KER 2937: Impaired, Spermatogenesis leads to Decreased, Viable Offspring Concordance Table

Species	Experimental design	Evidence of Impaired Spermatogenesis (IS)	Evidence of Viable Offspring, Decreased (VOD)	IS observed?	VOD observed?	Citation	Notes
Zebrafish (*Danio rerio*)	Two generation exposure to 1nM BPA	• Significant decrease in sperm density of F1 and F2 males compared to control • Decreased sperm quality as measured by motility, velocity, ATP content and lipid peroxidation in F1 and F2 males	• Delayed hatching at 48hpf and increased malformation and mortality were observed in the offspring from BPA-exposed F2; paternal-specific resulting from BPA-exposed males • No significant difference in egg production and fertilization of F1 and F2 females	Yes	No: F1 and F2 Yes: offspring of F2	Chen et al., 2015	Female-biased sex ratio observed in both F1 and F2 adults
Tilapia (*Oreochromis niloticus*)	CRISPR/Cas9 mediated mutation of *eEF1A1b*; F1 sampled at 90, 120, 150 and 180 days after hatch	• Significant downregulation of key genes involving spermatogenesis • Spermatogenesis arrested; reduced number of spermatogonia and spermatocytes • Altered morphology • Delayed spermatogenesis • Reduced motility	• Reduced in vitro fertilization rate (5% vs 80% in WT) due to abnormal spermiogenesis	Yes	Yes	Chen et al., 2017	eEF1A1b - elongation factor
Zebrafish (*Danio rerio*)	Adult males exposed to two concentrations of bis-(2-ethylexhyl) phthalate (DEHP; 0.2 or	• Areas of spermatogonial and spermatid cysts were larger in fish exposed	• Significant decrease in embryo production (up to 90%) observed in	Yes	Yes	Corradetti et al., 2013	Reproductive performance evaluated with untreated

	20 µg/L) for three weeks; 25 ng ethynylestradiol positive control	to 20 µg/L DEHP compared with controls • Testicular area of spermatocyte cysts was lower in males exposed to 0.2 µg/L DEHP • Testicular area occupied by spermatocytes was reduced in fish exposed to DEHP compared to controls, with a concomitant increase in the area occupied by spermatogonia	males treated with DEHP (0.2 and 20 µg/L) • Hatch rate of embryos significantly lower in DEHP-exposed males				females in clean water
Zebrafish (*Danio rerio*)	Targeted genetic disruption of *tdrd12* through TALEN techniques	• Reduced expression of germ cell markers *vasa, dnd, piwil1* and *amh* in mutants • Deformed and apoptotic spermatogonia at 35 dpf found in mutants • Lack of spermatozoa at adult stage	• Infertile under standard breeding despite being able to induce female egg laying (0% fertilization)	Yes	Yes	Dai et al., 2017	Tudor domain-related proteins (Tdrds) have been demonstrated to be involved in spermatogenesis and Piwi-interacting RNA (piRNA) pathway
Zebrafish (*Danio rerio*)	Fish were exposed from 2 to 60 days post-hatch (dph) to nonylphenol (NP; 10, 30, or 100 µg/L nominal) or ethinylestradiol (EE2; 1, 10, or 100 ng/l nominal); reared until	• Majority of fish exposed to 10 ng/l EE lacked differentiated gonadal tissue (undeveloped gonads) at 60 dph • One fish at NP-30 µg/l and two fish at NP-100 µg/l were observed to	• Zebrafish exposed to 10 ng/l of EE exhibited a significant reduction in the percent of viable eggs (clear vs opaque)	Yes	Yes	Hill and Janz, 2003	Due to high mortality in the 100 ng/l EE group, insufficient fish were available for analyses

	adulthood (120 dph) for breeding studies	have ovatestes at 60 dph	• Significant decrease in hatch and swim-up success observed with EE2 and 100 µg NP/L				
Roach (*Rutilus rutilus*)	Mature adult roach collected from both reference and river (effluent contaminated) sites during two consecutive spawning seasons; artificially induced to spawn in laboratory	• Volume of milt released from spermiating male fish significantly lower in the intersex fish than in the reference males • Most fish that did not spermiate had testes that were clearly immature	• Fertilization rate significantly reduced when sperm from intersex males used to fertilize eggs collected from females • Both proportion of fertilized embryos reaching eyed stage and hatching success decreased with increased feminization	Yes	Yes	Jobling et al., 2002	Embryo viability was determined after 24 h (fertilization success), at eyed stage and at swim-up stage (hatching success)
Japanese medaka (*Oryzias latipes*)	Adult medaka exposed for 21 days to 29.3, 55.7, 116, 227, and 463 ng/L 17β-estradiol (E2)	• In males exposed to 463 ng/l, a few oocytes were observed in testis, and testicular tissue almost completely replaced by connective tissue • Accompanied by presence of macroscopic atrophy and degenerated spermatozoa and spermatocytes suggest a lack of spermatogenesis	• Total number of egg spawned and fertility significantly reduced at 463 ng/l E2 compared to the control	Yes	Yes	Kang et al., 2002	
Zebrafish (*Danio rerio*)	Founder fish with originally *mlh1*	• Significant decrease in weight of spermatids	• Reduced fertilization rates	Yes	Yes	Leal et al., 2008	Mlh1 is a member of DNA mismatch

	mutation was crossed out twice to WT fish of the TL line from which the founder was generated	and spermatozoa); some spermatozoa were visible in testes of all mutant fish • Increased number and proportion of spermatogenic stages prior to spermatids compared to WT • Increase in apoptotic cells	under standard breeding conditions (0.4%) • Eggs fertilized from mutant sperm were malformed and and aneuploid				repair machinery and essential for stabilization of crossovers during first meiotic division
Zebrafish (*Danio rerio*)	3-month-old male fish exposed to 10 ug/L of DEHP for 3 months	• No effect	• No effect	No	No	Ma et al., 2018	Semi-static exposure; half water renewed daily and whole water renewed weekly; exposed males mated with WT females
	3-month-old male fish exposed to 30 ug/L of DEHP for 3 months	• No effect	• Concentration-dependent decrease in fertilization rate	No	No		
	3-month-old male fish exposed to 100 ug/L of DEHP for 3 months	• Percent of spermatocytes increased significantly by 27.4% • Significant decrease of 32.2% in spermatids	• Significant decrease in fertilization rate by 22% compared to the control	Yes	Yes		
Zebrafish (*Danio rerio*)	Multi-generational study to 0.5, 5 and 50 ng/L ethynylestradiol (EE2) or 5 ng/L 17β-estradiol (E2)	• None of the F_1 males exposed to 5 ng/L EE2 had normal testes; 43% had gonads not fully differentiated	• Time-related decrease in egg production and egg viability 14 hpf in F_0 generation at 50 ng/L EE2 and no survival of F_1 100 hpf; no eggs produced after 10 d exposure • Exposure to 5 ng/L EE2 in the F_1 caused	Yes	Yes	Nash et al., 2004	

			a 56% reduction in fecundity and no survival past 14 hpf • Proportion of nonviable eggs significantly higher for all treatments compared to control				
Zebrafish (*Danio rerio*)	Targeted genetic disruption of *fdx1b* using a TALEN approach	• Reduced sperm count compared to control (p=0.0097%) • Promale *sox9a* downregulated • Spermatogenic genes *igf3* and *insl3* downregulated	• Infertile under standard breeding despite being able to cause spawning of eggs (0% fertilization)	Yes	Yes	Oakes et al., 2019	fdx1b is an electron-providing cofactor for steroidogenic cytochrome P450
Zebrafish (*Danio rerio*)	• ENU mutagenesis screen to find mutations that lead to defects in gonadogenesis • 3 mutants focused on (*its, isa, imo*)	• Post meiotic germ cells absent at 3 months age (found aberrant germ cells instead) • Only spermatogonia and primary spermatocytes were present; no spermatids or sperm observed	• Decreased fertilization rates in cells from mutant testes (<2% vs 41.9-65.8 in WT) • Only 1 mutant embryo survived at 1 dpf compared to nearly 100% in WT	Yes	Yes	Saito et al., 2011	ENU= N-ethyl-N-nitrosourea
Zebrafish (*Danio rerio*)	*hsf5* mutants obtained by CRISPR/Cas9 technology targeting exon2	• Loss of spermatozoa along with increase in primary spermatocytes compared to WT • Decrease in sperm count and sperm motility • Altered morphology (microtubule arrangement, flagellar	• No viable offspring when mutants were crossed with any types of females • Lethality of embryos via in vitro fertilization with WT females (before 1 dpf)	Yes	Yes	Saju et al., 2018	Heat shock protein 5

		axoneme, sperm heads)					
Medaka (*Oryzias latipes*)	Mature fish exposed to 32.6, 63.9, 116, 261, and 488 ng ethinylestradiol (EE2)/L for 21 d under flow-through conditions	• Testicular tissue composed of abnormally developed connective tissue, with only a few sper-matozoa and spermatocytes compared to control	• Significant decrease in fecundity observed at 448 ng/L	Yes	Yes	Seki et al., 2002	
Zebrafish (*Danio rerio*)	• *ar* mutant line generated using TALENs	• Upregulation of *amh* and *gsdf* • Downregulation *igf3* • Reduced number of sperm • Reduction in number of germ cells observed in AR mutant fish • Increased proportion of pre-spermatids sperm cells • Small amount of mature spermatozoon still present in mutants	• Reduced in vitro fertilization rate ≤ 20% with WT female	Yes	Yes	Tang et al., 2018	Androgen receptor
Mice	• *mPCI* deficient mice	• Morphologically abnormal sperm (lacked tails and were degenerated) • Reduced motility (12.5%) compared to control (51.5%) • Apoptotic spermatocytes likely due to destruction of Sertoli cells	• Reduced in vivo fertilization rate (0.5%) vs control (94%) with WT females	Yes	Yes	Uhrin et al., 2000	• PCI - present in seminal plasma; inhibitor of activated protein C and a variety of proteases

Zebrafish (*Danio rerio*)	Adult males exposed to 0.5 mg DEHP kg-1 (body weight) for 10 days via intraperitoneal injection	• No effect	• No effect	No	No	Uren-Webster et al., 2010	DEHP is phthalate which is a plasticizer in many mass-produced products
	Adult males exposed to 50 mg DEHP kg-1 for 10 days via intraperitoneal injection	• Significantly lower proportion of spermatozoa and a significantly greater proportion of spermatocytes	• No effect	Yes	No		
	Adult males exposed to 5000 mg DEHP kg-1 for 10 days via intraperitoneal injection	• Significantly lower proportion of spermatozoa and a significantly greater proportion of spermatocytes	• Significant decrease in fertilization success of males, especially during the second 5-day period of exposure	Yes	Yes		
Mice (C57BL/6)	BRD7-deficient mice	• Irregular head shape • Deformed acrosome • Post-meiotic development of elongating spermatids disrupted • Abnormal morphology and degeneration of spermatids • Increased proportion of abnormal spermatids • Downregulation of various spermatogenic markers	• WT female mice coupled with homozygous mutant males did not produce any pups	Yes	Yes	Wang et al., 2016	• BRD7 is a bromodomain gene that inhibits cell growth and cell cycle progression and is a co-factor for p53 • BRD7 has high expression in mice testes
Zebrafish (*Danio rerio*)	*mettl3* mutant fish generated using TALENs	• Significantly increased proportions of spermatogonia (24.4% vs 7.5% in WT) and	• Decreased fertilization rate (48.8.% vs 91.4% in WT)	Yes	Yes	Xia et al., 2018	MEttl3 - multicomponent methyltransferase complex

		spermatocytes (56.1% vs 26.7% in WT) • Significantly decreased proportion of spermatozoa (10.4% vs 50.1% in WT) • Very little or no mature sperm • Sperm motility significantly reduced (average path velocity, curvilinear velocity, and straight-line velocity)	• 8.1% of mutant male x WT female spawned successfully vs 94.4% in WT				
Zebrafish (*Danio rerio*)	CRISPR/Cas9 gene targeting of E2f5	• Reduced number of spermatozoa compared to WT • Increased % of spermatocytes at leptotene and zygotene stages compared to WT • Suggests arrest of spermatogenesis at zygotene stage; later stages rarely observed • Increased germ cell apoptosis	• Decreased fertilization rates (3% vs 94% in WT) under standard breeding conditions	Yes	Yes	Xie et al., 2020	E2f5 is a transcriptional repressor during cell-cycle progression
Marine medaka (*Oryzias melastigma*)	0.1 mg/L of DEHP for 6 months from larval stage	• Contained mostly spermatocytes (Sp) and spermatids (Sd) with few spermatozoa especially in this treatment	• Significant decrease in fecundity compared to control (21.78 vs 29.89 eggs/f/d) • Significant decrease in fertilization success (84.12 vs 94.21%)	Yes	Yes	Ye et al., 2014	DEHP - phthalate MEHP - active metabolite of DEHP; fertilization success defined as proportion of fertilized eggs

	0.5 mg/L of DEHP for 6 months from larval stage	• Contained mostly Sp and Sd with few spermatozoa	• Significant decrease in fecundity compared to control (20.44 vs 29.89 eggs/f/d) • Significant decrease in fertilization success (81.61 vs 94.21%)	Yes	Yes		
	0.1 mg/L of MEHP for 6 months from larval stage	• Contained mostly Sp and Sd with few spermatozoa	• Significant decrease in fertilization success vs control (87.46% vs 94.21%)	Yes	Yes		
	0.5 mg/L of MEHP for 6 months from larval stage	• Contained mostly Sp and Sd with few spermatozoa	• Significant decrease in fertilization success vs control (82.16% vs 94.21%)	Yes	Yes		

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