Embryonic development and incidence of aneuploidy in two rabbit strains of different fecundity

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Summary. The correlation between strain fecundity and (i) development and (ii) rate of aneuploidy was studied in rabbit preimplantation embryos obtained from 2 strains of different fecundity. Embryos were investigated at Days 3–6 (preimplantation development) or Days 2, 4 and 6 post coitum (aneuploidy). Embryonic size and cell proliferation varied on the days of investigation, but with no consistent tendency in favour of one strain. The incidence of aneuploidy did not differ significantly between embryos from the 2 strains ($P > 0.05$). The multifactorially determined criterion of prolificacy was not selectively correlated with overall differences in embryonic preimplantation growth and rate of aneuploidy.

Keywords: preimplantation development; aneuploidy; fecundity; strain differences; rabbit

Introduction

The speed of preimplantation embryo development is genetically influenced. The most well-known example is a gene, *Ped*, located within the major histocompatibility complex (MHC) in mice (Goldbard & Warner, 1982; see Warner, 1986; Warner *et al.*, 1987a, b, 1988). In pigs an MHC-linked gene is associated with number of cells (Ford *et al.*, 1988) and survival (Conley *et al.*, 1988) of preimplantation embryos. Speed of development is supposed to be an essential expression of embryo viability. It has been shown, in a range of species, that rapidly dividing and advanced embryos have the best potential for further development and/or survival to term (human: Mohr *et al.*, 1983; Claman *et al.*, 1987; rhesus monkey: Bavister *et al.*, 1983; cattle: Renard *et al.*, 1977; Renard & Heyman, 1979; pig: Pope *et al.*, 1982, 1986; Wilmut *et al.*, 1985, 1986; Bazer *et al.*, 1988; rabbit: Hafez, 1962; Torres *et al.*, 1987a, b; mouse: Tsunoda *et al.*, 1985; Baunack *et al.*, 1986). Thus, differences in the rate of preimplantation development may contribute to the higher prolificacy of breeds or strains.

Findings in rabbits (Torres *et al.*, 1987a, b) indicate that embryo development during the first days of preimplantation development may be most directly linked to strain fecundity. Up to Day 4 post coitum (p.c.) a clear difference in ovulation rate between Californian (11.6 ± 0.2) and New Zealand (9.7 ± 0.2) rabbit strains became equalized to 8.9 and 8.2 embryos per female, respectively, the approximate litter size at birth (Torres *et al.*, 1987a). Diameters were statistically significantly larger in blastocysts from New Zealand does, the strain with the lower percentage of embryonic loss. Similar differences in ovulation rate and embryonic survival are known between Large White and Meishan pig breeds (Bazer *et al.*, 1988). The latter has fewer ovulations but larger and more uniformly sized conceptuses and less embryonic wastage, resulting in more piglets/litter in Meishan gilts than in Large White. In pigs, however, the significant differences in conceptus size between the breeds appeared in the second half of preimplantation development, closer to the time of implantation. The reports in rabbits and pigs were based on a limited number of observations. In

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the present study, employing more females and embryos, we tried to determine whether embryo development during later stages of the preimplantation period may also be related to higher fecundity in rabbits. We studied 2 strains which, after treatment of the females with follicle-stimulating hormone (FSH) differed in the mean number of preimplantation embryos by 10.

The incidence of aneuploidy was investigated in embryos from these strains. Aneuploidy is an important genetic disorder in mammalian embryos (see de Boer *et al.*, 1986 and Bolet, 1986) and may thus contribute considerably to embryonic loss and fecundity. Unlike previous studies (see Delbos-Winter *et al.*, 1987), the incidence of aneuploidy was studied in rabbit embryos of a fairly well-defined genetic background obtained on consecutive days of the preimplantation period. The percentages of aneuploid embryos and aneuploid cells per embryo were analysed.

Materials and Methods

Donors and embryos. Reproductive data were for 208 females. Embryos from 98 donor does were allocated for analysis of cell proliferation by incorporation of tritiated thymidine (in embryos 3–6 days old), diameter (in blastocysts 4–6 days old) and aneuploidy (in embryos 2, 4 or 6 days old). The high number of females was chosen to minimize donor effects. The does were killed at the various days indicated to obtain embryos. Not all embryos recovered were used in the present study: only those of normal morphology, randomly selected among litter mates and coded for blind reading, were analysed. Details regarding housing of the rabbits, superovulation treatment of the donors with FSH and embryo recovery are described by Fischer (1987) and Fischer & Meuser-Odenkirchen (1988); and regarding evaluation of morphology, measurement of diameter and thymidine incorporation by Fischer (1989).

Animals. The rabbits used for this study were commercial hybrids of high prolificacy (ZIKA-hybrids; basic seedstock: Dr Zimmermann, D-7083 Untergröningen, FRG) and outbreds from a fancy breed (Alaska) purchased from a local breeder. The hybrids were supplied by an intermediate seedstock producer. Typically, the females are sold to commercial farms as a maternal line (selected for maternal characters) for crossbreeding with a specific paternal line to produce offspring for meat production. The males used for the present study were also derived from the seedstock producer's maternal line. The male and female outbreds came from a stock from does bred within the colony and bucks from outside the colony. In non-FSH-primed females, there were 10.3 ovulations in the hybrids ($n = 48$ females) and 7.9 in the outbreds ($n = 8$; $P < 0.05$). As stated by the suppliers, the average number of live young at birth is 8.4 for the hybrids and ≤ 7 for the outbreds. The rabbits were bought at least 14 days prior to experiments and kept under the same conditions. At autopsy, corpora lutea were counted in isolated ovaries. Ovulations > 20 are macroscopically not accurately countable and were computed as 20 ovulations.

Analysis of DNA content. The method used for analysing the DNA content in embryonic cell nuclei is that described by A. Schumacher, T. Agorastos, B. Fischer and H. M. Beier (unpublished). Briefly, after dissolution of the zona pellucida with 0.5% pronase, the embryos were exposed to a hypotonic shock, spread individually on microscope slides, fixed, air dried for 24 h and Feulgen-stained according to Bolton *et al.* (1984). The amount of DNA was measured cytophotometrically against simultaneously stained vaginal smears as calibration standard. Cytometry was performed on a television-based image-analysis system combined with an automatic microscope (Miamed, Leitz, FRG; Aufermann *et al.*, 1984). Nuclei were considered aneuploid with $\geq 5c$ DNA. In Day 2 embryos, the nuclei of all blastomeres were measured and, in Day 4 and Day 6 blastocysts, nuclei of 100 cells/blastocyst were measured.

Statistical analysis. Diameters, thymidine incorporation and number of aneuploid cells/embryo were analysed by Student's *t* test; comparisons of strain prolificacy and incidence of aneuploid embryos by χ^2 test with Yates' correction (Sachs, 1984).

Results

Reproductive performance up to Day 6 p.c., i.e. immediately before implantation, was clearly different between females from the 2 strains (Table 1). The hybrid does yielded 10 preimplantation embryos more than the outbreds, mainly caused by a higher number of ovulations and better embryonic survival.

Size and thymidine incorporation in embryos from both strains, either from pure breeds or from reciprocal crossbreds, are summarized in Tables 2 and 3. Although variation occurred at the

Table 1. Reproductive performance of ZIKA hybrid and Alaska outbred rabbits after preovulatory treatment with follicle-stimulating hormone*

	Hybrid	Outbred
Females without ovulation (%)	2 ^a	10 ^b
<i>N</i>	116	92
No. of corpora lutea/female	32.7 ± 0.8 ^c	28.5 ± 1.1 ^d
<i>N</i>	114	83
Females without embryo recovery (%)	3 ^e	6 ^e
No. of embryos/female		
Total	28.0 ± 1.6 ^c	20.1 ± 1.7 ^d
<i>N</i>	111	78
Degenerated	3.5 ± 0.5 ^e	4.3 ± 1.0 ^e
Embryo survival (%)	75	55
No. of nondegenerated embryos/female†	23.3	13.3
<i>N</i>	116	92

*Cumulative data from Days 3 to 6 p.c.

†Mean number of embryos for all females in study.

Values are given as means ± s.e.m.; *N* = number of females. a vs. b $P < 0.05$; c vs. d $P < 0.01$; e $P > 0.05$.

different days of observation, there was no consistent tendency in favour of one strain or strain combination during the second half of preimplantation.

The incidence of aneuploid embryos was not statistically significantly different between the two strains, but it increased remarkably from Day 2 to Day 6 p.c. (Table 4). The mean number of aneuploid cells/embryo was generally low: 2.7 ± 0.9 ($\bar{x} \pm \text{s.e.m.}$) in hybrid and 4.1 ± 1.2 in outbred embryos ($P > 0.05$).

Discussion

Embryo viability is one determinant of prolificacy. In the present study, using rabbit strains differing in reproductive performance (see Table 1) and high numbers of females and embryos, an interrelation between growth of preimplantation embryos (Tables 2, 3), incidence of aneuploidy (Table 4) and strain prolificacy was not demonstrated.

In the experiments reported by Torres *et al.* (1987a, b), the strain with the most ovulations had the highest percentage of embryonic loss. The better prolificacy of the hybrid strain in the present investigation was based on more ovulations as well as on superior embryonic survival (Table 1). These results indicate that, in rabbits (i) higher ovulation rates are not always associated with a higher incidence of preimplantation embryonic loss and (ii) both criteria can obviously be improved, even simultaneously, by genetic selection.

Torres *et al.* (1987b) concluded that the major embryonic loss occurs early in pregnancy, as failure of fertilization and/or by retardation in cleavage. However, in a previous study, embryo mortality in FSH-primed rabbits was notably higher in blastocysts than in cleavage stages (Fischer & Meuser-Odenkirchen, 1988). In the present study, this finding was confirmed for early blastocysts at Day 4 p.c. but not for later stages, probably because of the limited number of females involved (data not given). In addition, a significant percentage of prenatal mortality occurs

Table 2. Diameters (mm) of blastocysts from crosses of ZIKA hybrid (H) and Alaska outbred (L) rabbits

Embryonic age (days p.c.)	H♂ × H♀		L♂ × H♀		H♂ × L♀		L♂ × L♀	
	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.
4	0.34 ^a	0.01	0.28 ^b	0.001	0.32	0.02	0.34 ^a	0.01
<i>n</i>	107		94		32		106	
<i>N</i>	5		12		4		6	
5	0.89 ^c	0.02	1.05 ^d	0.02	1.07 ^d	0.04	1.09 ^c	0.06
<i>n</i>	78		240		60		28	
<i>N</i>	3		9		2		2	
6	2.70	0.08	2.80	0.11	2.81	0.06	2.69	0.07
<i>n</i>	87		49		47		123	
<i>N</i>	4		2		3		5	

H = highly prolific, L = low prolificacy.

n = number of blastocysts, *N* = number of females.

a vs. b, c vs. d $P < 0.01$; c vs. e $P < 0.001$; all other comparisons $P > 0.05$.

Table 3. Thymidine incorporation (d.p.m./embryo) in embryos from crosses of ZIKA hybrid (H) and Alaska outbred (L) rabbits

Embryonic age (days p.c.)	H♂ × H♀		L♂ × H♀		H♂ × L♀		L♂ × L♀	
	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.
3	415 ^a	21	465 ^a	24	403 ^a	43	745 ^b	53
<i>n</i>	68		91		45		64	
<i>N</i>	3		8		4		7	
4	4301 ^c	215	3480 ^d	189	3767	380	4320 ^c	181
<i>n</i>	86		94		30		64	
<i>N</i>	3		12		4		3	
5	20 988 ^e	1383	33 182 ^f	1235	43 233 ^g	3006	33 340 ^h	3411
<i>n</i>	82		237		57		28	
<i>N</i>	3		7		3		2	
6	157 154 ⁱ	10 383	182 264	12 633	215 925 ^k	15 228	153 603 ^j	8075
<i>n</i>	57		46		40		66	
<i>N</i>	2		2		3		2	

H = highly prolific, L = low prolificacy.

n = number of embryos, *N* = number of females.

g vs. h $P < 0.05$; c vs. d, e vs. h, f vs. g, i vs. k $P < 0.01$; a vs. b, e vs. f, e vs. g, k vs. l $P < 0.001$; all other comparisons $P > 0.05$.

after implantation. Early work by Adams, including superovulated (1960a) and nonprimed rabbits (1960b), exhibited almost a doubling in mortality after implantation (~18%) compared with preimplantation loss (~10%), demonstrating the decisive importance of postimplantation development for the final number of young born.

Metabolic criteria may be more directly linked with developmental potential than growth and cell proliferation although, with notable exceptions, both properties seem to be related. Van der Meulen *et al.* (1989) recently reported a positive relationship between aromatase activity and diameter in pig blastocysts, but emphasized that there were considerable individual variations. In normal rabbit blastocysts, DNA and protein synthesis are highly correlated with diameter, but not in blastocysts damaged by culture in serum-supplemented media (Jung & Fischer, 1988). Among quantitative parameters, qualitative differences have to be noted. For example, blastocysts of the same size differ considerably in number of embryoblast cells (pig: Barends *et al.*, 1989; mouse:

Table 4. Incidence of aneuploidy (%) in embryos from ZIKA hybrid (H) and Alaska outbred (L) rabbits

Embryonic age (days p.c.)	H♂ × H♀	L♂ × L♀
2	11	16
<i>n</i>	19	31
<i>N</i>	3	4
4	29	20
<i>n</i>	28	40
<i>N</i>	8	8
6	58	46
<i>n</i>	24	26
<i>N</i>	3	4
2, 4 and 6	34	26
<i>n</i>	71	97
<i>N</i>	14	16

H = highly prolific, L = low prolificacy.

n = number of embryos, *N* = number of females.

All comparisons between H and L, *P* > 0.05.

Chisholm *et al.*, 1985). Studies in rabbit blastocysts, however, could prove that a threshold value for a minimal number of embryoblast cells has to be guaranteed to secure further development (Mootz, 1971). Therefore, valid criteria for judgement of embryo viability still have to be defined and might be more closely linked to strain prolificacy than the criteria presently investigated.

The percentage of aneuploid embryos found in the present study (Table 4) (i) was not influenced by maternal FSH treatment and (ii) was higher than in other studies (A. Schumacher, T. Agorastos, B. Fischer and H. M. Beier, unpublished). Apart from a few blastocysts, all from Day 6 p.c., the number of aneuploid cells per embryo was too low to be considered as a major reason for embryonic mortality during preimplantation development. We conclude that the higher reproductive performance in the hybrid strain studied cannot be traced back selectively to a generally advanced early embryonic development or reduced rate of aneuploidy. Other reasons, e.g. number of ovulations, maternally determined developmental conditions, implantation and postimplantation development, will have to be considered to define more closely the actual determinants of fecundity.

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