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Variation of Mouse Oocyte Sensitivity to Griseofulvin-Induced Aneuploidy and Meiotic Delay During the First Meiotic Division

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The effects of varying the time of chemical treatment on the induction of aneuploidy and meiotic delay in metaphase II (MII) oocytes were studied by administering 1,500 mg/kg griseofulvin (GF) at 0, 2, 4, 6, or 8 hr after an injection of human chorionic gonadotrophin (HCG). The results show that the oocytes have a different sensitivity to GF-induced aneuploidy and meiotic delay during the course of meiotic maturation. Although not restricted to a particular period of meiotic maturation, the frequency of aneuploidy was highest ($P < 0.05$) when GF was given at 2, 4, or 6 hr after HCG. The maximum frequency of hyperploidy (42.4%) occurred at the 4-hr treatment time. Also, GF treatment resulted in the induction of meiotic delay as demonstrated by ovulated metaphase I (MI) and polyploid MII oocytes. The meiotic delay data depict a period of relative resistance between two periods of sensitivity in that the percentages of ovulated MI oocytes were 53.3, 21.3, 3.5, 6.7, and 25.7 when GF was given at 0, 2, 4, 6, and 8 hr after HCG, respectively. Also, at these treatment times the percentages of polyploid oocytes were 0.6, 1.7, 7.7, 20.1, and 15.4, respectively. Therefore, the oocytes seem to be more sensitive to GF-induced meiotic delay during the periods preceding and following meiotic spindle assembly. In conclusion, the results demonstrate that the time of chemical treatment influences the frequency of aneuploidy and the degree of meiotic delay. Also, the results emphasize that to thoroughly characterize the aneugenic potential of a specific chemical several treatment times may be needed.

Key words: chromosome aberrations, meiosis, germ cell, mouse female, hyperploidy, meiotic spindle

INTRODUCTION

Despite the great burden that aneuploidy represents for humans [Hook, 1985; Hecht and Hecht, 1987], the etiology and mechanisms of this genetic disorder are still poorly understood. Even less information is available about the role that exogenous agents (chemical, physical) have on the genesis of human aneuploidy. Therefore, a need exists for developing and validating assays that can be used for estimating the genetic risk posed to humans by agents capable of increasing the frequency of aneuploidy. Since the vast majority (approximately 75%) of human aneuploidies originate during meiosis I in females [Gaulden, 1992], particular effort must be directed toward elucidating the factors leading to nondisjunction during oogenesis.

Recently, the female mouse germ cell assay has been shown to be a reliable system for investigating the mechanisms and causes of chromosome missegregation. To date, several chemicals have been identified as inducers of aneuploidy. These chemicals mainly interact with components of the spindle apparatus and include: colchicine [Tease and Fisher, 1986; Mailhes and Yuan, 1987a; Mailhes et al., 1990], vinblastine sulfate [Russo and Pacchierotti, 1988], griseofulvin [Tiveron et al., 1992; Mailhes et al., 1993], and benomyl [Mailhes and Aardema, 1992]. Also, an important finding for human health has been the demonstration that the majority of the aneuploid oocytes can be fertilized and produce aneuploid embryos [Mailhes et al., 1990; Marchetti et al., 1992]. However, additional information is needed to characterize those variables which can influence the experimental results.

Besides the pharmacokinetics and the mechanism of action of the chemical being studied, several experimental variables have been shown to influence the results from oocyte aneuploid studies, including: (1) the mode of treatment [Mailhes et al., 1990], (2) the availability of food during the treatment period [Tiveron et al., 1992; Mailhes et al., 1993], (3) the time between treating and harvesting the oocytes [Mailhes and Marchetti, 1993; Mailhes et al., 1993], and (4) the time of treatment relative to the stage of oocyte development [Rohrborn and Hansmann, 1971; Mailhes and Yuan, 1987a; Tiveron et al., 1992].

To date, only colchicine [Mailhes and Yuan, 1987a] and triaziquone [Rohrborn and Hansmann, 1971; Hansmann et al., 1974] have been administered at various times (more

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than two) relative to the human chorionic gonadotrophin (HCG) injection. Moreover, conflicting results have been obtained with triaziquone. In the present study, we wanted to further investigate the relationship between moment of treatment and aneuploidy induction. This was accomplished by administering griseofulvin (GF) at different times following HCG injection and analyzing metaphase II (MII) oocytes.

GF is an antibiotic fungicide that binds directly to both microtubule-associated proteins (MAPs) [Roobol et al., 1977] and to tubulin dimers [Wehland et al., 1977; Sloboda et al., 1982], thereby interfering with microtubule assembly. Since MAP incorporation into microtubules provides a stabilizing effect upon the spindle [Murphy et al., 1977], GF would be expected to cause a reduction or disruption of microtubules [Keates, 1981]. In recognition of the microtubule-disorganizing properties of GF, Leonard et al. [1979] have shown that GF did not increase the frequency of structural chromosome aberrations in germ and somatic cells of the male mouse. A comprehensive discussion of the pharmacokinetic and the genetic effects of GF on various cell systems can be found in De Carli and Larizza (1988).

Our results show that mouse oocytes vary in their sensitivity to GF-induced aneuploidy and meiotic delay during the first meiotic division. Also, the results demonstrate the presence of a period of relative resistance between two periods of sensitivity to the induction of meiotic delay as determined by the number of ovulated metaphase I (MI) oocytes and polyploid MII oocytes. However, this period of relative resistance to meiotic delay corresponds with the most sensitive period for aneuploidy induction.

MATERIALS AND METHODS

Animals

Virgin female ICR mice (Harlan Sprague-Dawley, Inc., Indianapolis, IN) between 8 and 12 weeks of age (25–32g), maintained under a 14-hr light/10-hr dark photoperiod (0600–2000) at room temperature of 21–23°C and relative humidity of 50 ± 5%, were induced to superovulate by an intraperitoneal (i.p.) injection of 7.5 I.U. pregnant mare's serum (PMS; Folligon, Intervet Ltd., Cambridge, UK) and 48 hr later, by an i.p. injection of 5.0 I.U. HCG (Ayerst, Inc., Philadelphia, PA). The animals were killed by CO_2 inhalation 17 hr after HCG, and the oocytes were collected and processed according to Mailhes and Yuan [1987b].

Timing of the First Meiotic Division

A preliminary experiment was performed to determine the time at which the first meiotic division occurred. Mice were given 2.0 mg/kg colchicine (CAS No. 64-86-8; Sigma Chemical Co., St. Louis, MO, No. C9754) by i.p. injection at 0, 7, 8, 9, or 10 hr after HCG. This dose has been shown to arrest 100% of the ovulated oocytes at the MI stage when given at the same time as HCG [Mailhes and Yuan, 1987a]. Food was removed from the animals 8–10 hr before colchicine administration. At each colchicine treatment time, the percentage of MII oocytes was used to estimate the number of oocytes that progressed beyond the first meiotic division prior to the time of colchicine injection.

TABLE I. Timing of the First Meiotic Division in ICR Mouse Oocytes

Time (hr) of colchicine[a] injection post-HCG	No. oocytes collected (avg/mouse)	No. oocytes on the slide	No. MI oocytes	No. MII oocytes	Percentage of MII oocytes
0	444 (14.8)	189	184	5	2.6
7	692 (34.6)	447	428	19	4.2
8	1,047 (36.1)	531	490	41	7.7
9	1,218 (40.6)	662	388	274	41.4
10	1,299 (43.3)	591	77	514	87.0

[a]2.0 mg/kg. Food removed 8–10 hr before treatment.

Cytogenetic Analysis of GF-Treated Oocytes

Mice were treated by oral gavage with 1,500 mg/kg GF (CAS No. 126-07-8; Sigma, No. G4753) suspended in olive oil (Sigma, No. 0-1500, Lot No. 40H-01232). A magnetic stirrer was used for at least 30 min to produce the suspension, which was given at a final volume of 0.5–0.64 ml per mouse. The suspension was administered at 0, 2, 4, 6, or 8 hr after HCG. Food was removed 8–10 hr before GF treatment to reduce the possibility of animal to animal variability resulting from the interference of food constituents with drug absorption. The proportions of MII and GF-arrested MI oocytes were determined prior to c-banding the cells according to the method of Salamanca and Armendares [1974]. C-banding was performed to allow an unequivocal identification of whole chromosomes and single chromatids. The cells were subsequently stained with 10% Giemsa in buffer solution (pH 6.8) for 90 min and analyzed for the frequencies of polyploidy and aneuploidy.

The frequency of polyploid cells was calculated relative to the total number of cells (MI, polyploid, and MII oocytes) analyzed. Aneuploidy was calculated by considering the number of hyperploid cells (N = 20½–29½ dyads) relative to the number of MII oocytes (hypoploid, haploid, hyperploid) analyzed. A cell with 20½ chromosomes contains 20 dyads and one unpaired chromatid. Also, the type and number of structural chromosome aberrations were recorded when observed.

Statistical Analysis

Chi-square analyses were used for comparing the frequency of MI oocytes and hyperploidy. However, a Fisher's Exact Test was used for evaluating the polyploidy data because the number of polyploid cells obtained when GF was given at 0 or 2 hr after HCG were very small. In such cases, the Fisher's Exact Test offers a better evaluation of the statistical variability than the Chi-square test.

RESULTS

Timing of the First Meiotic Division

The data relating to the timing of the first meiotic division in ICR mouse oocytes are reported in Table I. A reduction in the number of oocytes ovulated per mouse was noted when colchicine was given at the same time as HCG. A notable increase in the proportion of MII oocytes was found when colchicine was given 9 or 10 hr after HCG. At these times, the frequencies of MII oocytes were 41.4% and 87.0%, respectively. Considering that an unknown amount of time is required for colchicine to reach the oocytes, we estimated

TABLE II. Number of ICR Mouse Oocytes and Their Meiotic Stages After Griseofulvin Treatment

Griseofulvin mg/kg	Treatment time[a]	No. of mice[b]	No. oocytes (avg/mouse)	No. oocytes on slide before c-banding			
				Total	MI	MII	(%) MI
0[f]	0	34	(37.5)	476	0	476	—
1,500	0	60	(37.1)	1,071	571	500	(53.3)
1,500	2	34	(43.5)	816	174	642	(21.3)[c,d]
1,500	4	41	(39.4)	717	25	692	(3.5)[c,e]
1,500	6	57	(33.0)	894	60	834	(6.7)[c,e]
1,500	8	69	(35.9)	1,057	272	785	(25.7)[c]

[a]Hours after HCG injection.
[b]Mice were deprived of food 8–10 hr before GF administration.
[c]Statistically significant vs. 0-hr treatment time, $P < 0.001$ (Chi-square).
[d]Statistically significant vs. 8-hr treatment time, $P < 0.05$ (Chi-square).
[e]Statistically significant vs. 8-hr treatment time, $P < 0.001$ (Chi-square).
[f]Data from Mailhes et al. [1993].

TABLE III. Cytogenetic Analysis of ICR Mouse Oocytes After Administration of 1,500 mg/kg Griseofulvin

Treatment time[a]	No. oocytes analyzed					No. MII oocytes analyzed		
	Total	MI	MII	PP[b]	(%)	Hypoploid[c] (%)	Haploid[d]	Hyperploid[e] (%)
0[j]	230	0	230	0	—	15 (6.5)	214	1 (0.4)
0	685	477	204	4	(0.6)	40 (19.6)	119	45 (22.1)
2	403	143	253	7	(1.7)	77 (30.4)	91	85 (33.6)[h]
4	300	15	262	23	(7.7)[f]	104 (39.7)	47	111 (42.4)[i]
6	324	49	210	65	(20.1)[f]	96 (45.7)	43	71 (33.8)[h]
8	500	174	249	77	(15.4)[f,g]	77 (30.9)	140	32 (12.9)[h]

[a]Hours post-HCG.
[b]Polyploid oocytes containing 30–40 dyads.
[c]MII oocytes containing 10½–19½ dyads.
[d]MII oocytes containing 20 dyads.
[e]MII oocytes containing 20½–29½ dyads.
[f]Statistically significant vs. 0-hr treatment, $P < 0.001$ (Fisher's Exact test).
[g]Statistically significant vs. 6-hr treatment, $P < 0.05$ (Fisher's Exact test).
[h]Statistically significant vs. 0-hr treatment, $P < 0.05$ (Chi-square).
[i] Statistically significant vs. 0-hr treatment, $P < 0.001$ (Chi-square).
[j]Control data from Mailhes et al. [1993].

that the majority of oocytes undergo anaphase I between 9 and 10 hr after HCG injection.

Based upon these results, we administered GF at 0, 2, 4, 6, or 8 hr after HCG to insure that the oocytes were exposed prior to anaphase I.

Cytogenetic Analysis of GF-Treated Oocytes

The average number of oocytes collected per female and their meiotic stages are presented in Table II. The maximum level (53.3%) of GF-induced MI arrest occurred when GF was given immediately after HCG. Conversely, when ICR females received only olive oil immediately following HCG, ovulated MI oocytes were not recovered [Mailhes et al., 1993]. When the time of GF treatment was delayed relative to HCG, the frequencies of MI oocytes decreased to a minimum of 3.5% at 4-hr treatment time, but unexpectedly increased thereafter to 25.7% at 8-hr treatment time.

The aneuploid and polyploid data following administration of 1,500 mg/kg GF are presented in Table III. The frequency of polyploid cells (Fig. 1) was 0.6% when GF was given at the same time as HCG. Their frequencies, however, steadily increased to a maximum of 20.1% at 6 hr post-HCG and then decreased ($P < 0.05$) to 15.4% 2 hr later. The frequency of hyperploidy was 22.1% when GF was given along with HCG and reached a maximum of 42.4% 4 hr later. Subsequently, the percentage of hyperploid MII oocytes decreased to a minimum frequency of 12.9% at the latest (8 hr) treatment time (Table III). The variation in the frequencies of MI, polyploid, and hyperploid oocytes are presented in Figure 2.

Table IV shows the distribution of chromosome numbers as a function of treatment time. Generally, oocytes containing at least 10½ chromosomes were recorded. However, in a few cases an oocyte was associated with a polar body

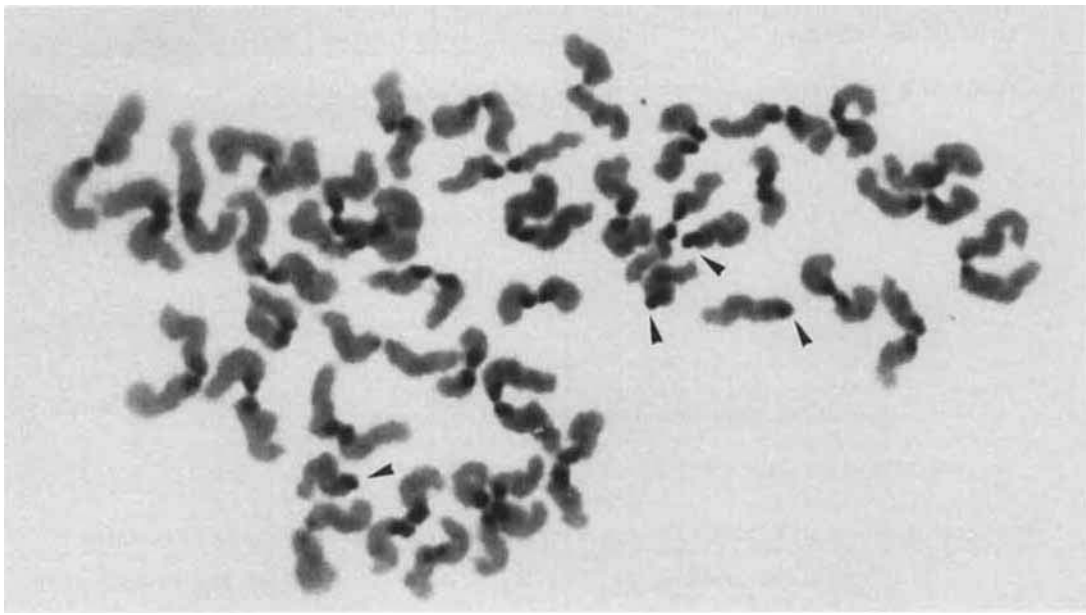

Fig. 1. Mouse metaphase II oocyte, polyploid (n = 40) with centromeric separation (arrowheads) in two dyads.

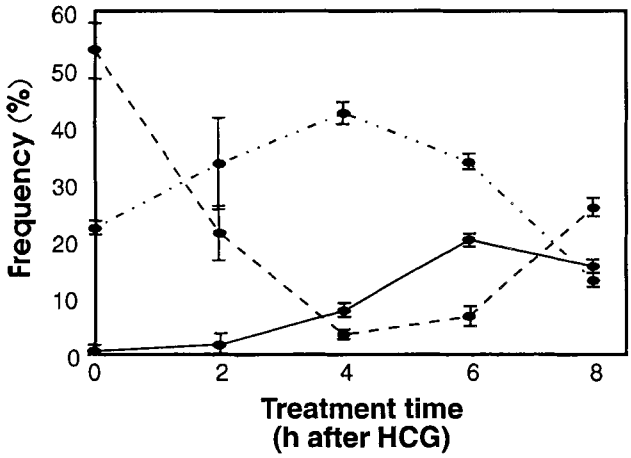

Fig. 2. The percentages of ovulated metaphase I oocytes (---), polyploid oocytes (——), and hyperploid oocytes (– · · –) after oral administration of griseofulvin at different times post-HCG injection (mean ± S.E.).

containing at least 30 chromosomes. The number of these cells is reported in the third column (<10) of Table IV, and an example is shown in Figure 3. This finding has two implications. First, it demonstrates that some grossly hypoploid oocytes did not result from technical artefacts involving excess chromosome loss. Secondly, it suggests that some of the oocytes with 30–39½ chromosomes (next to last column of Table IV) could be real hyperploid oocytes and not polyploid cells in which some chromosomes were lost during fixation. However, their number should not exceed the number of oocytes with less than 10 chromosomes, and should not significantly affect the observed frequencies of hyperploidy.

Finally, 3 of 858 (0.3%) MI oocytes and 4 of 1,354 (0.3%) MII oocytes contained structural chromosome aberrations. These aberrations were uniformly distributed among the different treatment times and consisted of chromatid acentric fragments in MII oocytes or chromosome reciprocal translocations in MI oocytes. The chromatid acentric fragments most likely represent spontaneous or technical artefactual events whereas the translocations may be representative of a particular animal(s). These data do not differ ($P > 0.05$) from those of Mailhes et al. [1993] in which no structural chromosome aberrations were found.

DISCUSSION

The objectives of this study were to investigate the effects of varying the time of GF treatment on the induction of aneuploidy and of meiotic delay during the first meiotic division in mouse MII oocytes. Since different strains of mice can vary in their rates of oocyte maturation [Polanski, 1986], a preliminary experiment was conducted to determine the timing of the first meiotic division. According to the results in Table I, the majority of the oocytes progress through the first meiotic division between 9 and 10 hr after HCG injection. This timing agrees with previous studies performed both in vitro [Donahue, 1968] and in vivo [Edwards and Gates, 1959; Tiveron et al., 1992].

The reason(s) for the decreased number of ovulated oocytes observed at 0 hr post-HCG may reflect a relatively rapid inhibitory effect of colchicine on HCG activity. Colchicine is known to affect various biochemical processes [Dus-

TABLE IV. Distribution of Chromosome Number in Mouse Metaphase II Oocytes After 1,500 mg/kg Griseofulvin Administered at Different Times After HCG

Treatment time[a]	Total	<10	10½[b]	11	11½	12	12½	13	13½	14	14½	15	15½	16	16½	17	17½	18	18½	19	19½	20
0	209	1				1			1					1		4		4	3	20	4	119
2	260					2	1	7		2	2	2		6		9		6	5	29	6	91
4	285	3		2		3	2	4		3		8	1	4	1	8	7	14	5	28	11	47
6	295	9		1	1	3	3	1		4	1	5	1	7	1	8	7	17	10	10	7	43
8	326							4	2	2		9		9		11	2	12	5	18	3	140

20½	21	21½	22	22½	23	23½	24	24½	25	25½	26	26½	27	27½	28	28½	29	29½	30–39½	40
5	23	4	8		3		1		1										2	2
7	32	1	14	4	17		3	1	1	2	1		1				1		4	3
10	37	8	13	4	17	3	3		5	1	3	1	2		2		2		14	9
5	15	6	9	1	7	3	8		7	1	5	1	2			1			35	30
1	6	2	7	1	2		3	1	1	2	5		1						10	64

[a]Hours after HCG injection.
[b]½ stands for a single chromatid.

Fig. 3. Mouse metaphase II oocyte, hypoploid, six dyads in the oocyte, about 34 in the polar body.

tin, 1984]. Variation of oocyte sensitivity to the induction of chemically-induced aneuploidy during oocyte maturation has been reported in mice [Rohrborn and Hansmann, 1971; Mailhes and Yuan, 1987a] and in hamsters [Hummler and Hansmann, 1985, 1988]. These investigators found that the frequency of hyperploidy decreased as the time between chemical treatment and the first meiotic division decreased. However, such a relationship was not found by Tiveron et al. [1992]. They found that the number of ovulated MI oocytes decreased and the number of hyperploid MII oocytes increased when GF was given 2 hr after HCG compared with GF given at the same time as HCG.

Since uncertainty exists regarding the relationship between the time of chemical treatment and aneuploidy induction, we administered GF at different times after HCG but prior to the first meiotic division. When MII oocytes are

studied, the chemical must be given prior to anaphase I to enable the potential damage to be expressed during MII. Consequently, the latest treatment time that we chose was 8 hr after HCG since 7.7% of the oocytes have completed anaphase I at this time (Table I).

The data in Table III show that the oocyte sensitivity to GF-induced aneuploidy is not restricted to a particular period during meiotic maturation. However, these data do point out that the oocytes are more prone to undergo an abnormal division when they are exposed to GF during the mid-portion (2–6 hr) of meiotic maturation. In fact, the highest frequency (42.4%) of hyperploidy was found when GF was given 4 hr after HCG injection (Table III). Although this frequency is not statistically significant ($P > 0.05$) from the frequencies detected at the 2- and 6-hr treatment times, it does differ ($P < 0.001$) from those values observed at the 0- and 8-hr treatment times.

An unexpected finding in this study was the presence of a period of relative resistance to the induction of ovulated MI oocytes located between two periods of relative sensitivity (Table II, Fig. 2). A possible explanation for such a response encompasses the interaction of GF with microtubules and MAPs during the course of meiotic maturation. We suggest that the relatively high frequencies of MI oocytes found at the early and late times of GF administration reflect non-assembly and non-functioning meiotic spindle, respectively. Conversely, the relative decrease in MI oocytes noted when GF was given 4–6 hr after HCG may indicate that the drug was given too late to damage the assembly of meiotic spindle and too early to interfere with anaphase movement.

Another variable that can influence the aneuploidy results is the availability of food during the course of chemical treatment [Tiveron et al., 1992; Mailhes et al., 1993]. This variable is especially relevant when the chemical is given orally because the food constituents can slow the rate of absorption and reduce the systemic level of the compound. In this regard, the results from this experiment (food was removed 8–10 hr before treatment) can be compared with those of Mailhes et al. [1993] (food not removed) in which the same strain of mice was used. When 1,500 mg/kg GF was administered at the same time of HCG injection, the frequencies of MI oocytes and hyperploid oocytes were 53.3% and 22.1%, respectively (Tables II and III), whereas Mailhes et al. [1993] reported 29.2% MI oocytes and 4.5% hyperploidy.

Our results qualitatively agree with those of Tiveron et al. [1992] in that the frequency of MI oocytes decreased while the frequency of hyperploid oocytes increased when GF was given 2 hr post-HCG. Quantitatively, however, we found a higher frequency of hyperploidy at the 0-hr treatment time (Table III), even though we used a lower dose of GF and calculated the frequency of hyperploidy in the the same manner as Tiveron et al. [1992]. In fact, they only considered oocytes with a chromosome number of 20½–24 as hyperploidy and did not include hypoploid oocytes in the denominator. Their frequencies of hyperploidy following 2,000 mg/kg GF were 1.2% and 56.0% at 0- and 2-hr treatment times. When we calculated the frequencies of hyperploidy following 1,500 mg/kg GF according to their approach, we obtained the values of 27.0% and 46.2%. This difference may be due to the different harvest time (18 hr post-HCG), technique, sample size, and scoring criteria used and highlights the need of clearly defining the criteria for cytogenetic analysis and data reporting.

In conclusion, our results indicate that the oocyte's sensitivity to GF-induced aneuploidy and meiotic delay varies throughout the first meiotic division. Specifically, an inverse relationship was found between hyperploidy and meiotic delay as a function of GF-treatment time (Fig. 2). Because the highest levels of meiotic delay and aneuploidy were induced when GF was given at two different times after HCG, our results highlight the importance that the treatment selection can have on the characterization of the aneugenic activity of a given chemical. Therefore, several treatment times may be required for some studies involving chemically-induced aneuploidy in mouse oocytes. We are currently studying the effects of varying the time of treatment during the second meiotic division of mouse oogenesis.

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