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Bisphenol A Effects on the Growing Mouse Oocyte Are Influenced by Diet[1]

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ABSTRACT

Growing evidence suggests that exposure to bisphenol A (BPA) has the ability to disrupt several different stages of oocyte development. To date, most attention has focused on the effects of BPA on the periovulatory oocyte, and considerable variation is evident in the results of these studies. In our own laboratory, variation in the results of BPA studies conducted at different times appeared to correlate with changes in mill dates of animal feed. This observation, coupled with reports by others that dietary estrogens in feed are a confounding variable in studies of endocrine-disrupting chemicals, prompted us to evaluate the effect of diet on the results of BPA studies of the periovulatory oocyte. Genetically identical females were placed on a high- or low-phytoestrogen diet prior to mating. Their female offspring were exposed to BPA, oocytes collected, and meiotic spindle and chromosome characteristics compared between control and BPA-treated females. We observed significant diet-related variation in both the frequency of abnormalities in oocytes from untreated females and in the response to BPA. Our results demonstrate that the impact of BPA on meiosis depends, at least in part, on diet. We suggest that variation in the conclusions of recent BPA studies reflects differences in the diets used, as well as other methodological differences. Because meiotic disturbances are a feature of all studies to date, however, we conclude that low levels of BPA adversely affect the meiotic process.

aneuploidy, bisphenol A, BPA, diet, gamete biology, meiosis, oocyte, phytoestrogens, toxicology

INTRODUCTION

Low-dose bisphenol A (BPA) exposure (i.e., daily doses below 50 mg/kg) during fetal and neonatal development has been reported to cause a variety of developmental defects in rodents (reviewed in Ref. 1). BPA is the building block of polycarbonate plastic and a component of resin coatings. Its use in a wide variety of consumer products, including food and beverage packaging, compact disks, eyeglass lenses, dental sealants, and "carbonless" paper used in receipts, makes BPA a ubiquitous part of daily life. Studies of human populations in several different countries have demonstrated low levels of BPA in the blood and urine of virtually all test subjects (reviewed in Ref. 2). In addition, detectable levels have been reported in breast milk and colostrum [3–5], follicular and amniotic fluid [6, 7], and umbilical cord blood and placental tissue [8]. BPA acts as a synthetic hormone, eliciting estrogenic responses in in vitro assays, and experimental studies in rodents suggest that the biological impact of the chemical on the developing fetus is significant [1]. Thus, the exposure of humans to BPA on a daily basis raises concerns about human health.

The results of rodent studies provide compelling evidence that low-dose BPA exposure adversely impacts the developing reproductive tract of both males and females. By comparison with fetal and newborn animals, the reproductive effects of BPA exposures of adult animals have received considerably less attention. Exposure of adult male rodents has been reported to reduce sperm production and quality [9–11], lower testicular testosterone levels [12, 13], and decrease testis, prostate, and seminal vesicle size [10, 13–15]. However, there has been considerable variation among studies in the occurrence and/or severity of the effects, presumably reflecting differences in the experimental approaches (e.g., use of different species [rat or mouse]) or strains of animals, or in the doses, duration, route or timing of the exposures.

Exposure of adult female rodents reportedly affects maternal behavior [16] and, in a growing number of studies, effects on the periovulatory oocyte have been observed [17–21]. Our laboratory reported the first such effect. As a result of an accident in our animal facility, we serendipitously identified an effect of BPA on the growing oocyte [17]. Inadvertent exposure of our mice to BPA affected control datasets in two different analyses; studies of spindle formation and chromosome alignment at the first meiotic division and cytogenetic studies of metaphase II (MII) arrested eggs. Specifically, we observed an increase in chromosome misalignment at metaphase I (MI) in the first study, and an increased level of aneuploidy among MII eggs in the second. The studies of chromosome alignment were being conducted to test the hypothesis that endocrine changes that affect the late stages of oocyte growth underlie the dramatic increase in aneuploidy that occurs with advancing age in the human female [22]. Thus, the BPA-associated changes in oocytes from control females seemed to support our hypothesis and provided further evidence that chromosome alignment defects at the first meiotic division predispose to aneuploidy.

After publishing our findings, we initiated studies to assess the effect of long-term BPA exposure on the growing follicle. To our surprise, levels of BPA that were sufficient to elicit an effect on meiotic chromosome dynamics during the previous 2 yr of study suddenly produced little or no effect. In an analysis of possible changes in experimental protocol, the only change

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identified was the lot of animal feed. Thus, we began to suspect that fluctuating levels of dietary estrogens might influence the effect of BPA on the growing follicle. The relocation of our laboratory from Case Western Reserve University (CWRU) to Washington State University (WSU) precluded immediate testing of this hypothesis. When we also encountered variable results in our new laboratory at WSU, we concluded that a detailed analysis of the effect of diet was essential. In this article, we provide evidence of significant variability in dietary estrogen levels in different lots of the same animal feed, and we demonstrate that the effects of BPA on the growing oocyte are modulated by phytoestrogens in the diet. In addition, we summarize all of the published data on BPA effects on the periovulatory oocyte, the conclusions that can be drawn, and the questions that remain.

MATERIALS AND METHODS

Animals and Diets

C57BL/6J mice obtained from the Jackson Laboratory were used for all studies. Pathogen-free animals were housed on Sani Chip bedding (Harlan Teklad) in polysulfone cages with polysulfone water bottles on a ventilated rack. Drinking water and feed were provided ad libitum. All animal experiments were approved by the WSU Institutional Animal Care and Use Committee. WSU is fully accredited by the American Association for Accreditation of Laboratory Animal Care, and all investigations were conducted in accordance with the Guide for the Care and Use of Laboratory Animals [23].

To test the effect of diet, breeding stocks were placed at least 1 wk prior to mating on 1 of 2 rodent diets: 1) TestDiet AIN-93G, a casein diet that does not contain soy as a protein source, but does contain soybean oil; and 2) Harlan Teklad Sterilizable Rodent Diet 8656, a soy-based diet. Males and females were paired for mating at 6 wk of age, and the female offspring of these matings were used to assess the effect of diet on BPA effects on the oocyte.

BPA Exposure, Oocyte Collection, Culture, and Fixation

All females used in these studies were reared in our breeding colony from C57BL/6J stock originally obtained from the Jackson Laboratory. The 21-day-old females were treated with BPA (CAS no. 08-05-7) in a corn oil carrier, as described previously [17]. Females received daily oral doses of 20, 40, 100, 200, or 500 μg/kg body weight for 7 days preceding oocyte collection. Oocytes at the germinal vesicle stage were obtained from the puncture of antral follicles, as described previously [17, 22], from ovaries of 28-day-old females. The only exception was for the analysis of oocytes from adult females on the soy-based diet, in which 6- to 11-wk-old females were used. Oocytes were cultured in 10-μl drops of Waymouth medium (Gibco BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum and 0.23 mM sodium pyruvate overlaid with Squibb mineral oil, and incubated for 16–17 h at 37°C in an atmosphere of 5% CO_2 in air. At the end of the culture period, oocytes exhibiting a first polar body were embedded in a fibrin clot (bovine fibrinogen type IV, Calbiochem, La Jolla, CA; bovine thrombin, Sigma, St. Louis, MO) attached to a microscope slide, as previously described [24], and immediately fixed in 5% formaldehyde, 2% Triton X-100, 0.1 M Pipes, 5 mM $MgCl_2$, and 2.5 mM EGTA for 30 min at 37°C. Following fixation, oocytes were washed for 15 min in 0.1% normal goat serum (NGS; Gibco BRL, Gaithersburg, MD)/PBS, blocked for at least 1 h at 37°C in PBS wash solution containing 10% NGS, 0.02% sodium azide, and 0.1% Triton X-100, and stored at 4°C.

Immunofluorescence Staining and Analysis

Slides were incubated with a 1:150 dilution of a direct-labeled α-tubulin antibody (Sigma), washed in 10% NGS/PBS, counterstained with 1 μg/ml 4′,6′-diamidino-2-phenylindole, and a coverslip applied with Bio-Rad antifade and sealed with rubber cement. Oocytes were analyzed by two independent observers with a Zeiss Axioplan epifluorescent microscope, and the meiotic stage of individual oocytes classified on the basis of chromosome configuration and spindle morphology, as described previously [17]. MII-arrested oocytes were classified as abnormal if: 1) the chromosomes failed to align on an otherwise normal meiotic spindle (congression failure); 2) the spindle exhibited serious malformations; or 3) both spindle and chromosome alignment were normal, but one more pair of chromosomes was far removed from the spindle equator, most commonly behind one spindle pole.

Statistical Analysis

For comparisons between different dietary groups, we used contingency table analyses or Fisher exact tests. Bonferroni corrections were used for multiple comparison procedures.

RESULTS

Isoflavone Levels Are Correlated with Meiotic Findings

The inadvertent exposure of our animals to BPA during the course of meiotic studies suggested that BPA exposure disrupts the first meiotic division, increasing the incidence of aneuploid eggs. Subsequent studies designed to test this hypothesis suggested that a daily low-dose (20 μg/kg) BPA exposure for a 1-wk period induced detectable disturbances in meiotic chromosome behavior [17]. In studies initiated after the publication of these findings, we found that the same 20-μg/kg exposure protocol produced little or no meiotic effect. When a careful analysis of all variables suggested that the only change was the lot of animal feed, we began to suspect that fluctuating levels of dietary estrogens were affecting the response of the growing follicle to BPA. The relocation of our laboratory from CWRU to WSU prevented us from immediately testing this hypothesis, but we collected samples of animal diet (Purina Laboratory Autoclavable Rodent Diet 5010 [5010]) and stored them at −4°C for subsequent analysis. When initial studies of the same low-dose exposure at WSU also failed to elicit the expected response to BPA, we decided that an investigation of the effect of dietary estrogens was warranted.

A previous study by Brown and Setchell [25] suggested that isoflavone levels are high in the 5010 diet (671 ± 14 μg/g by HPLC analysis). However, considerable lot-to-lot variation in dietary estrogen levels has been reported in some rodent diets [26–28]. Although it was impossible to determine the isoflavone levels in the lot(s) used in the studies that generated our initial BPA findings [17], we suspected that isoflavone levels in different batches of feed were responsible for the variation in the results of different experiments. Samples of autoclaved pellets from six different lots of diet were sent to Purina and analyzed by N.P. Analytical Laboratories. Table 1 shows the levels (parts per million) of the isoflavone component compounds, diadzein, genistein, and glycitein, as well as the total isoflavone levels for the six samples analyzed. Although the mean isoflavone level for the six samples is in good agreement with that reported by Brown and Setchell [25], our analysis demonstrates remarkable variation among lots, with a nearly two-fold difference between the lots with the lowest and the highest isoflavone values. Intriguingly, this was not merely a reflection of seasonal variation, as isoflavone levels were markedly different in two recent lots milled in the same month. Importantly, from the standpoint of our meiotic results, isoflavone levels were particularly high in the lots of feed corresponding to studies that failed to elicit a BPA effect in our laboratory at CWRU (lot date, 4 October 2004) and in the studies conducted in our new laboratory at WSU (lot date, 5 November 2005) that also failed to elicit a response.

BPA Response in Genetically Identical Animals on Different Diets

To assess the effect of dietary estrogens on the response of the periovulatory oocyte to BPA, we introduced variability in dietary estrogen levels by placing animals on two different diets: a purified diet developed by the American Institute of Nutrition that is commonly used in toxicology studies

TABLE 1. Isoflavone profiles of different lots of Purina 5010 diet.

Mill date	Total isoflavones*	Total daidzein compounds (%)	Total genistein compounds (%)	Total glycitein compounds (%)
10/04	660	267 (40.5)	322 (48.8)	71 (10.8)
11/05	664	297 (44.7)	293 (44.1)	74 (11.1)
08/07	882	386 (43.8)	437 (49.5)	59 (6.7)
06/08	486	194 (39.9)	238 (49.0)	54 (11.1)
10/08[a]	621	259 (41.7)	305 (49.1)	57 (9.2)
10/08[b]	680	288 (42.4)	331 (48.7)	61 (9.0)
Average	665.5 ± 127.5	281.8 ± 62.5 (42.3)	321.0 ± 65.5 (48.2)	62.7 ± 8.0 (9.4)

* Values given in μg/g (parts per million).
[a,b] There were two different mill dates for October.

("casein"), and a soy-based diet ("soy"). Although not completely isoflavone free, the casein diet is very low in dietary estrogens (e.g., using HPLC analysis with a 5-μg/g detection limit, Brown and Setchell [25] were unable to detect estrogenic compounds in the diet). In contrast, the soy diet is considered higher in isoflavones than the 5010 diet, although lot-to-lot variability is expected, and our analysis of two lots revealed total isoflavone levels of 591 and 707. A comparison of the feed components of these two diets and the 5010 diet used in all previous studies conducted in our laboratory is shown in Table 2.

To control for maternal effects, animals were placed on these diets at least 1 wk prior to mating, and female offspring were used for exposure studies. For each diet, we analyzed the spindle and chromosome alignment in MII-arrested eggs (Fig. 1), an approach that allows us to rapidly collect information on a large series of eggs. In initial studies, we were simply interested in generating control data (i.e., for each diet, determining spindle and alignment abnormality rates in eggs from females that had received vehicle [corn oil] supplements). Unexpectedly, striking differences were observed between the diets (Table 3). Specifically, abnormal MII eggs were identified in 2% of eggs from control females on the casein diet, but the abnormality rate increased to nearly 8% for the soy diet ($\chi^2 = 10.0$; $P = 0.002$).

TABLE 2. Comparison of feed components.

Parameter	Standard (5010 Purina)	Soy (8656 Teklad)	Casein (AIN-93G test diet)
Guaranteed analysis			
Protein (crude)	23.0%	24.0%	18.7%
Fat (crude)	4.3%	4.0%	7.0%
Fiber (crude)	6.0%	4.0%	7.0%
Metabolizable energy (kcal/g)	3.08	2.96	3.97
Primary ingredients*			
Ground corn	+	+	-
Soybean meal	+	+	-
Ground soybean hulls	+	-	-
Soybean oil	+	+	+
Wheat middlings	+	+	-
Fish meal	+	+	-
Ground wheat	+	+	-
Wheat germ	+	-	-
Brewers yeast	+	+	-
Ground oats	+	-	-
Alfalfa meal	+	-	-
Casein	-	-	+
Animal fat preserved with BHA[†]	+	-	-
Dried beet pulp	+	-	-
Corn starch	-	-	+
Maltodextrin	-	-	+
Sucrose	-	-	+

* +, present; -, not present.
[†] BHA, butylated hydroxyanisole.

In subsequent studies, we examined the effects of BPA exposure for each diet. As shown in Table 3 and Figure 2a, the casein diet produced an apparent linear dose response, with significant increases in spindle/chromosome alignment abnormalities at the highest exposure level (200 μg/kg; Fisher exact test P value = 0.03). However, for the soy diet, an apparent U-shaped dose response was observed, with only a slight, nonsignificant increase in the rate of abnormalities at the highest BPA exposure level; indeed, the two lowest BPA doses actually elicited a slight decline in abnormalities rather than an increase.

BPA, Dietary Estrogens, and the Oocyte: A Balancing Act

The unexpectedly high rate of abnormalities in the vehicle-only control group on the soy diet, coupled with the unusual response of these animals to BPA, suggested that further studies of this diet were warranted. Accordingly, we analyzed eggs from females on the soy diet that had received neither vehicle nor BPA ("baseline"), and from females exposed to a higher dose (500 μg/kg) of BPA (Fig. 2b). The addition of these data points provides further evidence that diet influences the rate of abnormalities, since the baseline rate of 6.2% (9/146) was significantly higher than all laboratory values for the 5010 diet (data not show) and for the control value for the casein diet (Fisher exact test, $P = 0.03$). Furthermore, these additional data support the idea that low doses of BPA have a normalizing effect on the oocytes of females on this diet, since both the baseline and vehicle categories yielded higher abnormality rates than did the 20- or 100-μg/kg exposure levels. At the highest exposure levels, BPA appears to increase the likelihood of errors, since both the 200- and 500-μg/kg doses had elevated rates of abnormality over both the baseline and vehicle values. However, this effect was relatively modest; for example, the 12.8% (18/141 cells) abnormality rate observed for the 500-μg/kg category was significantly higher than the combined data from the two control doses (baseline + vehicle; $\chi^2 = 4.2$; $P < 0.05$), but not from either dose when considered individually.

Abnormalities on Soy Diet Are Not Unique to Juveniles

For most meiotic studies, we utilize oocytes from the first cohort of follicles that initiate growth in the sexually immature female. These oocytes are both meiotically and developmentally competent (e.g., injection of immature females with exogenous hormones is routinely used in embryo freezing and rederivation protocols), but are destined to undergo atresia due to the lack of appropriate hormonal support. For our purposes, this cohort of growing follicles provides access to a large

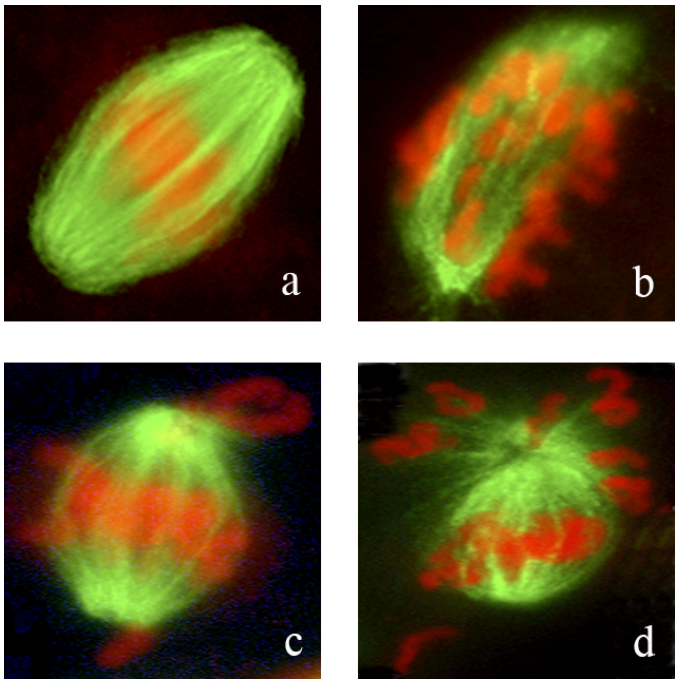

FIG. 1. Spindle and chromosome configurations in MII-arrested eggs. MII-arrested eggs immunostained with an antibody to α-tubulin to visualize the spindle (green) and counterstained with 4′,6′-diamidino-2-phenylindole to visualize the chromosomes (red). **a**) A normal MII configuration. **b**–**d**) Examples of eggs scored as abnormal: (**b**) failure of chromosome alignment on an otherwise normal spindle (congression failure); (**c**) normal alignment of all chromosomes except two that appear to be trapped behind the spindle poles; and (**d**) an abnormally shortened spindle with multiple chromosomes that appear to have been ejected from the spindle.

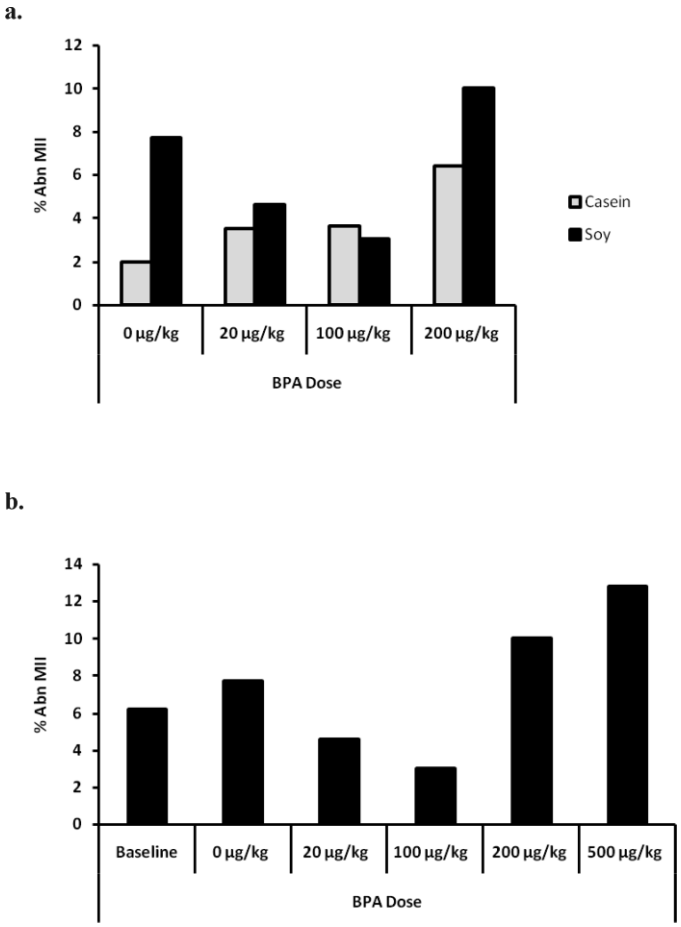

FIG. 2. BPA dose response in females on casein and soy diets. **a**) The frequency of abnormal (Abn) MII-arrested eggs shows a linear dose response in females on the casein diet (grey bars), but a U-shaped dose response in females on the soy diet (black bars). **b**) On the high-phytoestrogen diet (soy), levels of abnormal MII-arrested eggs appear elevated at both control doses (baseline and 0 µg/kg) and extremely high BPA doses (200 and 500 µg/kg), by comparison with low-dose (20 and 100 µg/kg) BPA exposures.

number of oocytes and eliminates the confounding effect of endogenous hormones in the sexually mature female. Furthermore, in studies comparing oocytes from juvenile and sexually mature females maintained on the standard diet, we have found few, if any, meiotic differences (Hunt, unpublished results). The high frequency of abnormalities observed in eggs from control females, however, raised questions about whether the effects of soy-based feed were a unique feature of growing oocytes in the sexually immature female. To address this concern, we analyzed eggs from 11 adult females between the ages of 6 and 11 wk. A total of 6 out of 85 (7.1%) MII-arrested eggs exhibited severe aberrations in chromosome alignment and/or spindle formation. This is in good agreement with the 6.2% baseline abnormality rate observed in eggs from juvenile females, and suggests that the aberrations observed in our studies of females on this diet are not a reflection of the immature female model used in these studies.

TABLE 3. Diet affects the frequency of chromosome alignment and spindle defects in oocytes from BPA-exposed females.

	No. abnormal MII eggs/total (%)	
Daily dose (µg/kg)*	Casein diet	Soy diet
0	6/300 (2.0)	19/248 (7.7)
20	5/142 (3.5)	4/87 (4.6)
100	4/112 (3.6)	3/99 (3.0)
200	8/125 (6.4)	10/100 (10.0)

* Doses were delivered over a 7-day period.

DISCUSSION

Exogenous estrogens are a serious confounding variable in rodent studies designed to assess the effects of endocrine-disrupting chemicals, and, because estrogenic contaminants can be present in food, bedding materials, water, and caging materials, controlling for their presence is a daunting task. Dietary estrogens have been best characterized, and it is clear that they can impact the developing female reproductive tract (e.g., affecting the timing of vaginal opening and uterine weight in immature females [26, 27]). The problem, however, is not limited to differences among animal diets. Considerable variation in dietary estrogen levels has been reported previously among different lots of the same laboratory diet [26–28], a finding confirmed by the analysis of six different lots of our standard mouse diet (Table 1). Indeed, the batch-to-batch variability in dietary estrogens in animal diets has led to the recent recommendation that investigators not only provide information on the diet used in their studies, but also the mill date and estrogenic content [28]. Our own experience has led us to conclude that the standard diet long used by our laboratory, Purina 5010, is not suitable for studies of endocrine-disrupting chemicals. Although the use of a diet

low in dietary estrogens has been suggested as a means of eliminating dietary effects [26], recent data suggest that animals born to mothers consuming such a diet exhibit "fetal estrogen syndrome" [29]. Given the complexity of this issue, it seems likely that diet alone may account for much of the variability in the results of studies of the estrogenic chemical, BPA. The results presented here provide evidence that diet influences one reported BPA-induced effect: disturbances in meiotic spindle formation and chromosome alignment in the periovulatory oocyte. Thus, our data underscore the need for manufacturers to provide levels of estrogenic activity in animal diets on a batch-by-batch basis, and for accurate reporting of this information by investigators. Furthermore, while diet is a potential confounder in reproductive research in general, the variation in results observed in our studies of BPA demonstrates that diet is an essential consideration in the design of studies of endocrine-disrupting chemicals.

Feed: A Confounding Variable

The initial suggestion that BPA exposure affects the oocyte resulted from an accidental exposure that occurred in the midst of meiotic studies in our laboratory [17]. We noted disturbances in spindle formation and chromosome alignment during the first meiotic division, and a corresponding increase in aneuploidy among MII-arrested eggs [17]. All subsequent studies have reported some level of BPA-induced disturbance in the oocyte [18–21], but, as discussed below, no study has precisely replicated our findings. Indeed, considerable variation in BPA-related effects in our own laboratory following the publication of our original paper prompted the present set of analyses.

Because dietary estrogens in feed are a recognized confounder, and the initial change in our results coincided with the introduction of a new lot of animal feed, we hypothesized that dietary estrogens were influencing the effect of BPA on the oocyte. To test the effect of diet directly, we conducted simultaneous studies of genetically identical animals on different diets. To minimize effects due to abrupt changes in feed, we placed animals on new diets prior to mating and conducted exposure studies on the offspring of these animals. The chosen diets allowed us to compare BPA effects in animals on a low dietary estrogen (casein) or a high dietary estrogen (soy) diet with the results of our previous studies. We anticipated that low dietary estrogens would elicit the strongest BPA response. However, while a BPA effect was evident on this diet, it was no stronger than that identified in our previously published studies using our standard diet [17]. Indeed, the most interesting aspect of our results was the high frequency of abnormalities observed in control females on the soy diet (Fig. 2b). This observation, coupled with the unusual dose response to BPA—a drop in the frequency of abnormalities at the lowest BPA doses and a substantial increase at higher doses (Fig. 2b)—suggests a complex relationship between dietary estrogens and BPA on the developing follicle. Specifically, each factor alone elicits an increase in meiotic aberrations. The effects, however, are not additive; at the lowest BPA doses (20 and 100 μg/kg), abnormality rates are actually lower than those in either placebo or untreated females. This finding, coupled with the loss of low-dose effects in studies using lots of our standard diet with high isoflavone levels (Table 1), suggests that high phytoestrogens protect the oocyte from low-dose BPA exposure. Ironically, this protection comes at a cost, as the diet alone induces meiotic aberrations.

BPA and the Periovulatory Oocyte: Can Diet Explain the Variation in the Results of Different Studies?

Following our initial report linking BPA exposure to meiotic abnormalities, several groups attempted to confirm this association. However, the results have been somewhat confusing. In an initial in vitro analysis, in which cumulus-enclosed oocytes were directly exposed to BPA, Can et al. [18] reported cell cycle delays and spindle aberrations. More recently, a consortium of investigators undertook three different approaches to reinvestigate the putative BPA effect, one study exposing cultures of preantral follicles during 12 days of in vitro growth [19], and two other studies attempting to replicate our in vivo exposures [20, 21]. In the in vitro study [19], aneuploidy levels were not determined, but unaligned chromosomes—thought to be a predictor of aneuploidy [17, 22, 30]—were observed at high frequency in oocytes at both meiosis I and II. In each of the other two studies, meiotic abnormalities were observed, but the results of chromosome analyses differed. Specifically, Eichenlaub-Ritter et al. [21] found no evidence of aneuploidy, while Pacchierotti et al. [20] reported an increase in premature sister chromatid aberrations at meiosis II.

How can we reconcile these disparate observations? Clearly, variation in diet is one possibility. The combined data from the present study provide evidence that—at least with respect to the periovulatory oocyte—diet is a serious confounder. Thus, different diets, with different levels of dietary estrogens, may explain at least some of the variation in results. However, significant differences in study design have almost certainly contributed as well. For example, both in vivo [20, 21] and in vitro [18, 19, 21] exposure paradigms were used, and attempts have been made to correlate the findings from these two different approaches [21]. This assumes that BPA acts via the same mechanism in both exposure paradigms, and we suggest that this is an invalid assumption. It seems likely that in vitro exposures of cumulus-enclosed oocytes [18], or of denuded oocytes [21], would act directly on the oocyte, inducing the type of disturbances in microtubule dynamics that have been reported in cultured somatic cells [31, 32]. In contrast, because the late stages of oocyte growth are estrogen dominated, and the ERβ receptor expressed by the granulosa cells plays a primary role in regulating follicle maturation and ovulation (reviewed in Ref. 33), it seems likely that the in vivo effects of BPA on the late-stage follicle are mediated through the somatic cells of the follicle. Thus, the relevance of the in vitro analyses to the in vivo situation is, at best, uncertain.

Furthermore, the two in vivo studies introduced new variables that were not present in our original analysis (e.g., one study used exogenous hormones [20] and the other used a different genetic background [21]). In addition, neither provided data on both of the variables that we examined (chromosome alignment and aneuploidy) in the same cohort of oocytes, further complicating comparisons among different studies.

The variation—both among experiments run at different times in our laboratory and among recently published studies conducted by the same consortium of investigators [19–21]—makes it difficult to draw a simple conclusion about the effect of BPA on the periovulatory oocyte. Clearly, future studies would benefit from standardization of protocols. Nevertheless, given the different approaches in the five studies published to date, and the potential confounding effects of diet, what is remarkable is not the differences, but the consistency among studies. That is, the common theme in the results of all in vitro and in vivo studies of the effects of BPA on the periovulatory

oocyte is that BPA induces detectable meiotic disturbances. On this basis alone, it seems prudent to conclude that BPA has the potential to disrupt the mammalian oocyte.

Lastly, there is growing evidence that BPA exposure has the potential to impact at least three different stages of oocyte development (reviewed in Ref. 34): 1) the earliest events of oogenesis in the fetal ovary [35]; 2) the packaging of oocytes into follicles (the second trimester in humans and in the perinatal period in mouse [36]); and 3) the growing oocyte in the sexually mature female. Thus, although the question of whether or not BPA exposure affects the periovulatory oocyte is an important one, the larger question of whether or not BPA affects oocyte quality cannot be addressed by simply analyzing the effects of exposure to the adult female.

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