SOME OBSERVATIONS ON THE EMBRYO'S ARRANGEMENT IN RABBIT UTERINE HORNS

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Embryo distribution in polytocous animals has been investigated by many workers (8–11, 24, 31, 38, 40, 44, 45). Distances between implantation sites are approximately even apparently due to uterine motility influenced in part by complex stimuli from the embryo. A disparity in the number of ova shed by each ovary introduces the phenomenon of transuterine migration. Embryo transuterine migration occurs not only by in bicornuate and bipartite uteri such as pigs (19–23, 28, 41, 42, 50, 52), cows (30) and mares (46), but also in simplex uteri as in primates reviewed by BOYD, HAMILTON and HAMMOND (12).

MARKEE (44) injecting sea urchin ova into the lumen of spinal rabbits demonstrated that distribution was quite uniform with transfer to estrous rabbits but became progressively poorer with transfer to 5 and 10 day pseudopregnant rabbits. Rabbit embryos pass from the Fallopian tube into the uterus approximately 72 hours *post coitum* (*p. c.*). They remain unattached in the uterus approximately 4 days and are initially scattered randomly throughout the lumen of the cornu. After 2 days when the blastocyst has greatly enlarged the embryos become progressively more evenly spaced from end to end of the horn (11). DZIUK, POLGE and ROWSON (23) observed intra-uterine migration in swine when embryos from a black breed were transferred to one Fallopian tube and embryos from a white breed were transferred to the opposite tube.

The purpose of this study was to observe the behavior of genetically-marked rabbit embryos transferred to the Fallopian tubes of recipients previously bred to fertile bucks.

MATERIALS AND METHODS

Japanese native white rabbits plus a strain bred several years at this

university for black color were used in this study. Thirty does were bred to 2 bucks of the same color and injected with 1 mg of P. L. H. (Armour) intravenously to insure ovulation. Eleven of these does (8 white and 3 black) served as donors and the remaining 19 (4 white and 15 black) served as recipients. Successful data, however, were obtainable from 14 recipients (only 1 white and 13 black in table 1) at the end of pregnancy. Ova were recovered *in vivo* from the donors via a mid-abdominal incision exposing the Fallopian tube. Five ml of saline: serum (1 : 1) was flushed from the uterine end of the tube to the fimbria and collected into a small test tube via a glass tubing fitted into the fimbria (cf. CHANG, 13). One or two recipients were bred synchronously with each donor. Anesthesia in both donor and recipient was induced with sodium pentobarbital (Nembutal, Abbott). Embryos were recovered at 48 hours *p.c.* (6 donors) or 60 hours *p.c.* (5 donors). Each recipient received 2 alien embryos from the opposite breed in each Fallopian tube. A midline laparotomy was performed 9 days *p.c.* to determine number, position and diameter of the uterine swellings and number of corpora lutea (CL). The recipients were autopsied 28 days *p.c.* and the reproductive tract removed for examination. Number of fetuses, placental scars, position in uterus, degenerate fetuses and color were recorded. Some degenerate fetuses could be identified as to origin by eye color. Particular attention was paid to the position of the alien fetuses in the uterine horn and this position was correlated with the 9 day *p.c.* data.

RESULTS

The results are summarized in table 1 and figures 1–2. Eleven donors had 95 ovulation points from which 83 embryos (87 per cent) were recovered. Most of the ova were in the 16 cell stage at 48 hours *p.c.* and in the morula stage at 60 hours *p.c.*

Six recipients were pregnant after transfer at 48 hours *p.c.* In this group total number of CL's, ova transferred and implants at 9 days *p.c.*, were 49, 20, and 54, respectively. Seventy eight per cent of the ova available were represented by implants. Eight recipients were pregnant after transfer at 60 hours *p.c.* The figures in this group for CL's, ova transferred and implants at 9 days *p.c.* were 80, 30, and 89, respectively. Eighty one per cent of the ova were represented by implantation sites at 9 days *p.c.* There was no significant difference between implantation rates after transfers made 48 or 60 hours *p.c.*

The 28 day data are shown in table 1. One recipient in the 48 hours group showed marked asthenia after laparotomy 9 days *p.c.* and therefore

TABLE 1. Results in recipients

Ova transferred	Recipient no.	Uterine horn	No. of CL's at 9 days *p.c.*	No. of alien embryos transferred	No. of implants	Fetuses at 28 days *p.c.* Viable	Degenerating	Total	Embryo arrangement (U.T.J.→Cervix)	Notes
48 hours *p.c.*	7034	L	1	1	2	2	0	2	NA	
		R	4	2	0	0	0	0		
	7040	L	3	2	5	3	2	5	NNNAA	
		R	7	2	8	5	1	6	N?ANNN?	One implant disappeared completely.
	7017	L	3	2	5	0	4	4	NANNA	Marked asthenia after
		R	5	2	7	0	4	4	N??N?NA	laparotomy.
	7038	L	5	2	7	5	1	6	NAANNNN	
		R	4	2	4	1	3	4	ANAN	
	6906	L	5	1	6	3	2	5	NNANNN	
		R	5	1	3	2	1	3	NNA	
	7009	L	4	1	4	1	0	1	ANNN	
		R	3	2	3	1	0	1	?A?	
	Total		49	20	54	23	18	41		
60 hours *p.c.*	7013	L	2	2	4	1	0	1	??A?	One crowded implant.
		R	5	2	5	1	0	1	??N??	
	7021	L	5	1	1	1	0	1	A	
		R	4	2	4	2	1	3	ANN?	
	6809	L	2	2	4	3	0	3	ANAN	
		R	10	2	9	4	0	4	NNNANNNNA	
	7005	L	4	2	6	3	0	3	NNNNAA	
		R	4	1	2	1	0	1	?N	Adhesion occurred.
	7041	L	9	1	8	5	0	5	ANNNNNNN	*
		R	7	2	7	5[1)]	1	6	NANNNAN	
	7016	L	3	2	5	4	0	4	NANAN	
		R	5	2	6	3	1	4	NN?AN?	
	7015	L	7	2	9	0	1	1	A??????????	Two fused implants.*
		R	4	2	6	2	0	2	???N?N	
	7032	L	7	2	8	6[2)]	0	6	ANNNN?N?	*
		R	2	3	5	3	0	3	ANAAN	
	Total		80	30	89	44	4	48		

CL—corpora lutea. U. T. J.—uterotubal junction. L—left side. R—right side. N—native embryo. A—alien embryo. *—one implant adjacent to cervix. ?—Placental scar or degenerate fetus which was not determined whether native or alien.

1) One fetus in the right uterine horn was a skull monster.

2) Two abnormal fetuses : One fetus had abdominal hernia, skull hernia and aplasia of the eyelid. The other fetus had aplasia of the eyelid and skull hernia; both were located in the left uterine horn.

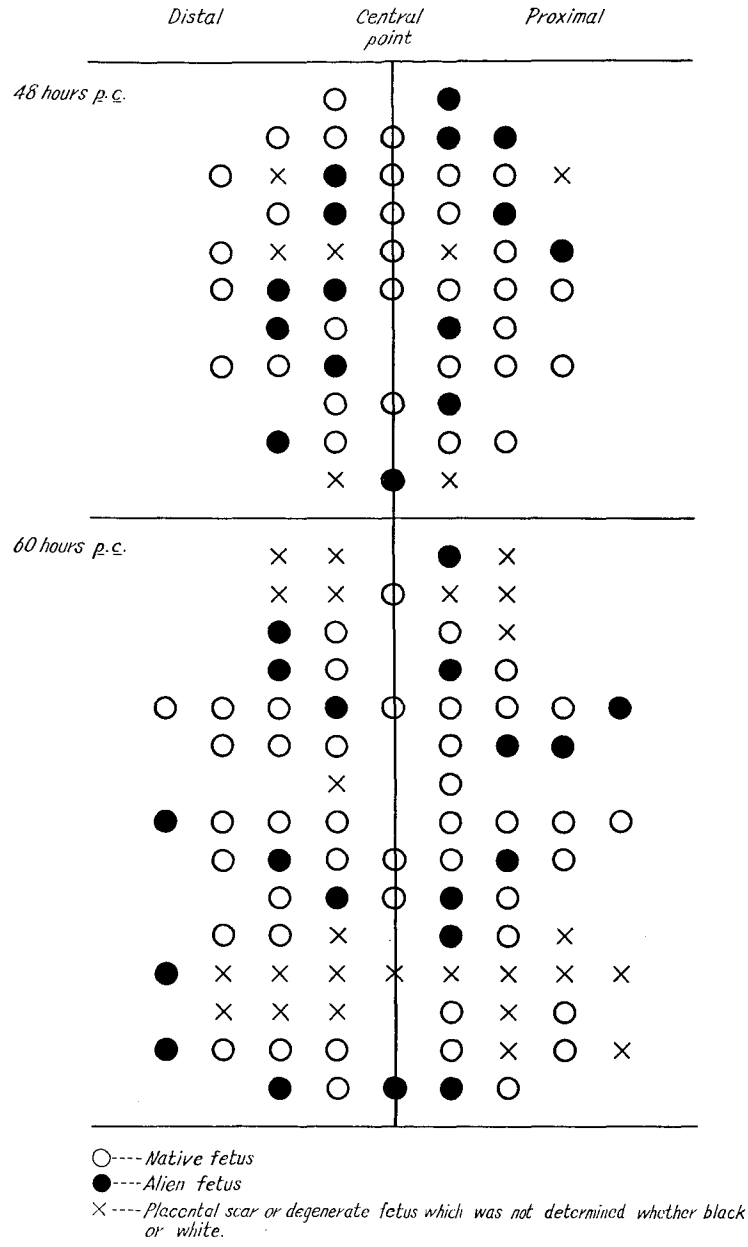

Fig. 1. Distribution of alien and native fetuses in the uterine horns with the distal end at the uterotubal junction and the proximal end at the cervix.

has been considered separately. The total number of implants were represented by 56 and 49 per cent viable fetuses at 28 days *p.c.* in the 48 hours and 60 hours groups, respectively. Viable fetuses represented 36 per cent of the total native ova (CL's) and 48 per cent of the alien ova (embryos transferred). The difference was significant by X^2-test ($P<.05$). When transfers were made at 48 hours *p.c.*, 29 per cent of the native ova and 69 per cent of the alien ova were represented by viable 28 day fetuses. For transfers at 60 hours *p.c.* the figures were 39 and 37 per cent, respectively. Only the difference at 48 hours *p.c.* was significant ($P<.01$). There was no difference in viability of native ova between the 48 and 60 hours group.

Figure 1 and table 1 show the distribution of alien implants in the

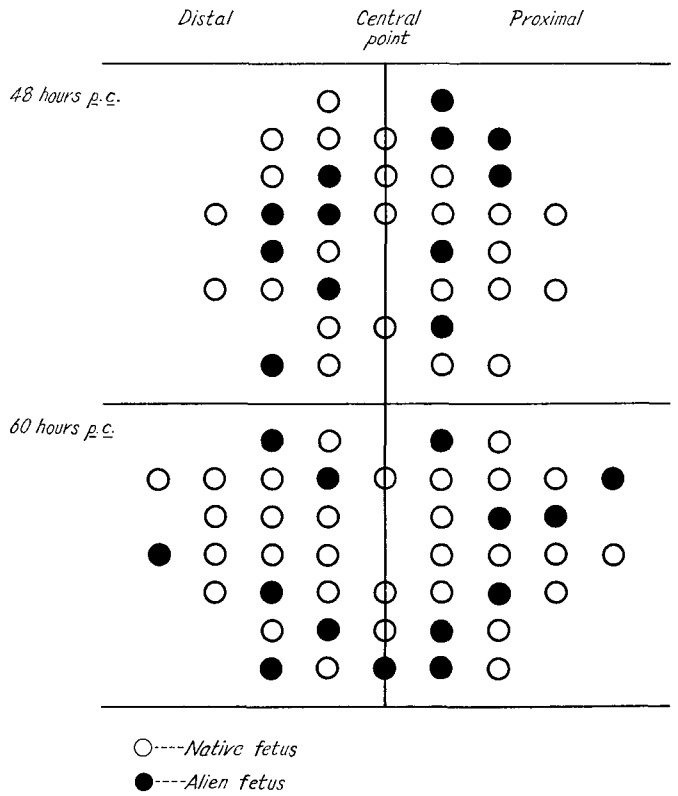

Fig. 2. Distribution of alien and native embryos in each uterine horn at 9 days *p.c.* Only those animals were used where all fetuses could be identified at 28 days *p.c.* Each litter in the uterine horn divided into a distal and proximal end as in Figure 1.

uterine horns. Alien embryos were not confined to either the distal or proximal end of the horn using the middle fetus in the horn as the dividing point of the horn. Figure 2 shows litters in which all fetuses could be identified as to origin. Again the distribution of alien fetuses appears to be random.

DISCUSSION

Implantation rates of from 47 to 89 per cent of embryos transferred are reported where from 5 to 20 embryos have been transferred (1, 29). The rates of 78 and 81 per cent reported here are comparable with these reported figures for rabbits. No apparent reason is seen for the significant difference between alien ova implantation (48 per cent) and native ova implantation (36 per cent) other than that the alien embryos were a selected group all of which were cleaving.

Normal embryo passage into the uterus occurs approximately 72 hours *p.c.* (4, 9, 25, 49, 51). This timing can be upset in a number of ways. Induction of a second ovulation with gonadotropin injections or induction of ovulation during pseudopregnancy or late pregnancy is reported to result in a shortening of the time required for the egg to enter the uterus by at least 12 hours (3, 54). On the other hand AUSTIN (5) reports ova transferred to the Fallopian tubes of late pseudopregnant rabbits are trapped there; and only during a critical period after ovulation (3–4 days) will transfer to the Fallopian tube result in transport into the uterus regardless of whether a second ovulation has been introduced or not. CHANG (17) found transport from the Fallopian tubes to the uterus was not consistent. The endocrine control of embryo transport through the Fallopian tube has received considerable attention (7, 14-16, 18, 26, 27, 34, 35, 37, 39, 43, 47, 48, 53) and has been reviewed by HAFEZ and BLACK (32) and BLANDAU (6). HARPER (33, 35, 37) and ADAMS (2) reported on egg transport in the cumulus clot. Estrogen stimulated transport and progesterone inhibited it.

Although embryo transfer in rabbits is a routine procedure, the relationship between native and alien ova in the tubes is not defined. The present study indicates alien and native ova are mixed randomly and that embryos transferred at 38 as compared to 50 hours after ovulation are equally mixed with native ova. This tends to support the concept that mixing occurs after entry into the uterus.

SUMMARY

To investigate embryo order in the rabbit uterine horn, genetically-

marked embryos were transferred to the Fallopian tubes. Implantations were observed at 9 days *p.c.* and fetal placement, alien and native, was determined at 28 days *p.c.* The distribution of alien embyros showed no special trends, whether transferred 48 or 60 hours *p.c.*, the alien embryos being randomly distributed throughout the uterus.

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Explanation of Plates

These photographs show pregnant uteri and fetuses from recipients at 28 days *p.c.* Litters were in numerical order from cervical end to tubal end in each uterine horn. No fetal number means no fetus was present at 28 days *p.c.* although the implantation site was recognizable at 9 days *p.c.* In pregnant uteri, B is black fetus, W is white fetus and a small circle is a degenerate fetus or placental scar. Black points lying under the fetal number indicates these fetuses are black. L···Left uterine horn, R··· Right uterine horn.

Plate 1

Fig. 3. Recipient No. 7038 (White). Donor No. 7016 (Black). Ovum transfer at 48 hours *p.c.*
Degenerate fetuses are identified as to color by inspection of eye.

Fig. 4. Recipient No. 6809 (Black). Donor No. 7049 (White). Ovum transfer at 60 hours *p.c.*

Plate 2

Fig. 5. Recipient No. 7041 (Black). Donor No. 7049 (White). Ovum transfer at 60 hours *p.c.*
Ova transferred are full sibs with the ova transferred in Recipient No. 6809 in Fig. 4.

Fig. 6. Recipient No. 7016 (Black). Donor No. 7052 (White). Ovum transfer at 60 hours *p.c.*

L
R
W
B
7038
6809
7 6. 5. 4 2 1
1 2. 3 4.
4 3. 1. 1 3. 5. 6

R
L
7041
R
L
7016
8 7 6 5 4 3 2 1
1 2 3 4 6 7
5 3 2 1
2 3 5 6