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Original Article

How many blastomeres of the 4-cell embryo contribute cells to the mouse body?

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ABSTRACT The aim of this study was to estimate how many blastomeres of the 4-cell mouse embryo contribute cells to the embryo proper and finally to the animal. To this end, 4-cell embryos of pigmented and albino genotypes were disaggregated and single blastomeres (henceforth called '1/4' or 'quarter' blastomeres) were reaggregated in the following combinations: one 'pigmented' blastomere + three 'albino' blastomeres or *vice versa* (henceforth called '1+3') and two pigmented blastomeres + two albino blastomeres (henceforth called '2+2'). The aggregations were cultured *in vitro* and transferred as blastocysts either to the oviduct or uterus of pseudopregnant females. Recipients were allowed to litter naturally, or the foetuses were removed by Caesarian section and raised by lactating foster mothers. Chimaerism was assessed on the basis of coat (adults) or eye pigmentation (dead neonates). Among 28 '1+3' animals, there were 13 chimaeric and 15 non-chimaeric individuals. The pigmentation of non-chimaeras was always concordant with the genotype of the three 1/4 blastomeres and not with the genotype of the single blastomere in the given aggregation. These results make rather unlikely the possibility that the mouse is built of cells derived either from one or all four 1/4 blastomeres. Both two remaining options (2 or 3 1/4 blastomeres) are conceivable but the observed ratio of chimaeras to non-chimaeras among '1+3' animals (13:15) fits better the assumption of two 1/4 blastomeres contributing cells to the animal body. This assumption finds additional support in the observation that among '2+2' animals there were non-chimaeras (5 out of 7) and these would not have been expected should three 1/4 blastomeres contribute cells to the mouse body.

KEY WORDS: *mouse embryo, 'quarter' blastomeres, cell lineage, chimaeras*

Introduction

One blastomere of a 2-cell mouse embryo can develop into a normal, fertile animal (Tarkowski, 1959a,b). Single blastomeres of a 4-cell embryo can develop into blastocysts (Tarkowski and Wróblewska, 1967) and implant but die soon afterwards at the egg cylinder stage (Rossant, 1976). It is believed that the early postimplantation death of these embryos is due to their small size and insufficient number of embryonic, i.e. inner cell mass (ICM)-derived cells at this critical stage of development. The evidence that isolated single blastomeres of the 4-cell embryos have the capability to develop into normal and fertile mice (and therefore are totipotent), has been provided by experiments of Kelly (1975, 1977) and of Tarkowski *et al.* (2001), who aggregated these blastomeres with genetically different diploid (Kelly) or tetraploid (Tarkowski *and cols.*) carrier blastomeres. In other mammalian species, like the rabbit (Moore *et al.*, 1968) and the sheep (Willadsen, 1981), single blastomeres of a 4- and occasionally even of a 8-cell embryo can develop into adult fertile animals. Willadsen (*loc. cit.*) has shown in addition that all four pairs of sister 2/8 blastomeres can develop into quadruplet lambs and, therefore, that in that species all four 1/4 blastomeres are totipotent. All these observations directly prove the totipotency of all blastomeres (or at least of some of the blastomeres) at these early developmental stages.

However, these and other studies on the developmental potential of single blastomeres developing either in isolation or in chimaeric aggregations, have not provided information on how many blastomeres at these first three developmental stages (2-, 4- and 8-cell) contribute cells to the embryo proper. The retention of totipotency by all blastomeres of the embryo up to any developmental stage does not imply that in the intact embryo all of them

Abbreviations used in this paper: BSA, bovine serum albumin; hCG, human chorionic gonadotrophin; ICM, inner cell mass; PMSG, pregnant mare's serum gonadotrophin.

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contribute to both the embryonic and the extraembryonic tisssues of the foetus. It is possible, for instance, that at the 2-cell stage either only one or both blastomeres contribute to the embryo (and animal) and that at the 4-cell stage the embryo and the resulting animal is derived from 1, 2, 3 or all 4 blastomeres. As far as the 2-cell stage is concerned the only relevant observations are those made by Kono *et al.* (1989) and Nakagawa *et al.* (1993), who found that not all of the 2-cell mouse embryos in which the nucleus in one blastomere was replaced by a nucleus from another strain, developed into chimaeric animals; this implies that the non-chimaeric animals must have been derived from only one blastomere of the 2-cell embryo.

The aim of this study was to answer this question as regards the 4-cell stage. To this end one blastomere of the 4-cell embryo of one genotype (henceforth denoted '1/4' or 'quarter' blastomere) was aggregated with three 1/4 blastomeres of another genotype which differed by a pigmentation factor, and the produced animals were assessed for chimaerism on the basis of coat or eye pigmentation. The observed incidence of chimaeras among produced experimental animals suggests that usually only 2 out of 4 blastomeres of the 4-cell mouse embryo contribute cells to the embryo proper.

Results

Eighty three '1+3' blastocysts were transferred to the uterus of 13 recipients: 11 females became pregnant and gave birth to 29 neonates (34.9%) out of which 26 survived and reached adulthood and 3 were found dead soon after delivery; in 2 out of these 3 neonates their constitution (chimaeric *versus* nonchimaeric) was assessed on the basis of the pigmentation of eyes. Altogether the constitution (chimaeric *versus* nonchimaeric) could be assessed in 28 '1 + 3' individuals.

Twenty three '2+2' blastocysts were transferred to the oviduct of 4 recipients: 3 females became pregnant and gave birth to 6 young; in addition one overgrown but dead foetus was found in the uterus of a pregnant female which had not littered by the 22nd day post mating and was killed (overall survival rate until birth - 30.4%). The number of individuals in which the constitution could be assessed thus increased to seven.

Somatic chimaerism

The frequency of the pigmentation chimaerism is shown in Table 1. Among twenty eight '1+3' born individuals there were thirteen chimaeras and fifteen non-chimaeras. It is worth drawing attention to the fact that the pigmentation of non-chimaeras was always concordant with the genotype of the three blastomeres and not with the genotype of the single blastomere in the given aggregation. As concerns chimaeras there was no clear correlation, however, between the degree of domination of albino or pigmented component in the coat and the initial number of albino and pigmented blastomeres (1:3 or 3:1). As can be seen in Table 2, in which the chimaeric animals were arranged according to the decreasing contribution of the albino component in the coat, albino contribution sometimes dominated in animals developed from embryos composed of one albino blastomere and three pigmented ones, and highly pigmented chimaeras sometimes developed from embryos that were composed predominantly of albino blastomeres (3:1).

TABLE 1

PHENOTYPE OF '1+3' AND '2+2' ADULT ANIMALS (32) AND DEAD NEONATES (3)

Composition and genotype of blastomere aggregations	Chimaeric	Non-chimaeric
'1+3': 1/4 albino + 3/4 pigmented	6	5 pigmented
'1+3': 3/4 albino + 1/4 pigmented	7[1]	10 albino[2]
Total: '1+3'	13	15
'2+2': 2/4 albino + 2/4 pigmented	2	5 albino[3]

[1]Six adult animals and one dead newborn. [2]Nine adult animals and one dead newborn. [3]Four adult animals and one dead newborn.

Germ line chimaerism

All twenty six '1+3' and six '2+2" animals were crossed with albino animals. Thirteen experimental albino animals produced only albino progeny thus demonstrating that the pigmented partner was absent also from the germ line. The analysis of the origin of progeny of the thoroughly pigmented experimental animals (i.e. those not displaying coat chimaerism) was slightly more complicated because the pigmented component was a hybrid developed from an albino egg and a pigmented hybrid (black x agouti) sperm. In animals in which the albino component was missing altogether (i.e. also absent in the germ line), 50% of the progeny should nevertheless be albino and 50% pigmented. Statistically significant predominance of albino progeny might suggest the contribution of the albino component (missing in the coat) to the germ line. Among five fully pigmented animals, four showed a ratio between albino and pigmented progeny of nearly exactly 1:1, and in one the pigmented progeny actually predominated (103 pigmented young among 173 born). There is no evidence, therefore, that the fully pigmented experimental animals were germ line chimaeras.

The phenotypic sex and the results of crossing overt chimaeras with albino animals are shown in Table 2. Among fourteen chimaeras (twelve '1+3' and two '2+2') there were four females and ten males. All females were fertile and produced both albino and pigmented progeny. However, in all four females albino progeny strikingly predominated over pigmented neonates (62:8, 108:2, 30:1, 22:1); this remained true even taking into account that among albino progeny of chimaeras some animals could have been derived from the hybrid pigmented component (see above). There was no correlation between coat chimaerism and germ line chimaerism: only in one female the albino component in the coat highly dominated, and this female had the highest proportion of pigmented progeny. In two others the albino component dominated but only slightly, and in the remaining chimaera it was the pigmented component that dominated. One might speculate that some of these females were sex chimaeras and that pigmented progeny developed from pigmented XY oocytes (Ford *et al.*, 1975; Evans *et al.*, 1977; also cf. Lovell-Badge and Robertson, 1990; Bronson *et al.*, 1995), but since we have no information as regards the genetic sex of both components such possibility is purely speculative.

Among ten chimaeric males one male was infertile (as revealed at autopsy at the age of 14 months it had an abnormal reproductive tract), six males had two-coloured progeny and three males had only albino progeny. Taking into account that the pigmented component alone should produce albino and pigmented progeny

in a 1:1 ratio, and that the greatest deviation from the theoretical ratio in favour of albino progeny (animal no. 12 in Table 2: 57% albino, 43% pigmented) was not statistically significant ($\chi^2 = 3.77$, $P > 0.05$) there is no evidence that these males were germ line chimaeras (although such a possibility cannot be entirely excluded). The three other males produced only albino progeny; since the number of offspring was quite large (123, 177, 182) it is very likely that they were not germ line chimaeras. Perhaps they were sex chromosome chimaeras with a genetically female pigmented component, which does not produce spermatozoa (Mystkowska and Tarkowski, 1968).

Discussion

As mentioned in the Introduction it is by no means clear whether at the first cleavage stages of the mouse embryo, all blastomeres, or only some of them, are founder cells of the future animal. The cell progeny of a given blastomere may happen to be located only in the embryo proper (and finally in the animal body) or only in the foetal membranes or in both. In this study we tried to answer the question of how many blastomeres of the 4-cell embryo contribute cells to the mouse body, and we have come to the conclusion that this number is usually two (sometimes, perhaps, three) rather than one or four. By stating this we do not imply that **all** descendant cells of these particular blastomeres are located in the mouse body and that none of the descendants had contributed also to the foetal membranes.

Our hypothesis is not based on direct evidence, such as could have been provided by examination of the constitution of a large group of mice constructed from four quarter blastomeres, each genetically different. Instead, it is indirectly inferred from the frequencies of experimental chimaeric and non-chimaeric animals, developed from embryos constructed from only two, genetically different, components. For this reason the hypothesis expresses only the probabilities of various developmental patterns: one, two, three or four 1/4 blastomeres as founder cells of a mouse, and does not exclude the possibility that any of them can ever occur. We believe, however, that the evidence presented in this study permits us to postulate that the likelihood of the pattern of two founder blastomeres is highest and that the likelihood of the patterns of one or four founder blastomeres is very small.

In aggregation experiments aimed at defining the number of founder blastomeres that make the body of a mouse embryo (animal), the descendant cells of the two types of aggregated blastomeres should have equal, or similar, chance to participate in the formation of the inner cell mass (ICM). Unequal chances may result from genetically determined different rates of cleavage or even slightly different developmental age of the two types of cells. There are observations that in intact embryos those blastomeres that cleave faster than the others tend to populate the ICM rather than trophectoderm (Graham and Deussen, 1978; Kelly *et al.*, 1978; Graham and Lehtonen, 1979; Surani and Barton, 1984; Garbutt *et al.*, 1987). Also in asynchronous chimaeric aggregations of embryos or of blastomeres, the more advanced partners contribute more cells to the ICMs and later to the embryos (animals) than the less advanced partners (Spindle, 1982; Willadsen and Fehilly, 1983; Fehilly and Willadsen, 1986). However, evidence to the contrary has also been presented (Prather and First, 1987). Although suggestive, the conclusions drawn from the latter studies may not apply to normal development because the volume of chimaeric aggregations was usually smaller or larger than the volume of normal embryos and the embryos were composed of blastomeres drawn from different cell cycle stages (eg. 8-cell *versus* 4-cell).

In view of the above studies and taking into account that inbred strains of the mouse often differ in the rate of preimplantation development [or in the timing of fertilization after mating (Krzanowska, 1964; McLaren and Bowman, 1973)] we decided to use embryos derived from genetically similar eggs (our colony of MIZ albino mice) and fertilized by genetically different sperm - either 'albino' (MIZ) or 'pigmented' [F1(C57BL/10xCBA/H)]. Although control intact MIZ (egg) x F1 (sperm) hybrid preimplantation embryos develop *in vivo* slighty faster than MIZ x MIZ embryos (data not shown) their potential advantage had no evident effect on the constitution of the aggregation chimaeras. We have not observed the dominance of this genotype in the phenotype of chimaeras and among non-chimaeras (Tables 1 and 2). In addition, in order to exclude the possible preference of the blastomeres of any of the two employed genotypes to populate the ICM we carried out the experiment in two variants: in one variant the ratio of albino blastomeres to pigmented blastomeres was 1:3, and in the other just opposite - 3:1. For the above reasons and taking into account that in this experiment - and in contrast to the majority of studies on aggregation chimaeras - the size of the embryos was kept normal, we believe that our system provided to a large extent equal developmental chances for both kinds of blastomeres and that the conclusions regarding the number of blastomeres of the 4-cell embryo that contribute cells to the mouse body are reliable.

Theoretically, in the 4-cell embryo either 1, 2, 3 or all 4 blastomeres could contribute to the ICM and the animal body. The results of the '1+3' experiment make rather unlikely the two extreme options: 1 and 4 blastomeres. If the first option were true all animals would be non-chimaeric, if the second one were correct all animals would be chimaeric. We are left therefore with the choice between 2 and 3 blastomeres as predecessors of the

TABLE 2

CHIMAERISM OF THE COAT (ALBINO *VERSUS* PIGMENTED) AND OF THE GERM LINE OF '1+3' AND '2+2' ADULT ANIMALS

Animal no.	Type of aggregate	Sex	Degree of coat chimaerism	Total no. of progeny	No. and % of albino progeny	No. and % of pigmented progeny
1	3A + 1P	female	A>>>P	70	62 (89)*	8 (11)
2	1A + 3P	male	A>>P	177	177 (100)	0
3	1A + 3P	male	A>P	144	59 (41)	85 (59)
4	2A + 2P	female	A>P	110	108 (98)*	2 (2)
5	2A + 2P	male	A>P	182	182 (100)	0
6	1A + 3P	female	A>P	31	30 (97)*	1 (3)
7	1A + 3P	male	A>P	123	123 (100)	0
8	1A + 3P	male	A=P	180	82 (46)	98 (54)
9	3A + 1P	male	A<P	infertile		
10	3A + 1P	female	A<<P	23	22 (96)*	1 (4)
11	1A + 3P	male	A<<P	177	93 (53)	84 (47)
12	3A + 1P	male	A<<P	208	118 (57)	90 (43)
13	3A + 1P	male	A<<<P	200	102 (51)	98 (49)
14	3A + 1P	male	A<<<P	88	43 (49)	45 (51)

Explanations: >>> very high predominance; >> high predominance; > slight predominance; = equal participation; A, albino; P, pigmented. *Among albino progeny some individuals (at the frequency similar to that of pigmented progeny) must have been derived from the pigmented component of chimaeras (see text for explanation).

mouse body. The incidence of chimaeras among animals developed from '1+3' aggregates was close to 50% (13:15) and better fits the assumption that the embryonic body (and eventually the animal body) is derived from cells originating from two rather than from three blastomeres of the 4-cell embryo. On statistical grounds the former option (2 blastomeres, theoretical frequency of chimaeras - 50%; $\chi^2 = 0.14$, $0.5<P<0.75$) is definitely more plausible than the latter (3 blastomeres, theoretical frequency of chimaeras - 75%; $\chi^2 = 12.19$, $P<0.005$). This conclusion is corroborated also by the fact that among '2+2' experimental animals there were non-chimaeras: if the embryo proper develops from three 1/4 blastomeres then all animals produced according to this experimental variant would have to be chimaeras.

Our conclusions find some support also in the observations on the incidence of chimaerism in animals produced by interchanging the nuclei in one blastomere of the 2-cell embryos belonging to two strains (Kono *et al*., 1989; Nakagawa *et al*., 1993). In both these studies some animals turned out to be non-chimaeras and therefore must have developed just from one 'half' blastomere. The incidence of chimaeras varied from 71.4% (Kono *et al., loc. cit.*) to only 15.8% (Nakagawa *et al., loc. cit.*). The data of the first study fit our presumption of two 'quarter' blastomeres being the founders of the mouse body: in the case of '2+2' type of aggregation theoretically 66.6% of animals should be chimaeric. The very low incidence of chimaeras in the second study was probably due to the extreme developmental advantage of the C3Hf genotype (nucleus) over BALB/c (for details see Nakagawa *et al., loc. cit.*).

The validity of estimating the number of founder 'quarter' blastomeres on the basis of the relative frequencies of chimaeras and non-chimaeras among experimental animals depends also on the reliability of methods used for their discrimination. In the context of the present study the main question is whether the lack of pigmentation chimaerism (pigmentation was the only marker used) suffices to classify the animal as a non-chimaera. In other words, how often the unicoloured animals were in fact chimaeras because the other component contributed only to the non-pigmented tissues and its presence has thus escaped notice. However, as demonstrated in previous studies, there is a high correlation between the pigmentation of the coat and eye (retinal pigment epithelium) and the composition of other tissues (bone marrow, cornea and blood) of animals developed from chimaeric blastocysts. According to Mystkowska and Tarkowski (1968) and Mystkowska *et al.* (1979) the bone marrow chimaerism of 26 animals was reflected in the variegation of the coat of 24 individuals (92%). In 14 overt chimaeras studied by Kelly (1977) 12 were blood chimaeras (86%) and all 25 unicoloured adult animals showed only 1-A form of GPI typical for the albino component. Among one hundred 12.5 day chimaeric foetuses derived from components differing in pigmentation and 1-A and 1-B forms of GPI, West and colleagues (West and Flockhart, 1994; West *et al*., 1995) encountered only five cases in which chimaerism of the trunk tissues was not reflected in the retinal pigment epithelium (concordance: 95%). In the light of the above studies we believe that our unicoloured animals were rightly classified as non-chimaeras. If, nevertheless, there were among them single 'hidden' chimaeras then the probability of three rather than two 1/4 blastomeres being the founder cells of the experimental mice would have been only slightly increased.

Finally, it seems important to refer our conclusions to what is known on the origin of the ICM in normal development of a mouse. Up to the 8-cell stage the blastomeres do not differ among themselves and the formation of inner and outer cells takes place mainly as a result of the differentiative division [i.e. producing one polar (outer) and one apolar (inner) cell] of some blastomeres during the fourth cleavage (Johnson and Ziomek, 1981; Balakier and Pedersen, 1982; Pedersen *et al*., 1986; Fleming, 1987). Few inner cells may be added occasionally later as a result of this type of division of outer cells (for review see Gueth-Hallonet and Maro, 1992). At the 16-cell stage, depending on the technique used for discriminating inner and outer cells and on the strain investigated, the number of inner cells was estimated as between 4.6 and 6.6 (Handyside, 1981), 5.2 (Fleming, 1987) and 5.7 inner cells (Boerjan and te Kronnie, 1993). Clearly, at the 16-cell stage the number of inner cells exceeds four, i.e. it is higher than one would expect had only two pairs of sister 1/8 blastomeres contributed cells to the ICM due to the differentiative division. This fact together with the possibility of few other inner cells being added at the fifth cleavage division fits better the assumption of more than two 'quarter' blastomeres contributing cells to the ICM. These observations do not necessarily disprove the option of two rather than three 1/4 blastomeres being predecessors of a mouse (as suggested by our experimental data) because the foetus is derived from the epiblast region of the ICM rather than the whole ICM and so the number of founder blastomeres may be secondarily reduced in the early postimplantation period. Nevertheless, taking into account that the number of '1+3" animals that we have produced is relatively small (close to 30), the option of three blastomeres cannot be definitely rejected. However, both the descriptive studies on the formation of the ICM and the results of the present experiment do not support the hypothesis of all four 'quarter' blastomeres contributing cells to the mouse body, as proposed by Kelly *et al.* (1978).

Our conclusions are not contradicted by the study of Markert and Petters (1978) who found that when aggregation mouse chimaeras are generated from three genetically different 8-cell embryos, some of them may be composed of cells derived from all three partners. It is not surprising that in such a large conglomeration of blastomeres built of three 8-cell embryos several cells from all three partners will contribute to the ICM and form the animal's body. However, because of the artificial circumstances - three times larger volume of the embryo at the time of formation of inner cells - these conclusions need not apply to normal development. From our observations it would follow that it would be difficult to construct a chimaeric mouse of triple or quadruple composition by aggregating genetically different 'quarter' blastomeres. However, such mice could, perhaps, be constructed by aggregating genetically different 1/8 blastomeres.

Materials and Methods

Animals and embryos

In order to obtain 'albino' embryos outbred MIZ albino females were caged with MIZ males. 'Pigmented' embryos were produced by MIZ females which were mated with F1(C57BL/10xCBA/H) agouti males.

4-cell embryos originated from females either induced to ovulate or ovulating spontaneously. In the first case the females were injected with pregnant mare's serum (PMSG) and hCG (human chorionic gonadotrophin) (Intervet; 10 i.u. of each given 38-49 h apart) and caged with males after the second injection. Embryos were recovered from the oviducts 54-56 h post hCG. In the second case cycling females were caged with males between 8.00 and 9.00 a.m. and checked for plugs within a 2 h period. The embryos were recovered 45-51 h later.

Manipulation of embryos

Embryos were flushed from the oviducts in M2 medium (Fulton and Whittingham, 1978). After the zona pellucida was removed in acid Tyrode solution (Nicolson *et al*., 1975), the embryos were placed in Ca^{2+}- and Mg^{2+}-free M2 medium for up to 20 minutes at 37ºC, and finally disaggregated by gentle pipetting. Three 1/4 'albino' blastomeres originating from one embryo and one 1/4 'pigmented' blastomere (or *vice versa*) were placed in phytohemagglutinin (Sigma, 0.3 mg/ml solution in BSA-free M2 medium) in an agar-coated Petri dish for 1-2 min where they were aggregated. The aggregates, henceforth called '1+3', were thoroughly washed and cultured in separate drops of KSOM or modified KSOM medium (respectively Erbach *et al*., 1994; Summers *et al*., 1995) under liquid paraffin at 37ºC in an atmosphere of 5% of CO_2 in air. In addition, in a small series of experiments, aggregates were also made between two 'albino' and two 'pigmented' 1/4 blastomeres (designated '2+2'). Aggregated blastomeres were observed once or twice daily to check whether all cells were incorporated into the chimaeric embryo and only such blastocysts were transferred to recipients.

Transfer of embryos

The embryos were cultured *in vitro* for 45-78 h and transferred as blastocysts either into the oviduct or into the uterus. The recipient females were F1(C57BL/10 x CBA/H) mated with vasectomized F1 males. Transfers to the oviduct were carried out on the first day of pseudopregnancy (1st day = day of plug) and transfers to the uterus on the 4th day of pseudopregnancy. Females were anaesthesized with 0.35 ml of aeqous solution of Nembutal (6 mg/ml; Serva) injected intraperitoneally.

Birth of young and assessment of chimaerism

Starting on the 7th day after mating vaginal smears were taken daily to verify that the recipient females were pregnant (abundant mucus and vaginal bleeding in the second half of pregnacy) and the females were permitted to litter naturally. Births took place after 19 or 20 days of pregnacy (young were usually found in the morning on the 20th or 21st day). Two females which were presumed to be pregnant and which did not litter by the morning of the 21st day after mating were killed and one living and one dead foetus were recovered. The living one was fostered by a female which gave birth two days earlier. The dead foetus and two dead neonates born by another female were assessed for chimaerism by inspecting the eyes (outer layer of retina). The chimaerism of animals which survived beyond birth was determined on the basis of coat pigmentation; the evaluation of the share of the two components in the coat was done by examination of flat skins and subjective evaluation of the participation of albino and pigmented hair (very high, high and slight predomination or equal participation of both types of hair).

All animals, whether overtly chimaeric or non-chimaeric, after attaining sexual maturity were crossed with albino animals of the opposite sex and the number and pigmentation of young were recorded to assess chimaerism of the germ line. These data were compared with somatic (coat) chimaerism.

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References

BALAKIER, H. and PEDERSEN, R.A. (1982). Allocation of cells to inner cell mass and trophectoderm lineages in preimplantation mouse embryos. *Dev. Biol.* 90: 352-362.

BOERJAN, M. and TE KRONNIE, G. (1993). The segregation of inner and outer cells in porcine embryos follows a different pattern compared to the segregation in mouse embryos. *Roux's Arch. Dev. Biol.* 203: 113-116.

BRONSON, S.K., SMITHIES, O. and MASCARELLO, J.T. (1995). High incidence of XXY and XYY males among the offspring of female chimeras from embryonic stem cells. *Proc. Natl. Acad. Sci.USA* 92: 3120-3123.

ERBACH, G.T., LAWITTS, J.A., PAPAIOANNOU, V.E. and BIGGERS, J.D. (1994). Differential growth of the mouse preimplantation embryo in chemically defined media. *Biol. Reprod.* 50: 1027-1033.

EVANS, E.P., FORD, C.E. and LYON, M.F. (1977). Direct evidence of the capacity of the XY germ cell in the mouse to become an oocyte. *Nature* 267: 430-431.

FEHILLY, C.B. and WILLADSEN, S.M. (1986). Embryo manipulation in farm animals. In *Oxford Reviews of Reproductive Biology* (Ed. J.R. Clarke) Vol. 8. Clarendon Press, Oxford, pp. 379-413.

FLEMING, T.P. (1987). A quantitative analysis of cell allocation to trophectoderm and inner cell mass in the mouse blastocyst. *Dev. Biol.* 119: 520-531.

FORD, C.E., EVANS, E.P., BURTENSHAW, M.D., CLEGG, H.M., TUFFREY, M. and BARNES, R.D. (1975). A functional "sex-reversed" oocyte in the mouse. *Proc. Roy. Soc. Lond. B,* 190: 187-197.

FULTON, B.P. and WHITTINGHAM, D.G. (1978). Activation of mammalian oocytes by intracellular injection of calcium. *Nature* 273: 149-151.

GARBUTT, C.L., JOHNSON, M.H. and GEORGE, M.A. (1987). When and how does cell division order influence cell allocation to the inner cell mass of the mouse blastocyst?. *Development* 100: 325-332.

GRAHAM, C.F. and DEUSSEN, Z.A. (1978). Features of cell lineage in preimplantation mouse development. *J. Embryol. Exp. Morph.* 48: 53-72.

GRAHAM, C.F. and LEHTONEN, E. (1979). Formation and consequences of cell patterns in preimplantation mouse development. *J. Embryol. Exp. Morph.* 49: 277-294.

GUETH-HALLONET, C. and MARO, B. (1992). Cell polarity and cell diversification during early mouse development. *Trends Genet.* 8: 274-279.

HANDYSIDE, A.H. (1981). Immunofluorescence techniques for determining the numbers of inner and outer blastomeres in mouse morulae. *J. Reprod. Immunol.* 2: 339-350.

JOHNSON, M.H. and ZIOMEK, C.A. (1981). The foundation of two distinct cell lineages within the mouse morula. *Cell* 24: 71-80.

KELLY, S.J. (1975). Studies of the potency of the early cleavage blastomeres of the mouse. In *The early development of mammals* (Eds. M. Balls and A.E. Wild). Cambridge University Press, Cambridge, London, New York, Melbourne, pp. 97-105.

KELLY, S.J. (1977). Studies of the developmental potential of 4- and 8-cell stage mouse blastomeres. *J. Exp. Zool.* 200: 365-376.

KELLY, S.J., MULNARD, J.G. and GRAHAM, C.F. (1978). Cell division and cell allocation in early mouse development. *J. Embryol. Exp. Morph.* 48: 37-51.

KONO, T., TSUNODA, Y., WATANABE, T. and NAKAHARA, T. (1989). Development of chimaeric two-cell mouse embryos produced by allogenic exchange of single nucleus from two- and eight-cell embryos. *Gamete Res.* 24: 375-384.

KRZANOWSKA, H. (1964). Time interval between copulation and fertilization in inbred lines of mice and their crosses. *Folia Biol. Krakow* 12: 231-244.

LOVEL-BADGE, R. and ROBERTSON, E. (1990). XY female mice resulting from a heritable mutation in the primary testis-determining gene, *Tdy. Development* 109: 635-646.

MARKERT, C.L. and PETTERS, R.M. (1978). Manufactured hexaparental mice show that adults are derived from three embryonic cells. *Science* 202: 56-58.

McLAREN, A. and BOWMAN, P. (1973). Genetic effects on the timing of early development in the mouse. *J. Embryol. Exp.Morph.* 30: 491-498.

MOORE, N.W., ADAMS, C.E. and ROWSON, L.E.A. (1968). Developmental potential of single blastomeres of the rabbit egg. *J. Reprod. Fert.* 17: 527-531.

MYSTKOWSKA, E.T., OZDZENSKI, W. and NIEMIERKO, A. (1979). Factors regulating the degree and extent of experimental chimaerism in the mouse. *J. Embryol. Exp. Morph.* 51: 217-225.

MYSTKOWSKA, E.T. and TARKOWSKI, A.K. (1968). Observations on CBA-p/CBA-T6T6 mouse chimeras. *J. Embryol. Exp. Morph.* 20: 33-52.

NAKAGAWA, S., SUMITA, K., HAMA, T. and MAYUMI, T. (1993). Production of chimeric mice by exchanging nuclei from blastomeres of the two-cell embryo. *Zool. Sci.* 10: 645- 651.

NICOLSON, G.L., YANAGIMACHI, R. and YANAGIMACHI, H. (1975). Ultrastructural localization of lectin-binding sites on the zonae pellucidae and plasma membranes of mammalian eggs. *J. Cell Biol.* 66: 263-274.

PEDERSEN, R.A., WU, K. and BALAKIER, H. (1986). Origin of the inner cell mass in mouse embryos: cell lineage analysis by microinjection. *Dev. Biol.* 117: 581-595.

PRATHER, R.S. and FIRST, N.L. (1987). Developmental fate in chimeras derived from highly asynchronous murine blastomeres. *J. Exp. Zool.* 242: 27-33.

ROSSANT, J. (1976). Postimplantation development of blastomeres isolated from 4- and 8- cell mouse eggs. *J. Embryol. Exp. Morph.* 36: 283-290.

SPINDLE, A. (1982). Cell allocation in preimplantation mouse chimeras. *J. Exp. Zool.* 219: 361-367.

SUMMERS, M.C., BHATNAGAR, P.R., LAWITTS, J.A. and BIGGERS, J.D. (1995). Fertilization in vitro of mouse ova from inbred and outbred strains: complete preimplantation embryo development in glucose-supplemented KSOM. *Biol. Reprod.* 53: 431-437.

SURANI, M.A.H. and BARTON, S.C. (1984). Spatial distribution of blastomeres is dependent on cell division order and interactions in mouse morulae. *Dev. Biol.* 102: 335-343.

TARKOWSKI, A.K. (1959a). Experiments on the development of isolated blastomeres of mouse eggs. *Nature* 184: 1286-1287.

TARKOWSKI, A.K. (1959b). Experimental studies on regulation in the development of isolated blastomeres of mouse eggs. *Acta Theriologica* 3: *191-267.*

TARKOWSKI, A.K., OZDZENSKI, W. and CZOLOWSKA, R. (2001). Mouse singletons and twins developed from isolated diploid blastomeres supported with tetraploid blastomeres. *Int. J. Dev. Biol.* 45: *591-596.*

TARKOWSKI, A.K. and WRÓBLEWSKA, J. (1967). Development of blastomeres of mouse eggs isolated at the 4- and 8-cell stage. *J. Embryol. Exp. Morph.* 18: 155-180.

WEST, J.D. and FLOCKHART, J.H. (1994). Genotypically unbalanced diploid↔diploid foetal mouse chimaeras: possible relevance to human confined mosaicism. *Genet. Res.* 63: 87-99.

WEST, J.D., FLOCKHART, J.H. and KISSENPFENNIG, A. (1995). A maternal genetic effect on the composition of mouse aggregation chimaeras. *Genet. Res.* 65: 29-40.

WILLADSEN, S.M. (1981). The developmental capacity of blastomeres from 4- and 8-cell sheep embryos. *J. Embryol. Exp. Morph.* 65: 165-172.

WILLADSEN, S.M. and FEHILLY, C.B. (1983). The developmental potential and regulatory capacity of blastomeres from two-, four- and eight-cell sheep embryos. In *Fertilization of Human Egg In Vitro.* (Eds. H.M. Beier and H.R. Lindner). Springer- Verlag, Berlin - Heidelberg, pp. 353-357.

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