than the control strains. On PDA, which increases proliferations in all studied strains, this increment was 1.8–2.7 times and concerns more the number of conidiophores with proliferations than the number of proliferations per conidiophore (table 1). On CM only *hpp* strain showed an increase of the frequency of proliferations (table 2). These data indicated interaction between strains and culture media, and we suggest that the study of the components of the culture media could be useful for the elucidation of the conditions that induce phialide proliferations.

Table 2. Effect of complete medium (CM) and potato dextrose agar (PDA) on the frequency of proliferations in *Aspergillus niger* strains

Strains	Culture media	
	CM	PDA
pur_1	+	++
pdx_1olv_1	+++	+++
pab_1fwn_1	+	++
$pur_1//pdx_1olv_1$	+	++
$pab_1fwn_1//pdx_1olv_1$	+	++
HCA	+	++

+, ++, +++ indicate low, media and high frequency of proliferations respectively, as observed with a stereomicroscope.

The diploids synthesized between hpp^+ (pur_1 or pab_1fwn_1) and *hpp* (pdx_1olv_1) strains showed that the characteristic is recessive (table 1). The segregants obtained from diploids suggested the existence of a pleiotropic effect of the olv_1 gene because all the olive ones were *hpp*. An alternative explanation could be strongly linked genes, and to test this hypothesis, revertants to wild type conidial color were isolated from the *hpp* strain. These revertants, including a mutant with light brown conidia (fawn), showed a frequency of proliferations similar to hpp^+ strains, indicating that the mutation in the olv_1 gene is responsible for the *hpp* phenotype.

To the authors' knowledge this is the first time that a mutant of this type has been reported, and the *hpp* strain could be used in order to understand better the morphogenetic processes of conidiation in *A. niger*.

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Preimplantation mouse embryos cultured in mouse, rat and human sera: differentiation and sister chromatid exchange

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Summary. Development of 4-cell and of 8-cell mouse embryos and of morulae and blastocysts is inhibited in vitro by mouse serum but not by rat or human sera which also do not influence sister chromatid exchange in cultured morulae and blastocysts.
Key words. Mouse embryo; preimplantation; in vitro culture; heterologous sera; sister chromatid exchange.

Rat and human sera have successfully been used in whole embryo culture during organogenesis both in the rat[1-3] and in the mouse[4]. However, there are no comparable data on the culture of preimplantation embryos in homo- or heterologous sera. A systematic evaluation of the effects of homo- and heterologous sera on the development of preimplantation mouse embryos in culture may help to establish test systems in which sera from patients or animals can be tested for embryotoxic effects on early embryos in vitro. We, therefore, cultured preimplantation mouse embryos in mouse, rat and human sera and we studied subsequent development of the embryos in culture during implantation[5] as well as the rate of sister chromatid exchanges (SCEs) in exposed embryos, since the SCE test has successfully been used to determine persistent DNA lesions at the earliest stages of embryonic development[6].

Materials and methods. Culture of preimplantation mouse embryos in homo- and heterologous sera. Nullipara NMRI mice were caged with males overnight. The morning on which a vaginal plug was found was considered to be day 0 of pregnancy. 4-cell and 8-cell mouse embryos flushed from the oviducts on day 2 were cultured for 48 h in Whitten's medium[7] supplemented with 0.3% BSA (W-BSA) to the blastocyst stage and then transferred to medium NCTC-109 (M.A. Bioproducts, Walkersville, Md, USA) supplemented with 10% fetal calf serum (NCTC). After 96 h of culture in NCTC, development and outgrowth of the inner cell mass (ICM) of the blastocyst were recorded as described previously[5,8]. Morulae and blastocysts flushed from the uteri on day 3 were cultured for 24 h in W-BSA to the late blastocyst stage and were then transferred to NCTC and cultured for 96 h[5,8].

To determine the effects of homo- and heterologous sera on development of preimplantation mouse embryos, 4- and 8-cell embryos and also morulae and blastocysts were cultured for 24 h in Whitten's medium supplemented with increasing concentrations of the sera to be tested. Thereafter, 4- and 8-cell embryos were transferred for another 24 h to W-BSA and then to NCTC whereas morulae and blastocysts were immediately transferred to NCTC. Development of the exposed embryos was recorded after 96 h of culture in NCTC[5,8] and compared to controls.

Preparation of sera. Homo- and heterologous sera from mice,

rats and from healthy human volunteers were prepared according to Steele and New[9] by delayed and immediate centrifugation of blood. Delayed centrifugation: Blood drawn from healthy human volunteers and from rats and mice was left at room temperature for 30 min before centrifugation at 500×g for 10 min. Immediate centrifugation (only performed on mouse blood): Blood drawn from mice was immediately centrifuged at 500 rpm for 10 min. After centrifugation all sera were heat inactivated (56 °C for 30 min), sterilized by filtration and stored frozen at −20 °C. The sera were thawed just before preparation of the culture media.

SCE determination in preimplantation mouse embryos. To analyze SCEs, morulae and blastocysts were cultured for 24 h in W-BSA containing 5-bromo-2-desoxyuridine (BrdU; Sigma AG, München, FRG; concentration 2×10^{-6} M) and for an additional 24 h in NCTC supplemented with thymidine (Sigma; 1×10^{-6} M). To compare SCE-frequencies in early embryos with adult tissues, mouse morulae, blastocysts and bone marrow cells were cultured in Eagle's Minimal Essential Medium (MEM) supplemented with 10% fetal calf serum (FCS) (MEM & FCS; Flow Laboratories, Meckenheim, FRG) and BrdU (concentrations: bone marrow = 1×10^{-5} M, embryos = 2×10^{-6}) for two cell cycles.

All cell cultures for SCE-frequency determination were carried out in the dark. 3 h before termination of the culture, colcemide (Serva, Heidelberg, FRG) was added to give a final concentration of 0.1 µg/ml. Chromosome preparations of preimplantation embryos were carried out according to Tarkowski[10]. Differential staining for SCEs was performed by the fluorescence-plus-Giemsa technique both in bone marrow cells and early embryos[6,11]. The same preparations were analyzed for micronuclei and structural chromosomal aberrations[6,11,12].

Statistics. The success rate of embryonic development in vitro was expressed as percentage of untreated controls. Groups were then compared by the Wilcoxon test. SCE frequencies were compared by Student's t-test.

Results and discussion. 4- and 8-cell mouse embryos as well as morulae and blastocysts are severely inhibited in development when cultured in homologous mouse serum for 24 h (concentration as low as 10%; table). This inhibitory effect could not be overcome by changing the procedure for serum preparation (delayed and immediate centrifugation; fresh serum and frozen serum). The two heterologous sera (rat and human), however, at concentrations up to 100% stimulate subsequent differentiation of 4- and 8-cell mouse embryos in NCTC as indicated by an increased ICM development in NCTC (table). Similarly in a recent investigation 4-cell and 8-cell mouse embryos cultured in Ham's F 10 supplemented with 10–30% human serum were more advanced in development than embryos cultured without any sera [12]. Furthermore the two heterologous sera had no inhibitory effects on morulae and blastocysts (table). According to our data, therefore, the presence of human and rat sera in culture media for preimplantation mouse embryos for 24 h seems to be beneficial rather than embryotoxic.

To further evaluate the effects of heterologous sera structural chromosomal aberrations, micronuclei and SCE frequencies were determined in mouse morulae and blastocysts. Culture in the presence of the heterologous sera did not induce an increase of chromosomal abnormalities or of micronuclei.

The spontaneous SCE-frequency in morulae and blastocysts was 25,4/chromosome set (25 metaphases evaluated) after culture for 24 h in W-BSA and for an additional 24 h in NCTC and it was 6,2/chromosome set in mouse bone marrow cells cultured under identical conditions (25 metaphases evaluated). The high SCE rate in morulae and blastocysts when compared to bone marrow cells is in agreement with the results of other investigators[6,12,13], although strain differences have been observed. In a study on radiation-induced SCEs in 2-cell mouse embryos the spontaneous SCE rate was considerably higher in 2-cell embryos of the BALB/c strain and the Dub:(ICR) strain (12/chromosome set) than of the C57BL/6J strain[6]. In an additional study from the same laboratory the SCE-frequency in blastocysts of the DUB:(ICR) strain was 12/chromosome set[13]. Other investigators found an even higher spontaneous SCE-frequency in 8-cell embryos of the Balb C/6 × C3H strain[12] (20–30/chromosome set). This is comparable to our results in morulae and blastocysts of the NMRI strain. The high SCE-frequency in early embryos compared to adult tissues may be related to differences in the sensitivity to DNA lesions and/or in repair capacity[6].

After culture of morulae and blastocysts of our NMRI strain for 24 h in rat or human sera, a slight but significant increase in the SCE-frequency was observed (table). However, it has to be

Effect of culture of mouse 4-cell and 8-cell embryos and of morulae and blastocysts for 24 h in homologous and heterologous sera (mouse; rat and human) on development in NCTC-109 and on SCEs

Concentration of serum in Whitten's medium	Differentiation in vitro in NCTC-109 medium					Sister chromatid exchange
	No. of embryos (A)	No. of experiments	Expanded blastocysts (% of A)	Inner cell mass (% of A)	Inner cell mass (% of control)	SCE/chromosome set (No. of chromosome sets evaluated)
0 3% BSA (control)	83 (4/8)	8	89 ± 15	50 ± 11	100%	
	124 (m/b)	12	97 ± 4	66 ± 14	100%	25.4 ± 1.9 (25)
10% mouse serum	101 (4/8)	9	46 ± 41*	5 ± 7*	10%	Determination impossible
	94 (m/b)	8	64 ± 29*	17 ± 21*	25%	due to growth inhibition
10% rat serum	44 (4/8)	4	98 ± 5	59 ± 8	117%	
	111 (m/b)	11	97 ± 6	61 ± 12	92%	38.8 ± 2.6** (10)
50% rat serum	49 (4/8)	5	97 ± 5	67 ± 21	134%	
	62 (m/b)	6	97 ± 8	60 ± 6	91%	38.8 ± 3.7** (10)
100% rat serum	37 (4/8)	4	98 ± 4	67 ± 12	132%	
	30 (m/b)	3	87 ± 15	60 ± 20	91%	38.2 ± 2.2** (10)
10% human serum	76 (4/8)	7	91 ± 17	55 ± 13	110%	
	81 (m/b)	8	95 ± 11	64 ± 14	97%	35.0 ± 2.0** (10)
50% human serum	56 (4/8)	6	96 ± 6	60 ± 15	118%	
	50 (m/b)	5	98 ± 5	62 ± 7	94%	36.4 ± 2.9** (10)
100% human serum	56 (4/8)	5	98 ± 4	76 ± 10*	150%	
	61 (m/b)	8	100 ± 0	62 ± 9	94%	37.2 ± 1.8** (10)

4/8 = 4-cell and 8-cell embryos. m/b = morulae and blastocysts. All values are expressed as mean ± SD. * Significantly different from the control ($p < 0.05$; Wilcoxon-test); ** significantly different from the control ($p < 0.001$; Student's t-test); A = total number of embryos.

taken into account that only an increase in the SCE rate of 100% is generally considered to be genetically important, since minor but statistically significant increases can be induced by changes in the culture conditions[14-16]. Furthermore, the SCE frequency did not increase when the concentrations of rat or human sera were raised from 10% to 100% (table). Since there were no dose-related effects of the sera on SCE frequency, micronuclei and structural chromosomal aberrations in exposed embryos, it may be assumed that the sera did not contain any genotoxic factors. Similarly, in a recent study no increase in SCE frequency, structural chromosomal abnormalities and micronuclei could be detected in 8-cell mouse embryos of the Balb C/6 × C3H strain during culture in media containing 10–30% human serum[12].

The present investigation proves that preimplantation mouse embryos can be cultured in the presence of media containing human and rat sera. There are several applications of such culture systems, e.g. the development of preimplantation mouse embryos in media containing human serum can be used to study the viability of different lots of human sera to be used in human in vitro fertilization programs[12]. Furthermore, in reproductive toxicology the culture of early mouse embryos in sera from exposed humans or rats holds promise for the detection of chemicals which are embryotoxic during the preimplantation period.

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Human serum as a culture medium for rat embryos

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Summary. A comparison was made between the development of post-implantation rat embryos in human serum and rat serum. Protein synthesis (growth) and somite number (differentiation) were retarded in human serum and there was an increased frequency of neural tube defects. Male and female human sera supported development equally well.
Key words. Embryo culture; human serum; neural tube defect.

Whole embryo culture techniques are now being used widely in the study of teratogenic mechanisms and are also being evaluated for possible applications in teratogen screening[1,2]. One approach is to use serum from an individual treated with the teratogens as the culture medium. This could combine any direct effects of an agent or metabolites of an agent on the embryo with effects resulting from changes in the serum produced by interactions between the agent and the maternal metabolism[3-5]. This procedure can also be used to study the effects on embryonic development of sera abnormal for reasons other than treatment with teratogenic agents (e.g. dietary deficiency[6]).

The potential value of this approach would be greatly increased if embryos could be grown in serum taken from humans dosed with a test substance. Unfortunately, human serum has not so far been shown to be satisfactory as a culture medium for rat embryos. In contrast, rat serum can support development in vitro comparable with that in vivo during the organogenetic phase[7].

Development in human serum can be improved by glucose supplementation[8]. However, when comparisons are made with other studies, using rat serum, it would appear that development in human serum is not as good as that in rat serum; there is

Table 1. Embryonic development in 90% human and 100% rat sera. The human sera were supplemented with glucose to 300 mg%. The rat sera were not supplemented

Group	No. of embryos	Heart-beat (at end of culture)	Yolk sac circulation (at end of culture)	Turning complete	Anterior neuropore closed	Optic vesicles	Otocysts	Two or more pharyngeal arches	Allantois fused with chorion	Limb buds	Somites $\bar{x} \pm SE$	Protein (μg) $\bar{x} \pm SE$
10 human sera	30	30 $p < 0.001$	30 $p < 0.001$	25 $p < 0.05$	14 $p < 0.001$	30	30	29	30	28	20 ± 1	139 ± 10
10 rat sera	30	18	18	18	28*	27	30	26	28	25	20 ± 1 ($n = 29$)	135 ± 13

*The somites in one of the two embryos with an open anterior neuropore were difficult to count accurately. Therefore, it is possible that the failure of the anterior neuropore to close may not have been abnormal for the stage of development.