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# Bisphenol S is present in culture media used for ART and cell culture

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**STUDY QUESTION:** Do plastic laboratory consumables and cell culture media used in ART contain bisphenols?

**SUMMARY ANSWER:** The majority of human embryo culture media assessed contained bisphenol S close to the nanomolar concentration range, while no release of bisphenols by plastic consumables was detected under routine conditions.

**WHAT IS KNOWN ALREADY:** The deleterious effect of the endocrine disruptor bisphenol A (BPA) on female fertility raised concerns regarding ART outcome. BPA was detected neither in media nor in the majority of plastic consumables used in ART; however, it might have already been replaced by its structural analogs, including bisphenol S (BPS).

**STUDY DESIGN, SIZE, DURATION:** Seventeen plastic consumables and 18 cell culture and ART media were assessed for the presence of bisphenols.

**PARTICIPANTS/MATERIALS, SETTING, METHODS:** Ten different bisphenols (bisphenol A, S, AF, AP, B, C, E, F, P and Z) were measured using an isotopic dilution according to an on-line solid phase extraction/liquid chromatography/mass spectrometry method.

**MAIN RESULTS AND THE ROLE OF CHANCE:** While the plastic consumables did not release bisphenols under routine conditions, 16 of the 18 cell culture and ART media assessed contained BPS. Six media exhibited BPS concentrations higher than 1 nM and reached up to 6.7 nM (1693 ng/l).

**LARGE SCALE DATA:** N/A.

**LIMITATIONS, REASONS FOR CAUTION:** Further studies are required to investigate a greater number of ART media to identify less potentially harmful ones, in terms of bisphenol content.

**WIDER IMPLICATIONS OF THE FINDINGS:** As BPS has already been reported to impair oocyte quality at nanomolar concentrations, its presence in ART media, at a similar concentration range, could contribute to a decrease in the ART success rate. Thus far, there has been no regulation of these compounds in the ART context.

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**Key words:** assisted reproduction / female infertility / oocyte quality / endocrine disruptors / cell culture / plastic consumables / bisphenols / culture media

## Introduction

Researchers have questioned the relation between the increase in human infertility observed in western countries and the impact of

environmental factors, especially endocrine disruptors. Previous studies reported positive correlations between the outcome of IVF and embryo transfer and the levels of pollutants in female follicular fluids

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(Al-Saleh et al., 2010; Jirsova et al., 2010). Bisphenol A (BPA) is a widespread plasticizer, mainly used in polycarbonate monomers, epoxy resins and thermal papers, owing to its heat resistance and elasticity properties (reviewed in Abraham and Chakraborty, 2020). BPA has been extensively used for several decades in the plastic industry, particularly to produce food containers, baby bottles and metal cans. However, it has also been used in medical devices, soaps, lotions, shampoo, nail polish, sunscreen and toys (Eladak et al., 2015; Giulivo et al., 2016; Andaluri et al., 2018; Kirchnawy et al., 2020). Humans are primarily exposed to BPA through diet, as a result of container-content transfer (Kang et al., 2006; Kubwabo et al., 2009; Andra et al., 2015), but also through indoor dust inhalation (Liao et al., 2012b) and the transcutaneous route (Thayer et al., 2016). Widespread BPA use has led to its detection in 95% of patient urine samples in the USA, at concentrations  $\geq 0.1$  ng/ml (0.44 nM; Calafat et al., 2005; Calafat et al., 2008), with an average urine and blood concentration of 1–3 ng/ml (4–13 nM; Eladak et al., 2015).

The deleterious effects of BPA on health have previously been reported (Richter et al., 2007; Wetherill et al., 2007; Rochester, 2013). Low BPA concentrations (in the nanomolar range) are associated with obesity, cardiovascular diseases (Lang et al., 2008; Rochester, 2013), type 2 diabetes (Grun and Blumberg, 2007; Lang et al., 2008) and alterations in reproductive function (Peretz et al., 2014). BPA is an endocrine disruptor. It indeed exhibits a weak oestrogenic activity (Nadal et al., 2018). Moreover, the highest urinary BPA concentrations in women undergoing ART are associated with decreased oocyte number and quality, and reduced oestradiol levels (Mok-Lin et al., 2010; Fujimoto et al., 2011; Ehrlich et al., 2012). BPA reportedly also disrupts steroid production in rat, ovine, porcine and human granulosa cells (Mlynarcikova et al., 2005; Zhou et al., 2008; Grasselli et al., 2010; Mansur et al., 2016; Banerjee et al., 2018; Bujnakova Mlynarcikova and Scsukova, 2018; Samardzija et al., 2018; Teteau et al., 2020).

The data suggesting a deleterious effect of BPA on female fertility raised concerns regarding the ART outcome. BPA was not detected in ART media and its presence in plastic consumables used in ART did not lead to a significant leaching into the media (Gatimel et al., 2016). Nevertheless, BPA might not have been detected because it has already been replaced by its structural analogs. Several studies demonstrated that BPA restriction in some countries [EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processings Aids (CEF), 2015; Usman and Ahmad, 2016] led to an increased human exposure to bisphenol S (BPS), an unregulated BPA structural analog (Ye et al., 2015). Therefore, BPS is now being derespectively detected in urine at the same concentration range as BPA [0.02–21 ng/ml or 0.09–91 nM; (Liao et al., 2012a)]. BPS and BPA shared a disruptive effect on ovine granulosa cell steroidogenesis (Teteau et al., 2020). Their structural analogy suggests that BPS and BPA might exhibit similar properties and adverse health effects (Eladak et al., 2015; Ahsan et al., 2018; Campen et al., 2018; Ijaz et al., 2020). BPS also disrupts steroid secretion in human granulosa cells and negatively affects ewe oocyte quality *in vitro*, even at nanomolar concentrations (Amar et al., 2020; Desmarchais et al., 2020).

There is a need for experimental data to support the hypothesis of the endocrine disrupting properties of bisphenols, but their ubiquitous use, even in research laboratories, makes it more difficult to perform robust assays. In Europe, bisphenols are regulated in food-grade

plastic, in which only BPA and BPS are authorized (European Union, 2019). In contrast, plastic-containing devices used for biological assays and for oocyte and embryo handling (for both research applications and ART) could contain other bisphenols that are poorly studied regarding their endocrine disrupting properties. The aim of the present study was, therefore, to investigate whether plastic consumables contained bisphenols, determine if they leach into media under conditions close to those used in routine practice and assess bisphenol presence in ART and cell culture media. Because several bisphenols that are structural analogs of BPA are already used in the industry, we decided to assess 10 different bisphenols: BPA, BPS, bisphenol AF (BPAF), bisphenol AP (BPAP), bisphenol B (BPB), bisphenol C (BPC), bisphenol E (BPE), bisphenol F (BPF), bisphenol P (BPP) and bisphenol Z (BPZ) (Table I).

## Materials and methods

The aim of this study was to assess the bisphenols released by plastic consumables or that are present in cell culture and ART media. Therefore, no biological materials were needed, and ethics committee approval was irrelevant.

### Plastic consumables

The 17 plastic consumables tested were those used in either a research center focused on granulosa cell culture and embryo production (INRAE Centre Val de Loire, UMR Physiology of Reproduction, Nouzilly, France) or in an ART center (Service de Médecine et Biologie de la Reproduction, CHRU de Tours, France). The list of plastic consumables assessed is defined in Table II. Both polystyrene- and polypropylene-based plastics were assessed. The consumables used to collect samples (oocyte or embryo) (tubes, tips), select samples (plastic dishes), cultivate them (cell culture plates or flasks, 4-well petri dishes) or store them (cryopreservation tubes) were evaluated. We also tested media plastic bottles for potential leaching of bisphenols.

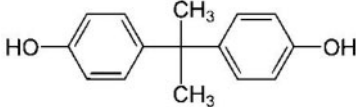
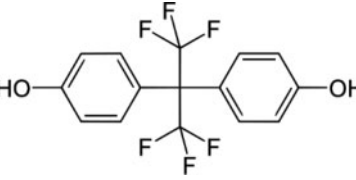
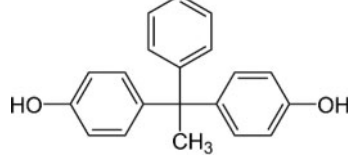
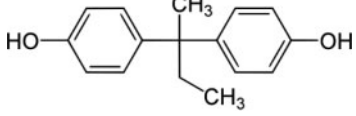
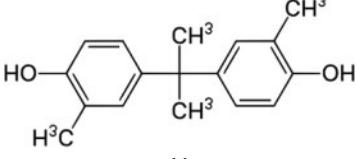
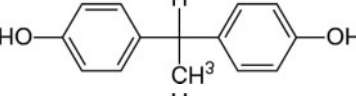
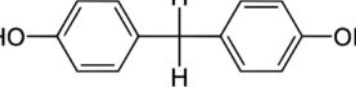
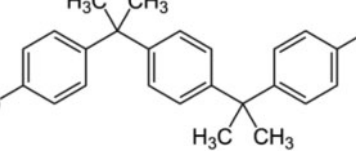
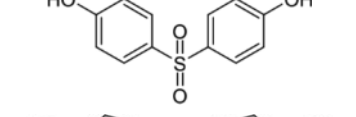
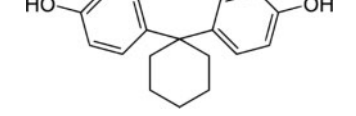
First, plastic consumables were either filled with methanol or cut into pieces and embedded in methanol for 24 h at 40 °C (Table III). These experiments are based on migration tests required for plastic food as stipulated in the Commission Regulation EU 10/2011. Ten bisphenols were then measured (BPA, BPS, BPAF, BPAP, BPB, BPC, BPE, BPF, BPP and BPZ, see below). Each measurement was performed in triplicate.

A second leaching test with pure water was implemented on materials having strong positive results in the leaching test with methanol. For each of these products, a second experiment mimicking the conditions close to routine practice, regarding duration of the experiment, were performed (Table IV). For tips, water was pumped through them 10 times before transfer into a glass vial. Controls were realized in parallel in glass vials, using the same water storage conditions used for the leaching tests. All 10 bisphenols were then measured (see below). Each measurement was performed in triplicate.

### Cell culture and ART media

Eighteen cell culture media that are usually used for oocyte collection (Medium 199 with Hepes, BO-HEPES-IVM), maturation (Medium 199, BO-IVM), fertilization (BO-IVF) and embryo development (BO-IVC) in

**Table 1** List and formula of the 10 bisphenols assessed in plastic consumables and ART/culture media.

Abbreviation and structural name	Structural formula	CAS number	MW g/mol
<b>BPA</b> —Bisphenol A 4,4'-Isopropylidenediphenol		80-05-7	228.3
<b>BPAF</b> —Bisphenol AF 4-[1,1,1,3,3,3-Hexafluoro-2-(4-hydroxyphenyl)propan-2-yl]phenol		1478-61-1	336.2
<b>BPAP</b> —Bisphenol AP 4,4'-(1-Phenylethylidene)bisphenol		1571-75-1	290.4
<b>BPB</b> —Bisphenol B 2,2-Bis(4-hydroxyphenyl)butane		77-40-7	242.3
<b>BPC</b> —Bisphenol C 2,2-Bis(4-hydroxy-3-methylphenyl)propane		79-97-0	256.3
<b>BPE</b> —Bisphenol E 1,1-Bis(4-hydroxyphenyl)ethane		2081-08-5	214.3
<b>BPF</b> —Bisphenol F Bis(4-hydroxyphenyl)methane		620-92-8	200.2
<b>BPP</b> —Bisphenol P 4,4'-(1,4-Phenylenediisopropylidene)bisphenol		2167-51-3	346.5
<b>BPS</b> —Bisphenol S 4,4'-Sulfonyldiphenol		80-09-1	250.3
<b>BPZ</b> —Bisphenol Z 4,4'-Cyclohexylidenebisphenol		843-55-0	268.4

CAS, Chemical Abstracts Service; MW, molecular weight.

**Table II** List of plastic consumables assessed for the presence of bisphenols.

Sample number	Product type	Brand	Origin	Name	Plastic	Use	Product reference
1	50 ml tubes	Clearline, Dominique Dutscher	France	Polystyrene centrifuge tube 50 ml sterile	PS	RL	380502
2	15 ml tubes	Falcon BD Biosciences	USA	Tube with conical bottom 15 ml (on base) Falcon®	PP	RL	352097
3	14 ml tubes	Vitrolife	Sweden	Oocyte collection tube 14 ml	PS	ART	16101
4	1.5 ml tubes	Eppendorf	Germany	Eppendorf Safe-Lock Tube 1.5 ml, Biopur, individually sealed	PP	ART	0030 121-589
5	Tips for oocyte holding and medium preparation	Fisher scientific	France	Fisherbrand™ SureOne™ 1000 µl Filter Tip	PP	RL	11977724
6		VWR	USA	Sterile Aerosol Pipet Tips	PP	ART	732-0560
7		Gilson	Germany	D1000ST Diamond Tipack	PP	ART	F171501
8	Plates for cell culture	Thermo scientific	Korea	BioLite 96 Microwell Plate	PS	RL	130188
9	Flask for cell culture	Falcon BD Biosciences	USA	Tissue Culture Flask 50 ml	PS	RL	353014
10	Culture medium bottles	Sigma-Aldrich	UK	plastic bottles of Medium 199	PP	RL	M4530
11	Plastic dishes for oocyte collection	VWR	Italy	Petri dish 90 mm	PS	ART	391-0556
12		Thermo scientific	Denmark	Nunc IVF Petri Dish 35 × 10mm Non treated	PS	ART	150255
13	Plastic dishes for oocyte maturation and fertilization/embryo culture	Falcon BD Biosciences	USA	EASY GRIP Petri dish—35 × 10 mm	PS	RL	353001
14		Thermo scientific	Denmark	Nunclon Delta surface treated 4-well dish	PS	ART	176740
15		Thermo scientific	USA	IVF ICSI Dish	PS	ART	150265
16		Vitrolife	Sweden	5 Well Culture Dish	PS	ART	16004
17	Tubes for gonadal tissue cryopreservation	Thermo scientific	USA	Nunc Cryotube vials		ART	375353

ART, consumables used in ART labs; RL, consumables used in research labs; PP, polypropylene; PS: polystyrene.

ruminants for granulosa cell culture (McCoy's 5A medium) and for ART in women (SAGE 1-step, Global, Sequential Fert, Sequential Cleav, Sequential Blast, etc.) were assessed for 10 bisphenols (Table V). For media assessment, each measurement (therefore each sample number) corresponded to sampling in separate bottles. In one case (Medium 199), because the BPS level was quite high, several measurements of the same bottle (sample number 19) and of separate bottles of the same batch (sample number 19-21) were assessed. Triplicate measurements were performed when possible and the complete dataset including all replicates is provided in Supplementary Tables SI and SII (in ng/l and nM, respectively). Each sample was analysed directly and after a 10-fold dilution in pure water to avoid a potential matrix effect caused by salts in the media. Two types of controls were associated with this measurement: controls with ultrapure water and each media spiked with a solution of bisphenols (Table VI).

### Bisphenol measurements

HPLC-grade methanol, acetic acid and water were purchased from Fisher Scientific (Illkirch, France). Analytical standards of bisphenols were purchased from Dr. Ehrenstorfer (VWR International, Fontenay sous Bois, France), CIL-Cluzeau (Sainte-Foy-La Grande, France),

Sigma-Aldrich (Saint Quentin Fallavier, France) and Techlab (Saint-Julien-lès-Metz, France) as powder (purity > 95%).

Bisphenols were measured using an isotopic dilution, according to an on-line solid phase extraction/liquid chromatography/mass spectrometry (LC/MS) method. One milliliter of sample was spiked with 100 µl of solution containing internal standards (2 µg/l). Of this, 500 µl was injected into the on-line extraction cartridge (Xbridge C18 Direct Connect HP 10 µm, 2.1 mm × 30 mm, 1.8 µm; Waters) coupled with UPLC/MS-MS equipped with an HSS T3 analytical column (2.1 mm × 100 mm, 1.8 µm; Waters). Detection was carried out using a triple quadrupole mass spectrometer (XEVO-TQXS; Waters), fitted with an ESI interface (negative mode) and controlled by MassLynx software (Waters Corporation, Milford MA, USA).

Specific and intense product ions of each target analyte were used for quantification, and a secondary product ion was used as a qualifier ion for confirmatory purposes. Method quantification limits are indicated in Tables III and VI.

The method has been validated on a linear range from the limit of quantification (LOQ) to 1000 ng/l (except for BPP limited to 300 ng/l). Recoveries ranged on water samples between 80% (BPP) and 100% with a high level of repeatability from 3% to 10% (for BPP). Absolute recoveries of bisphenols from media were lower (from 40% to 85%) but totally outweighed by the internal standards that confirmed the

**Table III Bisphenols detected after solvent leaching of the plastic consumables.**

Sample number	Product type	Solvent volume (ml)	Exposure frame	Bisphenols (LOQ in ng/l)														
				BPA (10)	BPS (2)	BPAF (5)	BPAP (10)	BPB (5)	BPC (5)	BPE (2)	BPF (5)	BPP (10)	BPZ (10)					
1	50 ml tubes	20	Direct filling in the vial	103 ± 79	13 ± 6	<b>96 ± 64</b>	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
2	15 ml tubes	10		108 ± 101	4 ± 1	45 ± 14	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
3	14 ml tubes	2		32 ± 15	2 ± 0	8 ± 4	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
4	1.5 ml tubes	1.5		65 ± 8	8 ± 4	7 ± 2	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
5	Tips	10	Cutting into pieces and transferred in glass vial	<b>308 ± 62</b>	<b>30 ± 7</b>	30 ± 3	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
6		4		118 ± 83	19 ± 9	55 ± 8	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
7		8		<b>736 ± 303</b>	<b>85 ± 5</b>	52 ± 19	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
8	Plates for cell culture	5	5 ml divided into 16 wells for each replicate	31(1)	4 ± 2	54 ± 16	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
9	Flask for cell culture	10		57 ± 36	5 ± 2	<b>195 ± 108</b>	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
10	Culture medium bottles	25	Direct filling in the vial	32 ± 24	<b>712 ± 1205</b>	<b>229 ± 35</b>	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
11	Plastic dishes for oocyte collection	5		93 ± 80	7 ± 1	16 ± 11	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
12		2		16 ± 5	3 ± 1	16 ± 10	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
13	Plastic dishes for oocyte maturation/embryo culture	2		34 ± 15	4 ± 2	11 ± 2	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
14		1.5	1.5 ml divided in 3 wells for each replicate	13 ± 3	2 ± 0	12 ± 1	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
15		2	Direct filling in the vial	48 ± 4	2 ± 1	5 ± 1	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
16		3	3 ml divided in 3 wells for each replicate	29 ± 4	3 ± 1	8 ± 1	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
17	Tubes for embryo cryopreservation	1	Direct filling in the vial	87 ± 67	4 ± 2	9 ± 2	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
ref1-2ml		2		71 ± 26	3 ± 1	8(1)	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
ref2-10ml		10		54 ± 16	4 ± 1	29 ± 3	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
ref3-10ml		10	Direct filling in the vial for 24 h at 40°C	25 ± 14	8 ± 1	17 ± 6	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ

LOQ, limit of quantification, mentioned below each bisphenols in parentheses; NQ: not quantifiable; bold text: significant difference with the controls  $P < 0.0001$ , non-parametric one-way ANOVA with the Tukey post hoc test were performed.

**Table IV** Routine conditions of our experiments applied to plastic consumables containing bisphenols.

Sample number	Brand	Name	Temperature	Duration	Volume of water
1	Clearline, Dominique Dutscher	50 ml Centrifuge tube	4°C	2 weeks	50 ml
5	Fisher scientific	Fisherbrand™ SureOne™ 1000 µl Filter Tip	Room temperature	30 s	1 ml
7	Gilson	D1000ST Diamond Tipack	Room temperature	30 s	1 ml
9	Falcon BD Biosciences	Tissue Culture Flask 50 ml	37°C	48 h	10 ml
10	Sigma-Aldrich	medium bottles	4°C	1 month	100 ml

**Table V** List of culture media assessed for the presence of bisphenols.

Sample number	Product type	Brand	Origin	Name	Vial	Use	Product reference
18-19	Cell culture medium	Sigma-Aldrich	UK	McCoy 5A Medium	PI	RL	M8403
20-21	Oocyte retrieval, holding and washing media	Origio, Cooper surgical	Denmark	Synvitroflush	PI	ART	15840125A
22-23		Origio, Cooper surgical	Denmark	Flushing medium	PI	ART	10840060A
24		Ivf Bioscience	UK	BO-WASH	GI	BEP	61008
25		Sigma-Aldrich	UK	Medium 199 with Hepes	PI	BEP	M7528
26	IVM media	Ivf Bioscience	UK	BO-IVM	GI	BEP	61002
27		Ivf Bioscience	UK	BO-HEPES-IVM	GI	BEP	61009
28	Sperm preparation and IVF media	Ivf Bioscience	UK	BO-IVF	GI	BEP	61003
29-30		Origio, Cooper surgical	Denmark	Gradient 40/80	PI	ART	84022060A
31-32		Origio, Cooper surgical	Denmark	Sequential Fert	PI	ART	83010060A
33		Origio, Cooper surgical	Denmark	Universal IVF medium	PI	ART	10310060A
34-36	<i>In vitro</i> development media	Origio, Cooper surgical	Denmark	SAGE I-Step™	PI	ART	67010010A
37		Origio, Cooper surgical	Denmark	Sequential Cleav	PI	ART	83040010A
38		Origio, Cooper surgical	Denmark	Sequential Blast	PI	ART	83060010A
39		LifeGlobal	USA	Global	PI	ART	LGGG-020
40		Ivf Bioscience	UK	BO-IVC	GI	BEP	61001
41-45		Sigma-Aldrich	UK	Medium 199	PI	BEP	M4530
46	Embryo washing and handling media	LifeGlobal	USA	Global with HEPES	PI	ART	LGGH-050

BEP, bovine embryo production; GI, glass vial; PI, plastic vial; RL, research laboratory.

need for using isotopic dilution for measurements. Owing to high specificity of each media regarding the matrix, controls concerning potential matrix interference effects have been systematically assessed and results confirmed by spiking or diluting samples when needed. All solvents and steps for the analytical procedure were checked separately to avoid systematic contamination: no pollution from the solvents has been highlighted.

## Statistical analyses

The levels of each bisphenol, found in either plastic consumables or cell culture media, were compared among the groups in Rcmdr [R package Rcmdr (Fox, 2005)], R version 4.0.0 (R Core Team, 2015), using non parametric one-way ANOVA [R package lmpPerm (Wheeler and Torchiano, 2010)], with the Tukey *post hoc* test (R package

nparcomp; Konietzschke et al., 2015). A difference of  $P \leq 0.05$  was considered significant.

## Results

### Plastic consumables

After 24 h of methanol action, the 17 plastic consumables listed in Table II were assessed for the presence of 10 bisphenols. BPA, BPS and BPAF were detected in all 17 plastic consumables (Table III). In contrast, BPAP, BPB, BPC, BPE, BPF, BPP and BPZ were detected in none of the consumables. Regarding the level of bisphenols detected compared to the LOQ, five consumables showed a systematic high

**Table VI Bisphenol assessment in cell culture and ART media (in ng/l).**

Sample number	Product type	Name	Reference number	Batch number	Use	Bisphenols (LOQ in ng/l)															
						BPA (10)	BPS (2)	BPE (10)	BPF (5)	BPAF (5)	BPAP (10)	BPB (5)	BPC (15)	BPP (10)	BPZ (10)						
18	Cell culture medium	McCoy's 5A Medium	M8403	SLCB0586	RL	NQ	74	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	
19				SLCB7211		NQ	395	NQ	NQ	NQ	5	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	
20	Oocyte retrieval, holding and washing media	Synvitroflush	15840125A	20250050	ART	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	
21				20150031		NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
22				20280036	ART	NQ	456 ± 3	12 ± 0.3	15 ± 0.6	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
23				20040044		5 ± 0.4	436 ± 3	28 ± 0.3	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
24		BO-WASH	61008	WASH1704	BEP	NQ	35 ± 0.2	NQ	6 ± 0.1	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
25		Medium 199 with Hepes	M7528	RNBG6724	BEP	28	308	15	NQ	NQ	NQ	14	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
26	In vitro maturation media	BO-IVM	61002	IVM1701N	BEP	28 ± 1.4	53 ± 0.9	NQ	17 ± 1.4	NQ	16 ± 0.5	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
27		BO-HEPES-IVM	61009	IVMH1602N	BEP	155 ± 2	23 ± 0.6	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
28	Sperm preparation and IVF media	BO-IVF	61003	IVF1702N	BEP	62 ± 1	112 ± 2	64 ± 1	NQ	NQ	38 ± 1.7	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
29		Gradient 40/80	84022060A	20110060	ART	NQ	67 ± 1	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
30				20180042		NQ	21 ± 1	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
31		Sequential Fert	83010060A	20190042	ART	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
32				20220035		NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
33		Universal IVF medium	10310060A	20270017	ART	NQ	750 ± 24	18 ± 0.6	20 ± 1.5	10 ± 0.4	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
34	In vitro development media	SAGE 1-step	67010010A	19370063	ART	NQ	283	16	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
35				19290061		12	394	18	NQ	11	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
36				20270046		NQ	337 ± 2	NQ	9 ± 1.3	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
37		Sequential Cleav	83040010A	20320064	ART	18 ± 0.7	179 ± 4	7 ± 1.3	8 ± 1.2	7 ± 0.5	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
38		Sequential Blast	83060010A	20330063	ART	4 ± 0.6	187 ± 20	NQ	3 ± 0.3	5 ± 0.1	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
39		Global	LGGG-020	LGGG-200824U	ART	NQ	10 ± 0.1	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
40		BO-IVC	61001	IVC1703N	BEP	34 ± 3.8	322 ± 4	119 ± 6	25 ± 2.6	NQ	16 ± 0.6	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
41		Medium 199—100 ml bottle	M4530	RNBH8521	BEP	NQ	278	16	5	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
42				RNBG5443		NQ	1188 ± 23	26 ± 4.6	18 ± 2.3	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
43				RNBG5443		NQ	1693	33	16	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
44				RNBG5443		NQ	1220	21	18	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
45				RNBH6994		NQ	233	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
46	Embryo washing and handling media	Global with HEPES	LGGH-050	LGGH-200820C	ART	NQ	5 ± 0.1	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ

NQ, not quantifiable; LOQ, mentioned below each bisphenols between brackets; each sample number corresponds to a separate vial; bold text: significant difference with the controls  $P < 0.0001$ , non-parametric one-way ANOVA with the Tukey *post hoc* test were performed; sample number 18, 19, 25, 34, 35, 41, 43, 44 and 45 were not analysed in triplicate.



level of bisphenols (at least 15 times more than the LOQ). BPAF was detected at a high level in sample number 1 (50 ml centrifuge tube) and 9 (cell culture flask). Both BPA and BPS were detected in tips (sample number 5 and 7). Both BPS and BPAF were detected in bottles of cell culture media (sample number 10). This experiment was based on plastic dissolution in methanol: the bisphenol level in and of itself is only a proxy of the plastic composition and has no real meaning on the potential leaching in culture media.

We therefore performed a leaching test on these five plastic consumables (sample numbers 1, 5, 7, 9 and 10) under conditions close to those used in routine practice (Table IV). No quantification of bisphenols, neither in leaching water nor in controls, was reported.

## Cell culture and ART media

We provided the level of bisphenols detected in both ng/l (Table VI) and in nM (Supplementary Tables SI and SII) to be able to compare our data to the literature. BPS was the main bisphenol detected in the cell culture media assessed (Table VI). Nevertheless, six bisphenols (BPA, BPS, BPAF, BPAP, BPE and BPF) were found among the 18 culture media assessed, while BPB, BPC, BPP and BPZ were never detected. BPAP was detected in four media and reached 38 ng/l (0.13 nM) in BO-IVF, BPAF was detected in five media and reached 10 ng/l (0.03 nM) in Universal IVF Medium, BPF was detected in nine media and reached 25 ng/l (0.12 nM) in BO-IVC, BPE was detected in eight media and reached 119 ng/l (0.55 nM) in BO-IVC, BPA was detected in nine media and reached 155 ng/l (0.68 nM) in BO-HEPES-IVM and BPS was detected in 16 of the 18 media assessed. BPS was the bisphenol detected at the highest level in all cases: up to 1693 ng/l (6.8 nM) in Medium 199. BPS exceeded 1 nM in six media: 308 ng/l in Medium 199 with Hepes (1.23 nM), 322 ng/l in BO-IVC (1.28 nM), 338 ng/l in SAGE-I Step (1.35 nM), 446 ng/l in Flushing Medium (1.78 nM), 750 ng/l in Universal IVF Medium (3.0 nM) and 922 ng/l in Medium 199 (3.69 nM).

## Discussion

This study aimed to evaluate the presence and/or leaching of 10 bisphenols in plastic consumables and in cell culture and ART media. For the first time, we reported that cell culture media contained bisphenols, notably BPS in nanomolar range concentrations. Moreover, while plastic consumables contain bisphenols, they do not leach detectable levels of bisphenols in conditions close to those used in routine practice. This study highlighted the need for assessing more ART media for the presence of endocrine disruptors.

The finding that raised the most concerns in the present study is the level of BPS detected in cell culture and ART media. Indeed, the BPS level was above 1 nM (0.25 ng/ml) in six media among 18 tested and reached up to 6.8 nM (1.7 ng/ml). BPA was also detected in nine media and reached up to 0.68 nM (0.15 ng/ml). Comparatively, undetectable BPA levels were reported for three and four ART media, respectively (Mahalingaiah et al., 2012; Gatimel et al., 2016). Undetectable meant below the respective LOQ of these studies, 0.27 (Mahalingaiah et al., 2012) or 0.5 ng/ml (Gatimel et al., 2016). To our knowledge, there are no data in the literature reporting BPS presence in cell culture media thus far. Regarding BPS exposure, the level

measured in culture media in this study (up to 1.7 ng/ml) is not above the level found in some human fluids (up to 21 ng/ml in urine) (Liao et al., 2012a). Nevertheless, in this study, the level of BPS was measured after glucuronidase treatment of the sample and, therefore, included both BPS (native form) and BPS glucuronide (metabolized form). Moreover, a recent study reported a deleterious effect of 10 nM BPS during 24 h oocyte maturation on ovine oocyte quality, measured in terms of blastocyst rate after *in vitro* embryo production (Desmarchais et al., 2020). Even a lower concentration of BPS (3 nM) during 48 h oocyte maturation in porcine was reported to significantly reduce the rate of oocytes reaching metaphase II (Zalmanova et al., 2017). In the present study, the BPS level measured in the media is in the same range of concentrations (nanomolar range) as the ones affecting oocyte quality in ovine and porcine. Moreover, ovine and porcine oocytes were exposed to BPS for 24 or 48 h, respectively, only during oocyte maturation. It is still possible that the oocyte maturation stage is more sensitive to the impact of BPS compared to the fertilization and/or early embryo development stages. Nevertheless, during ART, the embryo stayed up to 6 days in the culture medium and, therefore, can be affected by the BPS present in the medium. In addition, BPS was not in the glucuronide form in the medium. The cumulus-oocyte complex likely does not possess the required cellular machinery to transform BPS into its inactive form, BPS glucuronide, meaning that the effect of BPS will last for the duration of the culture. This is different from what happens *in vivo*, as the glucuronidation of bisphenols occurs rapidly after exposure. Therefore, even if the duration of exposure is short (up to 6 days), the effects might not be negligible compared to a similar *in vivo* exposure. Moreover, a BPA exposure during early embryo development in bovine (Choi et al., 2016) and murine (Pan et al., 2015) decreased the blastocyst rate and damaged blastocyst development, suggesting an effect not only on oocyte quality but also on early embryo development. These findings raise concerns about the effect of BPS contained in the media on the outcome of ART and suggested the importance of investigating a wider diversity of ART media. Moreover, the presence of bisphenols in cell culture media also raises concerns on results reported in the literature regarding bisphenol effects on cells, oocytes or embryos. These results should be analysed with caution, especially when dealing with nanomolar concentrations of bisphenols, or even lower.

Regarding a potential cocktail effect, even if other bisphenols are less abundant than BPS, the culture media still contained five other bisphenols (BPA, BPAF, BPAP, BPE and BPF). Their cumulative or potentially synergistic effects have not yet been studied on the oocyte. Such accumulation of exogenous molecules can strengthen their deleterious effects on oocyte quality. It is also important to keep in mind that the present study only focused on the bisphenol family. Therefore, only bisphenols have been detected and measured in the culture medium. It is likely that other exogenous molecules could be found in the ART medium, such as phthalates, as is the case in food containers (Gonzalez-Castro et al., 2011) and in IVF media (Takatori et al., 2012). Such combinations of compounds and their cumulative effects on oocyte quality are still poorly studied. Nevertheless, mixtures of endocrine disruptors, including BPA, have already been shown to have cumulative estrogenic effects on human endometrial cells (Aichinger et al., 2020). Other mixtures of compounds even showed synergistic endocrine disruption effects in human adrenocortical cells (Ahmed et al., 2019) or during embryo development in fish (Wu et al.,

2018). More studies are required to investigate the effects of mixtures of endocrine disruptors on oocyte quality.

In this study, bisphenols were detected in all the consumables assessed. Indeed, the addition of bisphenols to plastic consumables renders them hard to break, resistant to heat and easy to sterilize. These advantages can explain their widespread use in polypropylene or polystyrene-based plastic consumables. Here, we reported no leaching of the 10 bisphenols assessed from plastic consumables under routine practice conditions. This result is in line with previous studies that focused on BPA (Mahalingaiah *et al.*, 2012; Gatimel *et al.*, 2016). These data are reassuring. Despite the presence of BPA, BPS and BPAF in the plastic consumables used in ART, no additional bisphenol, over and above that detected in ART media, is expected to leach from the consumables. Nevertheless, the present study did not analyze all possible plastic consumables and cannot rule out the possibility that leaching may be observed at higher temperatures than in our conditions. Furthermore, the study from Gatimel *et al.* (2016) found BPA in the strippers used to remove the cumulus from the oocyte. It would be interesting to analyze these strippers for the 10 bisphenols measured in this study.

The source of the bisphenols measured in culture media is still unknown. Indeed, we still do not know whether the presence of bisphenols came from leaching in the medium's plastic bottle or from the media production process. Moreover, even media supplied in glass vials exhibited BPS, BPA and/or BPE levels. Our results did not demonstrate a leaching effect from the plastic bottle under routine practice conditions. It is nevertheless possible that leaching had already reached a plateau with the culture media originally present in the bottle and that the leaching experiment performed by replacing these media with water did not allow further leaching from the plastic. To answer this question, close collaboration with the companies producing ART medium would be required, so that media at different steps of the production process could be analyzed.

Regulation for plastic intended to come into contact with food is constantly evolving and integrating new knowledge on the endocrine properties of bisphenols. In parallel, water regulations have been implemented, through the European Union Water framework Directive (BPA and BPS) and European Union Drinking water Directive (BPA). To the author's knowledge, thus far no considerations are made for these compounds in the ART context.

In this study, analytical methods for bisphenol measurement were developed with many endpoints for quality control, considering the ubiquity and risks of sample contamination. Despite these precautions, some contamination can occur. This is why we chose to focus only on samples exhibiting bisphenol levels higher than 15 times the LOQ. Owing to the limited set of available samples, there is a need to replicate assays on a higher number of samples (both in terms of references and batch numbers assessed) to be more representative and to investigate whether some ART media could be less potentially harmful in terms of bisphenol content.

## Conclusion

In conclusion, we showed that the plastic consumables assessed in this paper and used in ART do not release bisphenols under routine conditions. Conversely, cell culture media, as well as media used in ART,

exhibited BPS in 16 of the 18 types of media assessed, six of them containing BPS in the nanomolar concentration range. As BPS was already reported to impair oocyte quality, its presence in ART media could contribute to a decrease in the ART success rate. Further studies are required to investigate a greater number of ART media to identify the less deleterious ones, in terms of bisphenol abundance.

## Supplementary data

Supplementary data are available at *Human Reproduction*.

## Data availability

The data underlying this article are available in the article and in its online [supplementary material](#).

## Authors' roles

A.T. and S.E. participated in the study design and the analyses and drafted the manuscript. A.D., O.T., C.B., C.V., V.M., F.G. and A.B. helped drafted the manuscript and participated in the critical discussion. S.B. participated in the execution of the experimental design, its analyses and the critical discussion.

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## Conflict of interest

The authors declare that they have no conflict of interest that could be perceived as prejudicing the impartiality of the reported research.

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**human reproduction** **SUPPLEMENTARY DATA**

**Supplementary Table S1 Bisphenol assessment in cell culture and ART media (in ng/l).**

Sample number	Product type	Name	Reference number	Batch number	Use	Bisphenols (BP)										
						(LOQ in ng/l)										
						BPA (10)	BPS (2)	BPE (10)	BPF (5)	BPAF (5)	BPAP (10)	BPB (5)	BPC (15)	BPP (10)	BPZ (10)	
18	Cell culture medium	McCoy's 5A Medium	M8403	SLCB0586	RL	NQ	<b>74</b>	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
19			SLCB7211		NQ	<b>395</b>	NQ	NQ	5	NQ	NQ	NQ	NQ	NQ		
20a	Oocyte retrieval, holding and washing media	Synvitroflush	15840125A	20250050	ART	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
20b						NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	
20c						NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	
21a							20150031	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
21b						NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	
21c						NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	
22a	Flushing medium	10840060A	20280036	ART	NQ	<b>459</b>	12	16	NQ	NQ	NQ	NQ	NQ	NQ		
22b					NQ	<b>457</b>	11	15	NQ	NQ	NQ	NQ	NQ			
22c					NQ	<b>451</b>	12	14	NQ	NQ	NQ	NQ	NQ			
23a						20040044	5	<b>437</b>	27	NQ	NQ	NQ	NQ	NQ		
23b					6	<b>432</b>	28	NQ	NQ	NQ	NQ	NQ	NQ			
23c					6	<b>441</b>	28	NQ	NQ	NQ	NQ	NQ	NQ			
24a	BO-WASH	61008	WASH1704	BEP	NQ	<b>34</b>	NQ	5	NQ	NQ	NQ	NQ	NQ			
24b					NQ	<b>35</b>	NQ	6	NQ	NQ	NQ	NQ				
24c					NQ	<b>35</b>	NQ	6	NQ	NQ	NQ	NQ	NQ			
25	Medium 199 with Hepes	M7528	RNBG6724	BEP	28	<b>308</b>	15	NQ	NQ	14	NQ	NQ	NQ			
26a	IVM media	BO-IVM	61002	IVM1701N	BEP	30	<b>53</b>	NQ	15	NQ	16	NQ	NQ	NQ		
26b						26	<b>51</b>	NQ	17	NQ	15	NQ	NQ	NQ		
26c						27	<b>54</b>	NQ	20	NQ	16	NQ	NQ	NQ		
27a	BO-HEPES-IVM	61009	IVMH1602N	BEP	<b>154</b>	<b>25</b>	NQ	NQ	NQ	NQ	NQ	NQ	NQ			
27b					<b>159</b>	<b>23</b>	NQ	NQ	NQ	NQ	NQ	NQ				
27c					<b>152</b>	<b>23</b>	NQ	NQ	NQ	NQ	NQ	NQ				
28a	Sperm preparation and IVF media	BO-IVF	61003	IVF1702N	BEP	<b>61</b>	<b>111</b>	<b>101</b>	NQ	NQ	35	NQ	NQ	NQ		
28b						<b>64</b>	<b>110</b>	<b>90</b>	NQ	NQ	38	NQ	NQ	NQ		
28c						<b>62</b>	<b>116</b>	<b>99</b>	NQ	NQ	41	NQ	NQ	NQ		
29a	Gradient 40/80	84022060 A	20110060	ART	NQ	<b>69</b>	NQ	NQ	NQ	NQ	NQ	NQ	NQ			
29b					NQ	<b>65</b>	NQ	NQ	NQ	NQ	NQ	NQ				
29c					NQ	<b>66</b>	NQ	NQ	NQ	NQ	NQ	NQ				
30a						20180042	NQ	<b>22</b>	NQ	NQ	NQ	NQ	NQ			
30b					NQ	<b>19</b>	NQ	NQ	NQ	NQ	NQ	NQ				
30c					NQ	<b>22</b>	NQ	NQ	NQ	NQ	NQ	NQ				
31a	Sequential Fert	83010060A	20190042	ART	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ			
31b					NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ				
31c					NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ				
32a			20220035		NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ				
32b					NQ	NQ	NQ	NQ	NQ	NQ	NQ					
32c					NQ	NQ	NQ	NQ	NQ	NQ	NQ					
33a	Universal IVF medium	10310060 A	20270017	ART	NQ	<b>799</b>	18	21	9	NQ	NQ	NQ	NQ			
33b					NQ	<b>728</b>	19	23	11	NQ	NQ	NQ	NQ			

(continued)

Supplementary Table S1 Continued

Sample number	Product type	Name	Reference number	Batch number	Use	Bisphenols (BP)									
						(LOQ in ng/l)									
						BPA (10)	BPS (2)	BPE (10)	BPF (5)	BPAF (5)	BPAP (10)	BPB (5)	BPC (15)	BPP (10)	BPZ (10)
33c						NQ	<b>724</b>	17	18	9	NQ	NQ	NQ	NQ	NQ
34	In vitro development media	SAGE I-step	67010010A	19370063	ART	NQ	<b>283</b>	16	NQ	NQ	NQ	NQ	NQ	NQ	NQ
35				19290061		12	<b>394</b>	18	NQ	11	NQ	NQ	NQ	NQ	NQ
36a				20270046		NQ	<b>342</b>	NQ	11	NQ	NQ	NQ	NQ	NQ	NQ
36b						NQ	<b>333</b>	NQ	10	NQ	NQ	NQ	NQ	NQ	NQ
36c						NQ	<b>336</b>	NQ	7	NQ	NQ	NQ	NQ	NQ	NQ
37a					Sequential Cleav	83040010A	20320064	ART	20	<b>174</b>	4	6	6	NQ	NQ
37b						18	<b>187</b>	9	9	8	NQ	NQ	NQ	NQ	
37c						18	<b>175</b>	7	10	8	NQ	NQ	NQ	NQ	
38a		Sequential Blast	83060010A	20330063	ART	3	<b>146</b>	NQ	3	5	NQ	NQ	NQ	NQ	
38b						3	<b>212</b>	NQ	2	5	NQ	NQ	NQ	NQ	
38c						5	<b>202</b>	NQ	4	4	NQ	NQ	NQ	NQ	
39a		Global	LGGG-020	LGGG-200824U	ART	NQ	11	NQ	NQ	NQ	NQ	NQ	NQ	NQ	
39b						NQ	11	NQ	NQ	NQ	NQ	NQ	NQ	NQ	
39c						NQ	10	NQ	NQ	NQ	NQ	NQ	NQ	NQ	
40a		BO-IVC	61001	IVC1703N	BEP	40	<b>326</b>	<b>129</b>	27	NQ	18	NQ	NQ	NQ	
40b						27	<b>313</b>	<b>117</b>	27	NQ	16	NQ	NQ	NQ	
40c						35	<b>326</b>	<b>110</b>	20	NQ	16	NQ	NQ	NQ	
41		Medium 199–100 ml bottle	M4530	RNBH8521	BEP	NQ	<b>278</b>	16	5	NQ	NQ	NQ	NQ	NQ	
42a				RNBG5443		NQ	<b>1228</b>	38	17	NQ	NQ	NQ	NQ	NQ	
42b						NQ	<b>1140</b>	21	23	NQ	NQ	NQ	NQ	NQ	
42c						NQ	<b>1210</b>	23	14	NQ	NQ	NQ	NQ	NQ	
42d						NQ	<b>1172</b>	23	17	NQ	NQ	NQ	NQ	NQ	
43				RNBG5443		NQ	<b>1693</b>	33	16	NQ	NQ	NQ	NQ	NQ	
44				RNBG5443		NQ	<b>1220</b>	21	18	NQ	NQ	NQ	NQ	NQ	
45				RNBH6994		NQ	<b>233</b>	NQ	NQ	NQ	NQ	NQ	NQ	NQ	
46a	Embryo washing and handling media	Global with HEPES	LGGH-050	LGGH-200820C	ART	NQ	5	NQ	NQ	NQ	NQ	NQ	NQ	NQ	
46b						NQ	5	NQ	NQ	NQ	NQ	NQ	NQ	NQ	
46c						NQ	5	NQ	NQ	NQ	NQ	NQ	NQ	NQ	

LOQ, limit of quantification, mentioned below each bisphenols between brackets; RL, research laboratory; BEP, bovine embryo production; NQ, not quantifiable; each sample number corresponds to a separate vial; bold text: significant difference with the controls  $P < 0.0001$ , non-parametric one-way ANOVA with the Tukey *post hoc* test were performed.

**human reproduction** **SUPPLEMENTARY DATA**

**Supplementary Table SII Bisphenol assessment in cell culture and ART media (in nM).**

Sample number	Product type	Name	Reference number	Batch number	Use	Bisphenols (nM)													
						BPA	BPS	BPE	BPF	BPAF	BPAP	BPB	BPC	BPP	BPZ				
18	Cell culture medium	McCoy's 5A Medium	M8403	SLCB0586	RL	NQ	<b>0.30</b>	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ				
19						NQ	<b>1.58</b>	NQ	NQ	0.01	NQ	NQ	NQ	NQ	NQ				
20a	Oocyte retrieval, holding and washing media	Synvitroflush	15840125A	20250050	ART	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ				
20b						NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ				
20c						NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ				
21a						20150031	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ			
21b							NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ			
21c							NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ			
22a							Flushing medium	10840060A	20280036	ART	NQ	<b>1.83</b>	0.06	0.08	NQ	NQ	NQ	NQ	NQ
22b						NQ					<b>1.83</b>	0.05	0.07	NQ	NQ	NQ	NQ	NQ	NQ
22c						NQ					<b>1.80</b>	0.06	0.07	NQ	NQ	NQ	NQ	NQ	NQ
23a						20040044	BO-WASH	61008	WASH1704	BEP	0.02	<b>1.74</b>	0.13	NQ	NQ	NQ	NQ	NQ	NQ
23b	0.03	<b>1.72</b>	0.13	NQ	NQ						NQ	NQ	NQ	NQ					
23c	0.02	<b>1.76</b>	0.13	NQ	NQ						NQ	NQ	NQ	NQ					
24a	NQ	<b>0.14</b>	NQ	0.03	NQ						NQ	NQ	NQ	NQ	NQ				
24b	NQ	<b>0.14</b>	NQ	0.03	NQ	NQ	NQ	NQ	NQ	NQ									
24c	NQ	<b>0.14</b>	NQ	0.03	NQ	NQ	NQ	NQ	NQ	NQ									
25	IVM media	Medium 199 with Hepes	M7528	RNBG6724	BEP	0.12	<b>1.23</b>	0.07	NQ	NQ	0.05	NQ	NQ	NQ					
26a						BO-IVM	61002	IVM1701N	BEP	0.13	<b>0.21</b>	NQ	0.08	NQ	0.06	NQ	NQ	NQ	
26b										0.11	<b>0.20</b>	NQ	0.08	NQ	0.05	NQ	NQ	NQ	
26c										0.12	<b>0.21</b>	NQ	0.10	NQ	0.06	NQ	NQ	NQ	
27a						BO-HEPES-IVM	61009	IVMH1602N	BEP	<b>0.67</b>	<b>0.10</b>	NQ	NQ	NQ	NQ	NQ	NQ	NQ	
27b										<b>0.69</b>	<b>0.09</b>	NQ	NQ	NQ	NQ	NQ	NQ	NQ	
27c	<b>0.67</b>	<b>0.09</b>	NQ	NQ	NQ					NQ	NQ	NQ	NQ						
28a	Sperm preparation and IVF media	BO-IVF	61003	IVF1702N	BEP	<b>0.27</b>	<b>0.44</b>	<b>0.47</b>	NQ	NQ	0.12	NQ	NQ	NQ					
28b						<b>0.28</b>	<b>0.44</b>	<b>0.42</b>	NQ	NQ	0.13	NQ	NQ	NQ					
28c						<b>0.27</b>	<b>0.46</b>	<b>0.46</b>	NQ	NQ	0.14	NQ	NQ	NQ					
29a						Gradient 40/80	84022060 A	20110060	ART	NQ	<b>0.28</b>	NQ	NQ	NQ	NQ	NQ	NQ	NQ	
29b	NQ	<b>0.26</b>	NQ	NQ	NQ					NQ	NQ	NQ	NQ						
29c	NQ	<b>0.27</b>	NQ	NQ	NQ					NQ	NQ	NQ	NQ						
30a	20180042	NQ	<b>0.09</b>	NQ	NQ					NQ	NQ	NQ	NQ	NQ					
30b		NQ	<b>0.08</b>	NQ	NQ					NQ	NQ	NQ	NQ	NQ					
30c		NQ	<b>0.09</b>	NQ	NQ	NQ	NQ	NQ	NQ	NQ									
31a	Sequential Fert	83010060A	20190042	ART	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ						
31b					NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ						
31c					NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ						
32a					20220035	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ					
32b						NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ					
32c						NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ					
33a	Universal IVF medium	10310060 A	20270017	ART	NQ	<b>3.19</b>	0.09	0.10	0.03	NQ	NQ	NQ	NQ						
33b					NQ	<b>2.91</b>	0.09	0.11	0.03	NQ	NQ	NQ	NQ						

(continued)

Supplementary Table SII Continued

Sample number	Product type	Name	Reference number	Batch number	Use	Bisphenols (nM)									
						BPA	BPS	BPE	BPF	BPAF	BPAP	BPB	BPC	BPP	BPZ
33c						NQ	<b>2.89</b>	0.08	0.09	0.03	NQ	NQ	NQ	NQ	NQ
34	In vitro development media	SAGE I-step	67010010A	19370063	ART	NQ	<b>1.13</b>	0.07	NQ	NQ	NQ	NQ	NQ	NQ	NQ
35				19290061		0.05	<b>1.57</b>	0.08	NQ	0.03	NQ	NQ	NQ	NQ	NQ
36a				20270046		NQ	<b>1.37</b>	NQ	0.06	NQ	NQ	NQ	NQ	NQ	NQ
36b						NQ	<b>1.33</b>	NQ	0.05	NQ	NQ	NQ	NQ	NQ	NQ
36c						NQ	<b>1.34</b>	NQ	0.03	NQ	NQ	NQ	NQ	NQ	NQ
37a		Sequential Cleav	83040010A	20320064	ART	0.09	<b>0.70</b>	0.02	0.03	0.02	NQ	NQ	NQ	NQ	NQ
37b				0.08		<b>0.75</b>	0.04	0.05	0.02	NQ	NQ	NQ	NQ	NQ	
37c				0.08		<b>0.70</b>	0.03	0.05	0.02	NQ	NQ	NQ	NQ	NQ	
38a		Sequential Blast	83060010A	20330063	ART	0.01	<b>0.58</b>	NQ	0.01	0.01	NQ	NQ	NQ	NQ	NQ
38b				0.01		<b>0.85</b>	NQ	0.01	0.01	NQ	NQ	NQ	NQ	NQ	
38c				0.02		<b>0.81</b>	NQ	0.02	0.01	NQ	NQ	NQ	NQ	NQ	
39a		Global	LGGG-020	LGGG-200824U	ART	NQ	<b>0.04</b>	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
39b						NQ	<b>0.04</b>	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
39c						NQ	<b>0.04</b>	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
40a		BO-IVC	61001	IVC1703N	BEP	0.17	<b>1.30</b>	<b>0.60</b>	0.14	NQ	0.06	NQ	NQ	NQ	NQ
40b						0.12	<b>1.25</b>	<b>0.54</b>	0.14	NQ	0.05	NQ	NQ	NQ	NQ
40c						0.15	<b>1.30</b>	<b>0.51</b>	0.10	NQ	0.06	NQ	NQ	NQ	NQ
41		Medium 199–100 ml bottle	M4530	RNBH8521	BEP	NQ	<b>1.11</b>	0.07	0.02	NQ	NQ	NQ	NQ	NQ	NQ
42a				RNBG5443		NQ	<b>4.91</b>	0.18	0.08	NQ	NQ	NQ	NQ	NQ	NQ
42b						NQ	<b>4.55</b>	0.10	0.11	NQ	NQ	NQ	NQ	NQ	NQ
42c						NQ	<b>4.83</b>	0.11	0.07	NQ	NQ	NQ	NQ	NQ	NQ
42d						NQ	<b>4.68</b>	0.11	0.08	NQ	NQ	NQ	NQ	NQ	NQ
43				RNBG5443	NQ	<b>6.76</b>	0.15	0.08	NQ	NQ	NQ	NQ	NQ	NQ	
44				RNBG5443	NQ	<b>4.87</b>	0.10	0.09	NQ	NQ	NQ	NQ	NQ	NQ	
45				RNBH6994	NQ	<b>0.93</b>	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	
46a	Embryo washing and handling media	Global with HEPES	LGGH-050	LGGH-200820C	ART	NQ	0.02	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
46b						NQ	0.02	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
46c						NQ	0.02	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ

Each sample number corresponds to a separate vial; bold text: significant difference with the controls  $P < 0.0001$ , non-parametric one-way ANOVA with the Tukey *post hoc* test was performed.