STUDIORUM PROGRESSUS

Maternal Effects on Implantation and Related Phenomena in the Rabbit

Extensive investigations have been conducted on the morphology, anatomy, and histology of implantation, but very little work has been done on the genetic aspects of implantation in vivo. The quantitative analysis of implantation in vivo is based on the experimental induction of overcrowding in utero. Overcrowding in utero can be produced by superovulation or by transferring an excessive number of embryos (fertilized ova) as shown in the rabbit[1,2] and mouse[3–6]. When rabbits are superovulated, most embryos are reabsorbed after implantation, and, in contrast to mice, fewer living fetuses survive than after natural breeding[2,7]. In superovulated sheep[8] and cattle[9] with the increasing number of implantations, the number of surviving fetuses reached a ceiling level somewhat at or under four per pregnancy. Every species seems to have a 'ceiling value' for the number of implantations that can be maintained successfully throughout pregnancy.

The rate of embryonic development is affected by the gene complement of the zygote, the gene complement of the maternal recipient and the various gene complements of the uterine litter mates, mediated by what appears to be intra-uterine competition[10]. Species, breed and race differences in fetal size are brought about by differences in rate of cell division. The maternal donor's contribution to variability in fetal size is of greater importance than the paternal contribution, and it has been estimated that 50 to 75% of the variability in fetal size is due to maternal factors[11–13].

Certain mothers have a characteristic ability to maintain more living embryos than others, as shown in mice[14,15] and swine[16]. The sire may exert a significant effect on the repeatability of embryonic survival in the successive litters of his daughters[17]. However, it is not known whether this is due to an inherent genetic factor or to maternal environment.

The purpose of this experiment was to study the effect of maternal factors on implantation, fetal survival and prenatal development. This was studied by reciprocal transfer of excessive number of embryos in two pure-bred breeds of rabbits.

Materials and methods. Pubertal and adult New Zealand white and Chinchilla rabbits were used in this investigation. Overcrowding in utero was induced in three groups using the embryo transfer technique. Reciprocal transfer of embryos between the two breeds was performed as follows: Group I, embryos from New Zealand donors were transferred into New Zealand recipients. Group II, embryos from New Zealand donors were transferred into Chinchilla recipients. Group III, embryos from Chinchilla donors were transferred into New Zealand recipients.

Donors: The donors were treated with 150 to 200 IU of pregnant mare's serum (PMS, Equinex, Ayerst) and 50 IU of human chorionic gonadotropin (HCG, Upjohn) to induce superovulation. PMS was injected subcutaneously, and the last dose was given 48 or 72 h before breeding, and HCG was injected intravenously at the time of breeding. The donors were bred to two fertile bucks of the same breed as the doe, and autopsied 40 to 50 h post coitum (pc). The uterine tubes were flushed with normal physiological saline and the embryos in the flushings were examined microscopically (× 99) for normality of development; the main criterion being that they should have reached the 8- to 16-cell stage. Unfertilized ova and embryos showing retarded cleavage were discarded. Two to four donors were autopsied at one time and all the normal embryos collected in one covered watch glass. Pending transfer, the embryos were held at room temperature; the interval between embryo recovery and subsequent transfer ranged from 15 to 60 min.

Recipients: At the same time as the donors were mated, the recipients were induced to ovulate with an injection of 15 IU of HCG. Two days after the HCG injection, 20 to 40 two-day-old embryos were transferred to each of the uterine tubes via a flank laparotomy. Aseptic techniques were used in all operative procedures.

At 9 days pc, a midline laparotomy was performed on the recipients and the number, diameter, and spacing of uterine swellings recorded. The recipients were autopsied at 29 days pc and the weight and sex of viable fetuses were recorded. The site of viable and degenerating fetuses was identified with respect to the implantation sites recorded at 9 days pc. The weight of the placenta was recorded for each viable and mummified fetus.

Results. The number of corpora lutea in recipients autopsied at 29 days pc varied from 8 to 25 and the average number of corpora lutea per group was 12 with no significant difference in the ovulation rate of the two breeds.

Implantation capacity: Implantation capacity is indicated by the number of implantations, per uterine horn or per litter, as determined by laparotomy at 9 days pc (Table I). The number of implantations per uterine horn varied from 3 to 27, whereas the number of implantations per litter varied from 11 to 50. The average number of implantations per litter in groups I, II, and III was 33, 42, and 34 respectively. The Chinchilla breed had significantly higher implantation capacity as compared with the New Zealand breed.

The percentage of implantations with a diameter of 11 mm or less on day 9 pc varied from 27 to 39. There was a trend to indicate that the percentage of these undersized implantations was higher when Chinchilla embryos were transferred into New Zealand recipients. In general, the diameter of implants was significantly reduced in overcrowded uteri.

The diameter of implants at 9 days pc which were represented by viable fetuses at 29 days pc varied from 8.3 to 19.0 mm irrespective of the breed and degree of crowding of the uterus.

[1] C. E. ADAMS, J. Reprod. Fertil. *1*, 36 (1960).
[2] C. E. ADAMS, J. Endocrin. *19*, 325 (1960).
[3] A. SATO, Misc. Rep. Yamashina Inst. Orn. Zool., Tokyo *13*, 27 (1959).
[4] A. SATO, Jap. Genet. *34*, 226 (1959).
[5] A. MCLAREN and D. MICHIE, J. exp. Biol. *36*, 281 (1959).
[6] A. MCLAREN and D. MICHIE, *Implantation of Ova* (P. ECKSTEIN, Ed., Mem. Soc. Endocrin., University Press, Cambridge).
[7] A. S. PARKES, J. Endocrin. *3*, 268 (1942).
[8] T. J. ROBINSON, J. agric. Sci. *41*, 6 (1951).
[9] E. S. E. HAFEZ, Anat. Rec. *148*, 203 (1964).
[10] R. A. BEATTY, Genet. Res., Cambridge *1*, 39 (1960).
[11] S. WRIGHT, USDA Bul. no. 1121 (1922).
[12] J. L. LUSH, H. O. HETZER, and C. C. CULBERTSON, Genetics *19*, 329 (1934).
[13] O. VENGE, Acta Zool. *31*, 148 (1950).
[14] M. N. RUNNER, J. exp. Zool. *116*, 1 (1951).
[15] R. G. EDWARDS and R. E. FOWLER, J. exp. Zool. *141*, 299 (1959).
[16] L. N. BAKER, A. B. CHAPMAN, L. N. GRUMMER, and L. E. CASIDA, J. Anim. Sci. *17*, 612 (1958).
[17] J. S. PERRY, J. Reprod. Fertil. *1*, 71 (1960).

Fetal survival: The number of viable fetuses as a percentage of total number of recovered fetuses at 20 days pc varied from 66 to 85 (Table II). The percentage of fetal survival was significantly higher in the New Zealand recipients than in the Chinchilla ones. The number of viable fetuses per litter varied from 6.5 to 7.9 with no significant difference between breeds. The maximum number of viable fetuses was 11 per uterine horn and 16 per litter; these values are slightly higher than those usually obtained in naturally bred does of corresponding breeds of recipients. It would appear that transferring a large number of embryos may cause an increase in the total litter size at 29 days pc.

The majority of prenatal deaths occurred following implantation. However, there was a certain percentage of early and late fetal death. Early embryonic mortality is calculated as the number of placental remnants at 29 days pc as a percentage of implantations at 9 days pc. Most of the early embryonic deaths seemed to have occurred 10 to 13 days pc. Early embryonic death varied from 64 to 73%. There was no special pattern for the distribution of viable fetuses in relation to degenerating fetuses (Figure 1).

Fetal and placental weight: Fetal weight at 29 days pc which ranged from 11.3 to 64.0 g showed wide variation within the same litter and between the two breeds (Figure 2). The weight of the placenta of viable fetuses ranged from 2.0 to 8.8 g. There were no significant breed differences in fetal and placental weights. There was a trend to indicate that more males than females survived in the overcrowded uteri.

Discussion. The results indicated that in the Chinchilla breed, the implantation capacity was higher and the percentage of fetal survival was lower than in the New Zealand breed. The litter size and fetal weight were not significantly different in the two breeds. It seems that differences in implantation capacity are partly due to genetic differences in the morphological and physiological integrity of the uterus, e.g. the length of the uterine horns and the degree of development and secretory activity of the endometrium. The number of implantations, in an overcrowded uterus, is controlled by the available uterine space[18].

The wide variability in implantation capacity of individuals in the same group may be due to maternal genetic factors. FEKETE[19] studied implantation in two inbred strains of mice and found that there was no difference in the viability of eggs of dba and C_{57} black strains and that the uterine environment of the C_{57} black mice was more favorable for development. In mice, inbreeding causes a reduction in pre-implantation loss without any deleterious effect on ovulation rate or post-implantation mortality[20]. Individual variation may be also due to variability in the

[18] E. S. E. HAFEZ, J. exp. Zool. *156*, 269 (1964).
[19] E. FEKETE, Anat. Rec. *98*, 409 (1947).
[20] D. S. FALCONER and R. C. ROBERTS, Genet. Res. *1*, 422 (1960).

Table I. Effect of breed on implantation and related phenomena in overcrowded uteri as measured 9 days post coitum

Group	I		II		III	
Breed of donor	New Zealand		New Zealand		Chinchilla	
Breed of recipient	New Zealand		Chinchilla		New Zealand	
Items	Range	Mean	Range	Mean	Range	Mean
No. of corpora lutea (R)	4–14	7	3–9	6	3–9	5
No. of corpora lutea (L)	3–11	6	5–9	6	3–9	6
No. of corpora lutea (both)	8–25	12	8–16	12	9–14	11
Total no. of embryos transferred	–	749	–	874	–	880
No. of implantations (R)	3–26	15.8	15–27	22	8–22	17
No. of implantations (L)	5–24	17.3	15–25	20	8–23	17
No. of implantations (both)	11–50	33.1	30–50	42	23–44	34
% Implantation	–	44	–	57	–	47
% Implantation with diameter less than 11.1 mm	–	27	–	20	–	39
Diameter of all implants (mm)	3.4–25.5	11.8	3.2–26.0	13.0	3.0–20.1	11.7
Diameter of implants producing viable fetuses at 29 days post coitum	8.4–16.1	13.1	10.1–17.9	14.2	8.3–19.0	14.0

Table II. Fetal survival, prenatal mortality and fetal development as measured 29 days post coitum

Group	I		II		III	
Items	Range	Mean	Range	Mean	Range	Mean
Total no. of fetuses	–	85	–	131	–	97
% Viable fetuses	–	85	–	66	–	80
No. of viable fetuses/doe (R)	0–7	3.8	1–11	3.4	0–11	4.0
No. of viable fetuses/doe (L)	1–8	3.4	1–6	4.5	0–7	2.5
No. of viable fetuses/doe (both)	3–15	7.2	2–16	7.9	0–14	6.5
% of early embryonic mortality/doe[a]	9–94	64	44–100	73	35–100	71
% of total prenatal mortality/doe[b]	27–94	69	59–100	82	39–100	76
% Males within viable fetuses	–	67	–	62	–	51
Fetal weight (g)	29.3–42.3	36.4	20.3–53.1	36.2	11.3–64.0	33.5
Placental weight (g)	3.8–5.8	4.9	2.4–7.9	4.7	2.0–8.8	4.5

[a] Implants degenerating following implantation represented at 29 days pc as placental remnants. [b] Implants not represented by viable fetuses at 29 days pc.

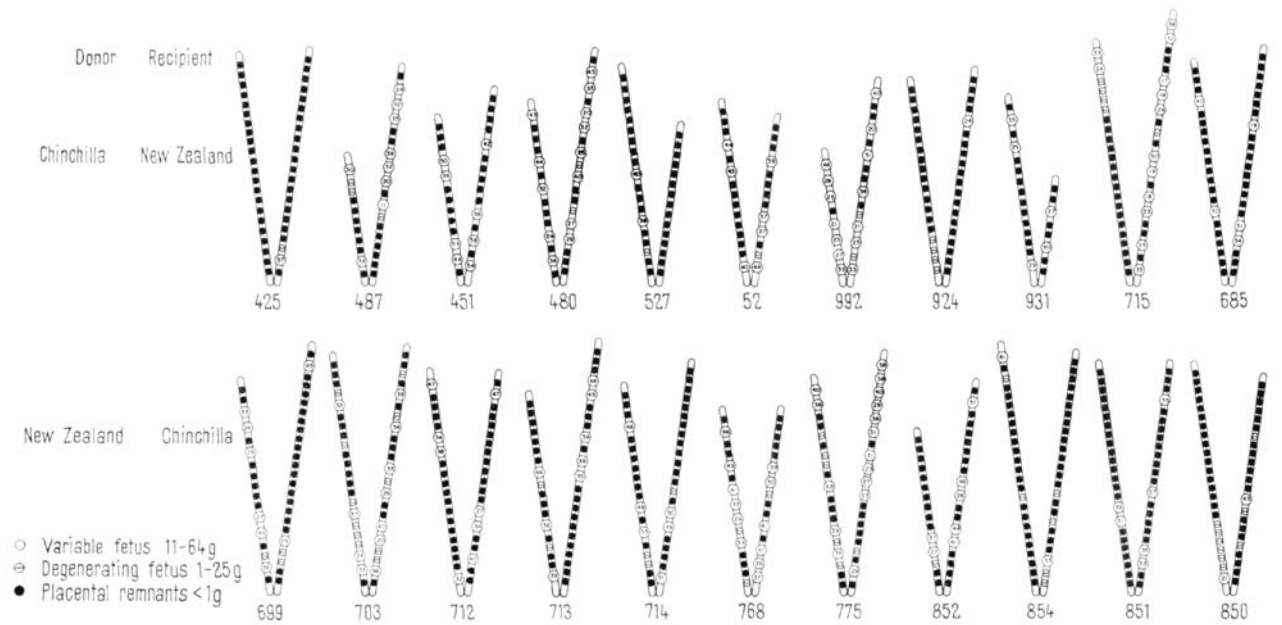

Fig. 1. Diagrammatic illustration to show the relationship between overcrowding in utero implantations at 9 days post coitum and fetal survival at 29 days post coitum in groups II and III. There was reciprocal transfer of embryos between the Chinchilla and New Zealand breeds. Note that the location of the fetus along the uterine horn had no effect on the survival or weight of fetuses. The figures within the circles indicate the fetal weight at 29 days post coitum.

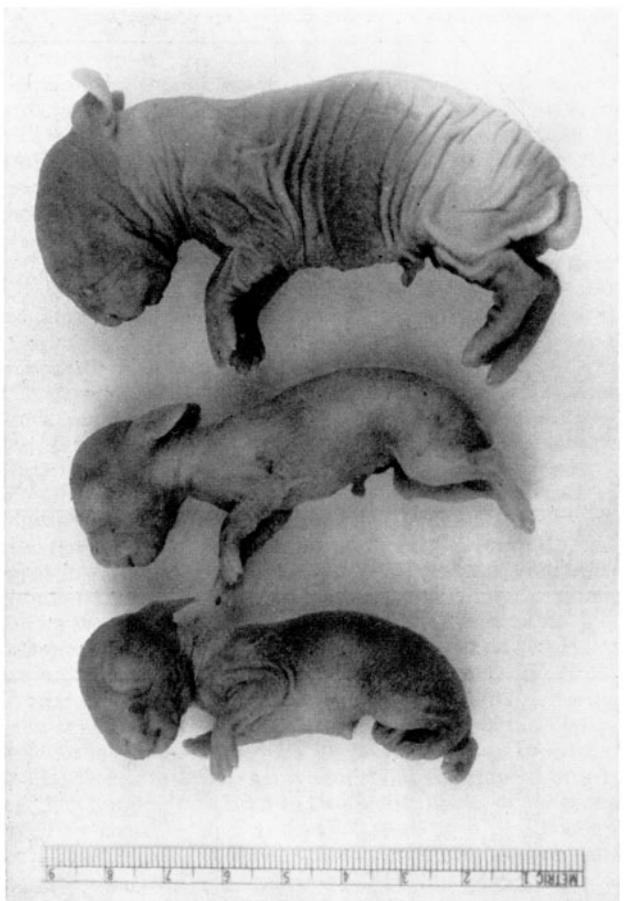

Fig. 2. Viable fetuses recovered from Group II at 29 days post coitum from one uterine horn. Note the great variability in weight.

degree of development of endometrial glands or uterine vasculature. MYERS and POOLE[21] reported that the domestic rabbit exhibits estrous cycle patterns every four to six days. This variability may be responsible for individual differences in implantation capacity; the response at one stage of the cycle may be different from that at a different stage.

Each uterine horn can sustain a limited number of viable fetuses to full term. The number of live fetuses per uterine horn showed little difference between two breeds, there being a marked rise in embryonic mortality as crowding increased. The maximum number of live fetuses observed in one horn was eleven. Individual differences in the capacity of the uterine horn to carry viable fetuses may be related to morphological or biochemical differences in the uterus which undergoes profound changes in order to accommodate the rapidly growing fetuses during pregnancy. Since most of the prenatal loss occurred immediately following implantation, the effect of overcrowding on fetal weight at 29 days pc had been reduced. In general the fetal weight is positively related to placental weight and negatively related to litter size. Thus the degree of overcrowding affected fetal weight. On the other hand, the location of the fetus in utero and the degree of proximity of viable fetuses in utero did not influence fetal weight.

The contribution of a particular donor female or male was not considered in the experimental design. The magnitude of variance in implantation and fetal development might have been influenced by an 'enhancement' effect to fetuses developing in separate uterine horns of a given recipient. During an experiment with the rabbit[22,23], it was noted that when genetically large and genetically

[21] K. MYERS and W. E. POOLE, Nature, Lond. *195*, 358 (1962).
[22] R. A. BEATTY, Res. Report 1952–54 (Ed.: C. H. WADDINGTON; Inst. Anim. Genet., Edinburgh 1955), p. 25.
[23] R. A. BEATTY, J. Genet. *55*, 325 (1957).

small offspring were born in the same litters after heterospermic insemination, the average difference in birth weight between them was greater than when they were conceived in different litters after homospermic insemination. The enhancement effect is presumed to be due to in utero competition between fetuses resulting in a disproportionately large gain in weight by those having a slight advantage over their litter-mates.

The influence of litter size on fetal size is manifested through a local effect and a general effect[24]. The local effect refers to an influence on the growth of a fetus determined by the presence of other individuals within the same horn. General effect refers to an influence of other individuals in the uterus; it is independent of their distribution between the horns. Thus, an increase in the number within the same horn could have both local and general effects on the fetus, whereas an increase in the number in the other horn could have only a general effect.

McLaren and Michie[25] presented evidence which shows the inadequacy of the classical explanation of the effect of litter size on fetal weight, viz. that there is only a limited pool of nutrient in the maternal blood for which the fetuses compete, so that the more fetuses there are, the less nutrient there is for each. In species in which the young are born at a relatively advanced stage of development, however, competition for nutrients may still play a part, particularly in the later stages of pregnancy. In this respect, the results of Healy et al.[26] were explained by the theory that the chief factor regulating fetal growth is the pressure at which maternal blood is supplied to the placenta. In the rabbit, the number of maternal arterial vessels which penetrate into the maternal placenta varies from 4 to 12 with an average of 8, whereas the number of maternal venous vessels varies from 1 to 6 with an average of 3[27]. This may explain the marked inter- and intra-litter variability in fetal weight[28].

Zusammenfassung. Intrauterine Embryonenüberfrachtung wurde experimentell durch Übertragung von Neuseeländer-Spenderkaninchen in Neuseeländer- und Chinchilla-Empfängerkaninchen, von Chinchilla-Spenderkaninchen in Neuseeländer-Empfängerkaninchen erreicht. Die Empfängertiere wurden 9 Tage nach Begattung zur Prüfung der Implantation und 29 Tage danach zur Beurteilung des fötalen Entwicklungszustandes eröffnet bzw. abgetötet. Chinchilla-Kaninchen zeigten erhöhte Implantationskapazität und verringertes fötales Überleben gegenüber den Neuseeländern.

E. S. E. Hafez

Department of Animal Sciences, Washington State University, Pullman (Washington USA), October 7, 1964.

[24] P. Eckstein, T. McKeown, and R. G. Record, J. Endocrin. *12*, 108 (1955).
[25] A. McLaren and D. Michie, Nature, Lond. *187*, 363 (1960).
[26] M. J. R. Healy, A. McLaren and D. Michie, Proc. Roy. Soc. Lond. B *153*, 367 (1961).
[27] Y. Tsutsumi and E. S. E. Hafez, Proc. 5th Inter. Congr. Anim. Reprod. A. I., Trento (Italy) *2*, 195 (1964).
[28] This investigation was supported in part by U.S. Public Health Service research grant HD-00585-03 of the National Institute of Child Health and Human Development. Drugs were kindly donated: PMS (Equinex) by Ayerst Laboratories Inc., New York (N.Y.); HCG by Upjohn Co., Kalamazoo (Mich.); Nembutal by Abbott Laboratories, North Chicago (Ill.). Thanks are due to Mr. R. E. Mauer for technical assistance. Scientific Paper no. 2556, Washington Agricultural Experiment Stations, Pullman (Washington). Project no. 1698.

Extraction and Analysis of Histone Protein from the Roots of Three Plant Species

Introduction. There is little doubt that histone is an important, although possibly non-permanent, component of chromosomes. Staining techniques[1] and extraction procedures[2] support its presence in the nucleus. Several recent investigations have implicated histone in cell metabolism, and particularly in the regulation of gene action[3], indicating that it plays more than a purely structural role in chromosome organization. Whereas methods for the extraction of histone have been developed for animal cells, its location in plant cells has been revealed primarily by cytochemical methods, and although histone has been characterized in a number of animal species[4], less information of a comparable nature is available for plants.

Consequently, and because of their advantages for chromosomal studies, three plant species, *Allium cepa*, *Pisum sativum*, and *Vicia faba*, have been examined for their histone content. In attempting to determine whether methods used for the isolation of whole histone from animal cells were applicable to plant cells, several techniques were employed. Procedures involving extraction with various concentrations of NaCl or dilute alkali were used to isolate nucleoprotein. Following removal of nucleoprotein, histone was separated from the complex by 0.2 *N* HCl, by chloroform (producing a chloroform-protein gel), or by saturating an aqueous solution of nucleoprotein with NaCl. One of the several methods originally used with calf thymus was found to be best suited for extraction of histone from plant cells[5]. The procedures were mild, rapid, and relatively straightforward.

Materials and methods. Histone extractions were made from seedling roots of *Allium cepa* var. Southport White Globe[6], *Pisum sativum* var. Alaska[6], and *Vicia faba* var. Seville Longpod. Root tips averaging 5 mm in length were excised, weighed, and placed in distilled water at 4°C. (It is essential that extraction steps be performed at temperatures under 5°C.) They were ground in 90 ml distilled water for 30 sec in a blendor at low speed, and disruption of the cells was completed in a glass homogenizer. The method of Crampton et al.[5] was followed in all extractions summarized in Figure 1. For the initial step homogenized roots of pea and onion were treated

[1] E. Rasch and J. W. Woodard, J. Biophys. Biochem. Cytol. *6*, 263 (1959).
[2] R. C. Huang and J. Bonner, Proc. Nat. Acad. Sci., Wash. *48*, 1216 (1962).
[3] V. G. Allfrey, V. C. Littau, and A. E. Mirsky, Proc. Nat. Acad. Sci., Wash. *49*, 414 (1963).
[4] L. Hnilica, E. W. Johns, and J. A. V. Butler, Biochem. J. *82*, 123 (1962).
[5] C. F. Crampton, S. Moore, and W. H. Stein, J. biol. Chem. *215*, 787 (1955).
[6] Courtesy Asgrow Seed Co., New Haven (Conn.).