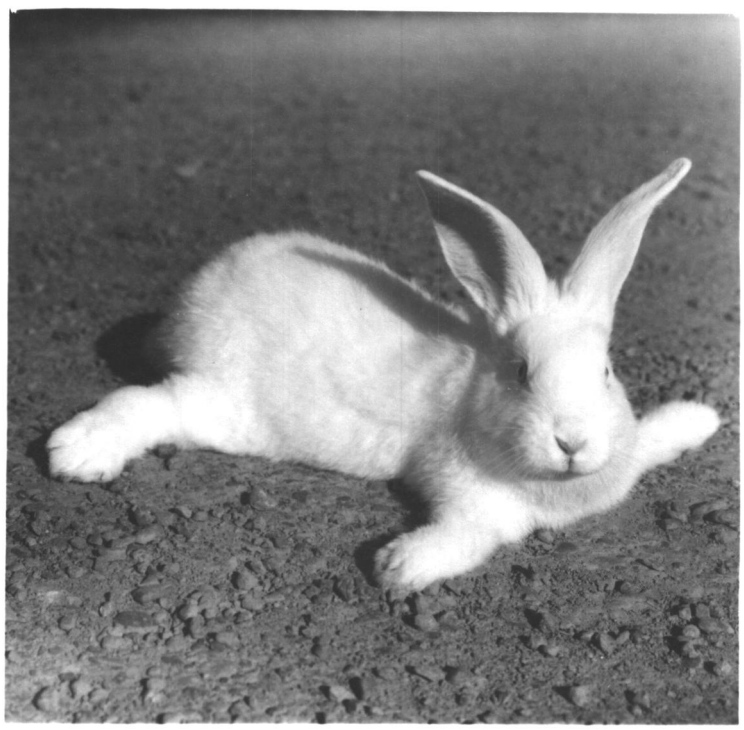

Figure 10. Male rabbit of Figure 9 at 74 days of age. Ovum irradiated 61.2 rads.

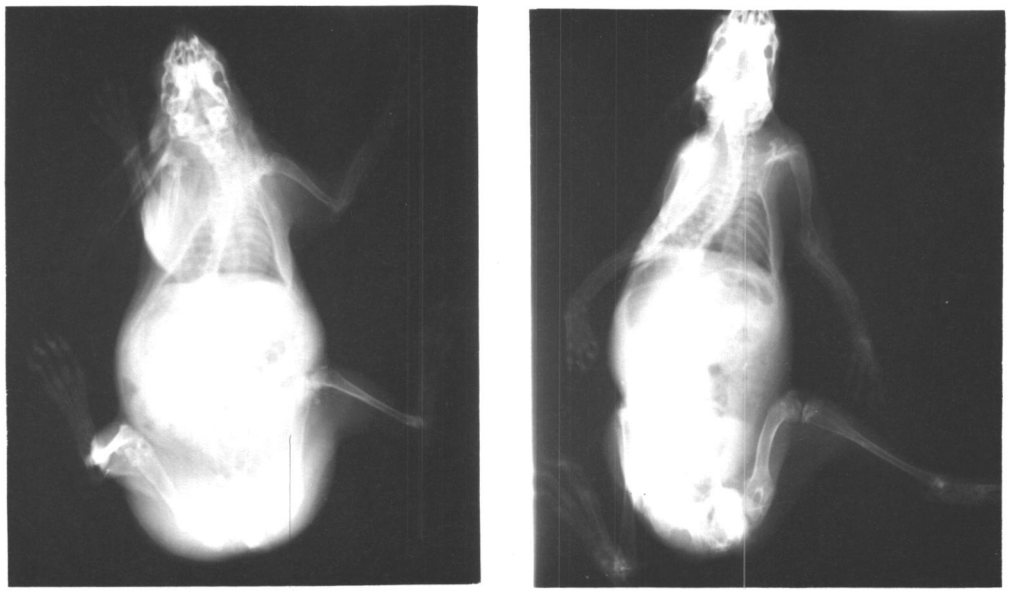

Figure 11. Radiographs of normal rabbit (left) and deformed rabbit which developed from ovum irradiated 61.2 rads (right). Note lack of dislocations, but abnormally positioned limbs. Intestine of irradiated animal is interpositioned between liver and diaphragm. Animal on left is 113 days old and animal on right is 127 days old.

days younger than the abnormal rabbit, it had a larger body and considerably more muscle mass. In comparing the bone structures, the irradiated animal appeared to have an abnormally shaped right acetabulum which caused the right leg to flail. There was no dislocation visible, however, some displastic changes were present. Scoliosis was visible in both animals, however, the condition was temporary in the control and permanent in the deformed animal. The irradiated animal showed extension at the elbows of the forelegs while the normal animal's elbows were flexed. It was also apparent that the intestinal tract of the irradiated animal was interpositioned between the liver and diaphragm. Since the deformed animal refused to eat and was progressively weakening, a midline laparotomy was performed and the intestine was repositioned. Recovery was good and the animal lived another four months. Repeated attempts to obtain a semen sample from the deformed male with an electro-ejaculator failed.

Histological Examination of Embryos

Two recipients each of which contained non-irradiated ova in one uterus and irradiated ova in the other uterus, were slaughtered at seven days after both groups of ova had been transferred to them. The level of irradiation of the treated ova was 61.2 rads. Uteri

containing implantations were fixed, sectioned and the slides were stained with hematoxylin and eosin. Embryos of the non-irradiated groups appeared to be at a later stage than the irradiated embryos (Figure 12). Embryonic knobs were present only in the non-irradiated groups. No distinguishing abnormal developments other than the apparent delay was observed in the embryos which had been irradiated as two-cell eggs. The trophoblastic layer of each embryo was carefully examined under oil immersion and no distinct variations were revealed between the two kinds of embryo preparations.

Weights and Measurements

The mean of the average gain per day for the first 50 days was calculated to be 34.6 grams for the thirteen offspring which survived radiation of either 61.2 or 91.8 rads. A similar mean calculated for the first 50 days of weight increase in the controls was 28.7 grams per day.

Measurements, which included head diameter and crown to rump, revealed only normal variation among all living fetuses obtained in this experiment.

Sex ratios of irradiated newborn were approximately the same as that in the controls which were 45 percent males. This appeared to be within normal ranges for the small number of available offspring.

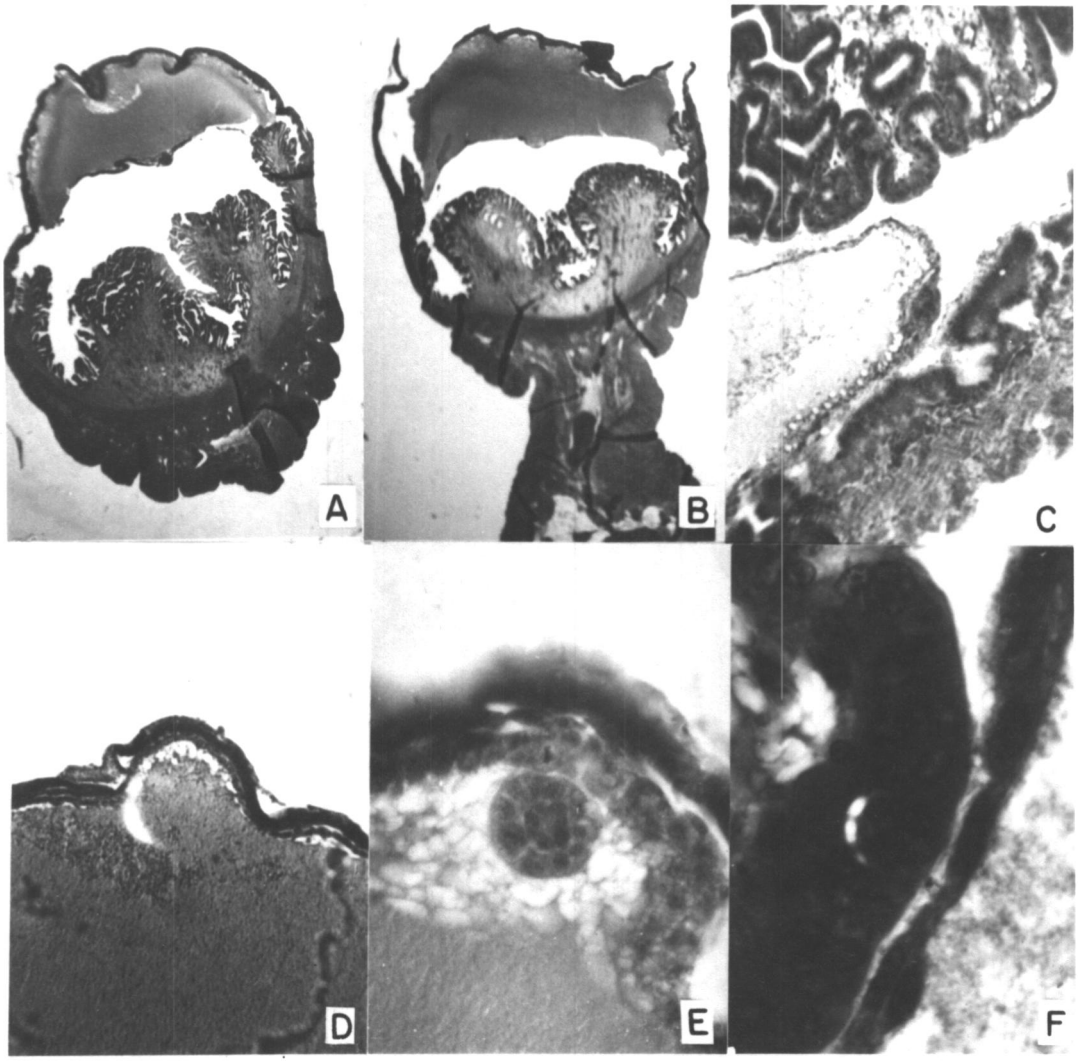

Figure 12. Transverse sections of rabbit uteri and eight-day embryos.

A. Uterine mesometrium is downward and the developing embryo from a non-irradiated ovum is oriented toward it. (X 8)

B. Less development of an embryo which arose from an irradiated (61.2 rads) ovum is noted. (X 8)

C. Non-irradiated embryo exhibits inner cell mass near uterine mesometrium. (X 100)

D. Three layers of an irradiated (61.2 rads) embryo can be observed extending outward from the embryonic pole. (X 100)

E. An embryonic knob with the yolk-sac endoderm and trophoblast above indicates later stage of development in the non-irradiated embryo. (X 430)

F. The trophoblast layer lies in apposition to the uterine epithelium with attachment at several points. (X 970)

DISCUSSION

Several major points in the foregoing data deserve discussion since the interpretation of these new findings may suffer if adequate comparisons with other results and conditions are not considered. The concept of radiosensitivity by Bergonie and Tribondeau (5, p. 984) states, in general, that cells which have the greater reproductive activity ahead of them are more radiosensitive. The newly fertilized ovum represents an extreme in this classification, and is described as highly sensitive to radiation (66, p. 176; 69, p. 310). Exencephalia and anencephalia have been observed in mouse embryos which received 15 roentgen at 1. 5 days (62, p. 575). However, most of these abnormalities resulted in increased mortality rates and no data have been found concerning latent abnormalities developing in individuals which survived ova irradiation. If the procedures of the present experiment had included cessation of data collection at birth or even up until three weeks following birth, no latent effects of radiation in the surviving individuals would have been observed. In most embryo radiation experiments to date the dose levels are expressed in roentgen, as reviewed by Rugh (60), and many times the radiation dose is derived by placing the target materials at a given distance from radioactive cobalt (11; 30; 31).

In order to delimit the dose variation and present more exact measurements of the energy absorbed, the absorbed dose or rad was used for this experiment. These superior radiation measurements plus the use of an x-ray machine, which provides an extremely accurate source of radiation in comparison to radioactive cobalt, are possible reasons for the malformations being found at only one level of radiation.

The spreading leg condition of two males, one colored and one white of the same litter, compares in appearance with a similar condition reported in inbred rabbits (10, p. 197). The spreading condition of the inbred rabbits was limited to the hindquarters and these rabbits died within two months after the deformity was noted. The irradiated "phenocopy" of the present experiment exhibited involvement of the front legs as well. The actual lifespan may have been prolonged through surgery. No measurable inbreeding existed in the deformed rabbits of this experiment.

Pregnant uteri rates increased unexpectedly at the ova radiation level of 91.8 rads. This involved a 28 percent increase in pregnant uteri over those uteri which received ova given 61.2 rads of irradiation. Implantation and parturition responses do not vary widely between these dose rates (Table 12), however, both stillborn and malformed fetuses and the late sequelae, which developed in the

apparently viable fetuses, occurred in ova receiving 61.2 rads. It is suggested that 91.8 rads is enough radiation to kill quickly all embryos which are not genetically hardy, while 61.2 rads damages the ova, some of which will die at a later stage and cause other developing embryos to be resorbed, and some of which will develop to birth and even survive to maturity. Thus it is in these few damaged but surviving embryos that malformations and late sequelae due to radiation will develop. Among uteri which exhibited implantations at nine days followed by 100 percent resorption, seven received ova irradiated 61.2 rads and one received ova irradiated 91.8 rads. Therefore 91.8 rads appears to be a qualitative selector at an early stage, thus producing an "all or none" effect. On the other hand 61.2 rads appears to cause delayed effects, both postimplantation with interactions and postnatal.

After irradiation of the uteri, the decrease in pregnant uteri, number of implantations, and fetuses developing to term was evidence of uterine damage. X-irradiated uteri are just beginning to show an effect on implantation and development to term at 122.5 rads, while the same exposure to ova in vitro produces 100 percent mortality. The postulate that the ovum is responsible for radiation induced death of embryos (23, p. 58) appears well grounded at these levels. Approximately a four fold increase in ionizing radiation was

needed to cause complete inhibition of pregnancy by uterine irradiation over that needed to produce a similar result by means of ova irradiation. The x-ray was applied directly to the uterus in this experiment, and therefore the result cannot be compared with recent work on whole body irradiation of recipient mice (23, p. 57).

The additive injury shown by the dose levels selected for irradiated ova in irradiated uteri provides an indication of the accuracy of the x-ray dose. The fraction of cells damaged must be fairly constant per unit dose in order for the uterus to limit postimplantation fetal development to the extent shown.

It is possible that among the irradiated two-cell ova, one blastomere was killed and the other formed the embryo. The slower segmentation and gastrulation of the irradiated embryos could be explained in this manner, however, gestation periods were approximately the same. More likely the low radiation levels damaged key molecules but repair mechanisms were able to reduce the damage to a non-lethal action with an accompanying delay in development.

A recent concept of radiosensitivity divides the responses associated with external factors, such as dose rate, oxygen effect, and volume, into one category called apparent radiosensitivity, from the responses due to alteration of the nuclear desoxyribonucleic acid (DNA), or inherent radiosensitivity (3, p. 602). Modification of DNA

is believed to be the only means of actually altering radiosensitivity of a cell. Experiments have demonstrated that the cytoplasm of a cell is highly resistant which is conceivably due to its many structures which are duplicated many times (33, p. 96). This line of reasoning suggests that the ova radiation altered the nuclear DNA and began the long series of complex processes which resulted in an altered rabbit. Uterine injury from radiation consists in all probability of many dead and non-reproducing cells. The mesometrium may become too overloaded with its own cellular debris to support the rapidly growing fetus.

That the surviving offspring from irradiated ova grew more rapidly than controls may be theorized as being the result of x-ray selection. These data suggest that perhaps the x-ray might be used as a very early selective tool. With the information available at present, such phenomena will need extensive research before application.

SUMMARY AND CONCLUSIONS

The effects of in vitro x-irradiation of fertilized mammalian ova on their subsequent in vivo development were investigated by means of rabbit ova transfer. Non-irradiated as well as irradiated two-cell ova were transferred to non-irradiated and irradiated uteri of recipients to discriminate between injurious effects to the ova and to the uterus. Numbers of implantations were recorded at nine days post coitum, surviving offspring were recorded at parturition, and observations were continued until the newborn reached the weaning age of 56 days.

The decreasing incidence of pregnant uteri at nine days post coitum which was due to increased levels of ova radiation ranged from 100 percent with no irradiation to 57 percent with 122.5 rads. Parturition in these same uteri occurred at a rate of 67 percent for non-irradiation to zero percent for 122.5 rads. Although exposure to 122.5 rads meant 100 percent mortality for embryos, an equal dose applied directly to the exposed uterus, which later received non-irradiated ova, allowed about one-half or 58 percent of the uteri to undergo a full term pregnancy. X-irradiation of uteri with 530.5 rads prevented all pregnancies while 91.8 rads produced no obvious effect. The decrease in implantation numbers which was due to

increase in ova radiation from zero to 122.5 rads was significant; evidence showed that ova receiving 61.2 rads or more produced the significant decrease. A highly significant decrease in numbers of offspring developing to term was found as ova radiation increased from zero to 122.5 rads. A level of 91.8 rads killed all but the most hardy embryos which repaired their injuries and developed with no apparent anomalies while 61.2 rads damaged egg cells so that death and abnormalities were observable at later stages.

Uteri which were irradiated at 91.8 rads exhibited no effects. Only stillborn fetuses were obtained at 265.3 rads and no implantations were found after 530.5 rads.

Transfer of irradiated ova into irradiated uteri showed that additive effects of radiation on the ova and uterus resulted in zero survivals if either the ova level was stepped up 15.1 rads or the uterus level was stepped up 30.6 rads over the ova/uterus levels of 61.2/250.2 rads.

The 100 percent lethal dose of radiation for development of rabbit embryos was approximately 4.4 times greater when only the uteri were irradiated as when two-cell ova were irradiated.

Irradiation of ova in vitro with 61.2 rads of x-ray caused viable, stillborn, malformed, and resorbed fetuses. One stillborn fetus exhibited cranium bifidum. Two apparently normal newborn

developed late sequelae in the form of spreading limbs which became obvious at one month and progressed to extreme deformation at four months. Bone structure of legs was abnormal but there were no dislocations.

Histological examination of eight day embryos, which had implanted after receiving 61.2 rads or no irradiation at the two-cell stage, revealed that the non-irradiated embryos had developed to a greater extent than the irradiated. Embryonic knobs were observable in the non-irradiated embryos, but not in the irradiated, although the trophoblastic layer and implantation sites appeared similar in both preparations.

An increased growth rate for the first 50 days of the surviving newborn of the 61.2 and 91.8 rads irradiation treatments over that of the controls indicated a possible selection of hardy and rapidly growing animals by the x-ray.

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