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REVIEW



Analgesic use in pregnancy and male reproductive development



Pablo *Hurtado-Gonzalez* and Rod T. *Mitchell*

Purpose of review

Male reproductive disorders are common and increasing in incidence in many countries. Environmental factors (including pharmaceuticals) have been implicated in the development of these disorders. This review aims to summarize the emerging epidemiological and experimental evidence for a potential role of in-utero exposure to analgesics in the development of male reproductive disorders.

Recent findings

A number of epidemiological studies have demonstrated an association between in utero exposure to analgesics and the development of cryptorchidism, although these findings are not consistent across all studies. Where present, these associations primarily relate to exposure during the second trimester of pregnancy. In vivo and in vitro experimental studies have demonstrated variable effects of exposure to analgesics on Leydig cell function in the fetal testis of rodents, particularly in terms of testosterone production. These effects frequently involve exposures that are in excess of those to which humans are exposed. Investigation of the effects of analgesics on human fetal testis have also demonstrated effects on Leydig cell function. Variation in species, model system, dosage and timing of exposure is likely to contribute to differences in the findings between studies.

Summary

There is increasing evidence for analgesic effects on the developing testis that have the potential to impair male reproductive function. However, the importance of these findings in relation to human-relevant exposures and the risk of male reproductive disorders remain unclear.

Keywords

analgesics, male reproduction, NSAIDs, paracetamol, testis

INTRODUCTION

Development of the male reproductive system is dependent on normal formation and function of the testis during fetal life. Failure of normal development may result in disorders that manifest in the neonatal period (cryptorchidism and hypospadias), or in adulthood (testicular cancer and poor semen quality) [1,2].

In humans, cryptorchidism occurs in 1–4.6% of newborns, although it will often resolve naturally leading to a prevalence of ~1% at 1 year [3,4]. Cryptorchidism is associated with an increased risk of testicular cancer, the commonest malignancy amongst young men, which is believed to arise from aberrant development of a population germ cells, known as gonocytes, during fetal life [5]. Cryptorchidism is also associated with impaired spermatogenesis resulting in a 30–60% risk of infertility in adulthood [6]. Hypospadias is also a relatively common disorder occurring in approximately 0.2–0.6% of male newborns [7]. The term Testicular

Dysgenesis Syndrome is frequently used to describe the association of these disorders as a result of events that occur during fetal life and their relationship with deficient androgen production or action [8]. Indeed, a critical period from embryonic day (e)15.5–e18.5, known as the “masculinization programming window” (MPW), has been described in fetal rats during which reduction in androgen production or action leads to the subsequent development of cryptorchidism and hypospadias [9]. A similar period of sensitivity has been postulated to

MRC Centre for Reproductive Health, The University of Edinburgh, The Queen's Medical Research Institute, Edinburgh, Scotland, UK

Correspondence to Rod T. Mitchell, MRC Centre for Reproductive Health, The University of Edinburgh, The Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, Scotland, UK. Tel: +44 131 242 9288; fax: +44 131 242 6197; e-mail: rod.mitchell@ed.ac.uk

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Androgens

KEY POINTS

- Analgesics are used by the majority of women during pregnancy
- Use of mild-analgesics during pregnancy has been associated with an increased risk of cryptorchidism in some studies
- Exposure to analgesics results in Leydig cell effects in a number of experimental animal models of fetal testis development
- The importance of human-relevant in-utero exposures to analgesic in relation to male reproductive health remains to be determined

occur in the first trimester in humans based in part on the timing of divergence in anogenital distance (AGD; an indicator of fetal androgen exposure) between males and females [10].

These common male reproductive disorders have increased in incidence over recent decades indicating that in addition to genetic abnormalities, environmental factors such as life style, diet, and chemical (including pharmaceutical) exposures are likely to play a role in their development [1,11]. Recently, there has been an increasing literature on the potential role of in utero exposure to analgesics, including paracetamol and nonsteroidal anti-inflammatory drugs (NSAIDs; e.g., ibuprofen or aspirin), on male reproductive development. This review will describe the emerging epidemiological and experimental evidence in relation to analgesic exposure and its potential effects on male reproductive development.

ANALGESIC USE DURING PREGNANCY

Women are generally advised to avoid taking medications during pregnancy where possible. However, despite this, the majority of women take one or more analgesics such as paracetamol or NSAIDs, at some point during pregnancy [12]. A **Large** Danish study ($n = 46,500$) reported analgesic use in 55% of pregnant women [13], whereas a US study ($n = 10,533$) reported 65% of pregnant women used paracetamol (15% in combination with ibuprofen) [12]. A smaller ($n = 895$) French study reported an even higher frequency of analgesic use (81%) in pregnant women [14]. Furthermore, the overall consumption of analgesics has increased significantly in the majority of European countries during the past 20 years [15]. These analgesics are able to cross the placenta and hence have the potential to cause direct effects on the fetus [16–18]. It is not ethical

to test the effects of analgesics on pregnant women directly and hence the evidence for associations between analgesic use during pregnancy and the development of male reproductive disorders derives from a combination of epidemiological and experimental studies conducted largely over the past two decades.

EPIDEMIOLOGICAL STUDIES RELATING TO ANALGESIC EXPOSURE AND DEVELOPMENT OF MALE REPRODUCTIVE DISORDERS

Association between analgesic use and cryptorchidism

A significant association between the overall use of mild analgesics during pregnancy and cryptorchidism in the offspring has been demonstrated in three studies with adjusted odds ratio (OR) of 1.93 (confidence interval [CI]: 1.03–3.62) [19], 2.12 (CI: 1.17–3.83) [20], and 2.30 (CI: 1.12–4.73) [21]. Another study did not demonstrate a significant association (OR: 1.1; CI: 0.31–3.6) [14] (Table 1). In two of the studies that demonstrated an association this only reached statistical significance for analgesic use during the second trimester with no significant association during the first trimester [20,21]. Interestingly, Kristensen *et al.* describe data on two separate Scandinavian populations. The significant associations were restricted to the Danish cohort, whereas in the Finnish cohort, there were no statistically significant associations [13]. Differences between cohorts may relate to variations in methodology, prevalence of cryptorchidism, or study power. Duration of exposure may also be important with a significant association between prolonged (>2 weeks) use of mild analgesics during pregnancy (includes first and second trimester) and cryptorchidism (OR: 2.47; CI: 1.02–5.96) [21]. Several of these studies have also investigated the effects of specific agents on the development of cryptorchidism.

Paracetamol exposure and risk of cryptorchidism

Three studies investigating associations between paracetamol use during pregnancy and cryptorchidism have described adjusted OR > 1.0; however, these do not reach statistical significance (Table 1) [13,19,20]. In one of these studies, exposure during the second trimester was significantly associated with cryptorchidism (OR: 1.89; CI: 1.01–3.51), similar to their results for mild analgesics overall [20], whilst this was not the case in the other studies [13,21]. Timing of exposure is also likely to be

Table 1. Association between analgesic exposure during pregnancy and male reproductive disorders

	Study period	Cohort	Publication	Gestational period	Analgesics adjusted OR (95% CI)	Paracetamol adjusted OR (95% CI)	Ibuprofen adjusted OR (95% CI)	Aspirin adjusted OR (95% CI)
Cryptorchidism	1996–2002	47,400	Jensen <i>et al.</i> , 2010 [13]	Pregnancy ^b	n/a	1.33 (1.00–1.77)	0.88 (0.64–1.19)	1.18 (0.93–1.49)
				First trimester	n/a	0.94 (0.75–1.17)	n/a	n/a
				Second trimester	n/a	1.17 (0.89 –1.54)	n/a	n/a
	2002–2006	3,184	Snijder <i>et al.</i> , 2012 [20]	Pregnancy	n/a	n/a	n/a	n/a
				First trimester	0.94 (0.36–2.46)	1.38 (0.52–3.64)	n/a	n/a
				Second trimester	2.12 (1.17–3.83)	1.89 (1.01–3.51)	8.93 (1.84–43.24) ^a	
	1997–2001	491	Kristensen <i>et al.</i> , 2011 [21] (Danish cohort)	Pregnancy ^b	1.43 (0.73–2.79)	1.337 (0.70–2.55)	1.82 (0.50–6.61)	2.22 (0.86–5.76)
				First trimester	1.48 (0.66–3.34)	1.61 (0.66–3.90)	n/a	5.60 (1.83–17.1)
				Second trimester	2.30 (1.12–4.73)	1.97 (0.94–4.12)	4.59 (1.10–19.0)	3.76 (1.15–12.3)
	1997–1999	1463	Kristensen <i>et al.</i> , 2011 [21] (Finnish cohort)	Pregnancy ^b	0.74 (0.35–1.57)	n/a	n/a	n/a
First trimester				0.77 (0.26–2.27)	n/a	n/a	n/a	
Second trimester				1.21 (0.53–2.76)	n/a	n/a	n/a	
2003–2006	903	Philippat <i>et al.</i> , 2011 [14]	Pregnancy ^b	1.10 (0.31–3.6)	n/a	n/a	n/a	
1987–1990	6,699	Berkowitz & Lapinski. 1996 [19]	Pregnancy	1.93 (1.03–3.62)	n/a	n/a	n/a	
Hypospadias	2002–2006	3,184	Snijder <i>et al.</i> , 2012 [20]	First trimester	2.05 (0.64–6.58)	2.24 (0.60–8.32)	1.65 (0.21–13.08) ^a	
				Second trimester	0.53 (0.12–2.34)	0.54 (0.12–2.41)	n/a	n/a
	1997–2007	5851	Lind <i>et al.</i> , 2013 [24]	First trimester	n/a	1.00 (0.80–1.10)	1.20 (1.00–1.30)	1.30 (0.90–1.70)
	1982–1989	56,037	Correy <i>et al.</i> , 1991 [25]	First trimester	n/a	n/a	n/a	3.50 (1.40–8.80)
	1959–1965	50,282	Slone <i>et al.</i> , 1976 [23]	First trimester	n/a	n/a	n/a	No association
	1997–2004	14,915	Hernandez <i>et al.</i> , 2012 [22]	First trimester	n/a	n/a	No association	

^aApplies to "other" painkillers including NSAIDs.^bRefers to exposure during first and/or second trimester of pregnancy.

Androgens

important based on the evidence for an MPW in rodents, postulated to occur between 8 and 14 weeks in humans [9]. Analysis of data from 8 to 14 weeks demonstrated a hazard ratio (HR) of 1.14 (0.97–1.34), which was significant for exposure > 4 weeks duration; OR: 1.38 (CI: 1.05–1.83) [13]. A second study also demonstrated a significant association OR: 2.78 (1.13–6.84) following prolonged (>2 weeks) exposure to paracetamol, similar to that described for mild analgesics in general [21]. Again, this association was restricted to the Danish and not the Finnish cohort.

NSAID exposure and risk of cryptorchidism

The association between cryptorchidism and exposure to NSAIDs, for example, ibuprofen and aspirin, has also been investigated (Table 1) [13,20,21]. No significant associations were demonstrated for overall use of ibuprofen or aspirin during pregnancy [13,21]. However, Kristensen *et al.* [21] demonstrated a significant increase in cryptorchidism following exposure to ibuprofen (OR: 4.59; CI: 1.10–19.0) and aspirin (OR: 3.76; CI: 1.15–12.3) during the second trimester. A similar significant association between exposure specifically during the second trimester has also been demonstrated in relation to “other” analgesics (i.e., analgesics excluding paracetamol) [20].

These results may indicate the importance of simultaneous use of more than one analgesic during pregnancy. The use of ≥ 2 agents was associated with a significant increase in the risk of cryptorchidism (OR: 7.72; CI: 2.09–28.6) in one study [21], whilst a second study did not demonstrate a significant association (OR: 1.07; CI: 0.82–1.40) [13].

Association between analgesic exposure and risk of hypospadias

The majority of studies investigating analgesic use during pregnancy and the incidence of hypospadias have not shown significant associations (Table 1) [20,22,23]. A study assessing analgesic exposure from one month prior until 4 months after conception showed a significantly increased risk of hypospadias for ibuprofen (OR: 1.20; CI: 1.00–1.30), whereas no association was reported for paracetamol or aspirin [24]. Another study reported a significant association between the use of aspirin and hypospadias (OR: 3.5; CI: 1.4–8.8) [25].

Analgesic exposure and anogenital distance

Cryptorchidism and hypospadias are associated with a reduction in androgen production or action during fetal life [9]. AGD has been shown to be a

reliable and robust measure of fetal androgen exposure in rodents [13,20,21] and reduced AGD has been associated with cryptorchidism and hypospadias in humans [26,27]. It should be emphasized that measurement of AGD in humans can be technically challenging and it is important that those performing the measurements are sufficiently skilled to avoid inter- and intra-observer bias. A recent study investigated 1027 mother-child pairs, recruited from 10 to 27 weeks of gestation, to determine the association between analgesic exposure in mothers and AGD in the offspring at 3 months of age [28^{***}]. No association was found between exposure to paracetamol or NSAID and AGD; however, exposure to a combination of paracetamol and “other” analgesics (including NSAIDs) was associated with reduced AGD_{AS} (ano-scrotal AGD; 32.3 vs. 36.2 mm; $P=0.03$) but not for AGD_{AP} (ano-penile AGD) which may relate to technical issues in conducting these measurements. In addition, this group included relatively small numbers ($n=20$). Further investigation of the association between AGD and in utero exposure to analgesics are warranted.

Taken together, the epidemiological evidence indicates that there may be an association between in utero exposure to analgesics, particularly during the second trimester, and cryptorchidism. The evidence for such an association with hypospadias is less convincing. There are a number of limitations to these studies relating to obtaining accurate information regarding the dosage, timing, and duration of analgesics exposure. This is particularly important for retrospective studies in which recall bias is a potential limitation [19,20].

EXPERIMENTAL STUDIES RELATING TO ANALGESIC EXPOSURE AND DEVELOPMENT OF MALE REPRODUCTIVE DISORDERS

Effect of analgesics on Leydig cell function in the fetal testis

Testicular descent requires the action of two hormones produced by Leydig cells, namely testosterone and Insulin-like growth factor 3 (InsI3) [29]. Several studies have investigated the effect of analgesic exposure on Leydig cell function in the fetal testis. This includes in vivo, ex-vivo and in vitro model systems of paracetamol (Table 2) and NSAID (Table 3) exposure using rodent and human tissues.

Analgesics and testosterone production

Testosterone production can be measured directly (e.g., serum or intratesticular) or indirectly (e.g.,

Table 2. Leydig cell function following exposure to paracetamol

	Species	Model	Duration (days)	Dose ^a	Age (start of treatment)	Result	Publication
Testosterone	Rat	In vivo	7	350	GD13	↔	Kristensen <i>et al.</i> , 2011 [21]
		In vivo	3	350	e13.5	↓ (~40%)	van den Driesche <i>et al.</i> , 2015 [30 ^{***}]
		In vitro	2	1 μmol/L	e14.5	↓ (~50%)	Kristensen <i>et al.</i> , 2011 [21]
		In vitro	2	1 μmol/L	e14.5	↓ (~25%)	Kristensen <i>et al.</i> , 2012 [31]
	Human	In vitro	1	10 μmol/L	8–12 GW	↔	Mazaud-Guittot <i>et al.</i> , 2013 [34]
		Xenograft	7	60	14–20 GW	↓ (~50%)	van den Driesche <i>et al.</i> , 2015 [30 ^{***}]
AGD	Rat	In vivo	7	150	GD13	↓ (~10%)	Kristensen <i>et al.</i> , 2011 [21]
		In vivo	6	350	GD13	↔	Axelstad <i>et al.</i> , 2015 [33]
		In vivo	3	350	e13.5	↓ (~10%)	van den Driesche <i>et al.</i> , 2015 [30 ^{***}]
	Mouse	In vivo	13	150	GD7	↓ (~15%)	Holm <i>et al.</i> , 2015 [32 [*]]
INSL3	Rat	In vivo	3	350	e13.5	↔	van den Driesche <i>et al.</i> , 2015 [30 ^{***}]
		In vitro	3	100 μmol/L	e14.5	↔	Kristensen <i>et al.</i> , 2012 [31]
	Human	In vitro	3	10 μmol/L	10–12 GW	↓ (~40%)	Mazaud-Guittot <i>et al.</i> , 2013 [34]

For significant effects (↑/↓), the result shown represents the minimum dose and shortest duration showing significance. For nonsignificant effects (↔), the highest dose and longest duration is shown. e, embryonic day; GD, gestational day; GW, gestational weeks.

^aDoses are given by mg/kg/day unless otherwise stated.

AGD). Paracetamol exposure has been linked to a reduction of fetal testicular testosterone production in several studies [21,30^{***},31] (Table 2). In-vivo exposure of fetal rats to paracetamol (350 mg/kg/d) during the MPW significantly decreased AGD by up to 10% in late fetal life [21,30^{***}], whereas another study only demonstrated a significant effect on AGD (15% reduction) at 10 weeks postnatally with no effect at 4, 6, or 8 weeks [32^{*}]. One of these studies

also described a significant reduction (40%) in intra-testicular testosterone and in mRNA expression of two key steroidogenic enzymes (CYP17a1 and CYP11a1) indicating a potential mechanism for the effect on steroidogenesis [30^{***}]. Another study in rats did not demonstrate an effect of exposure during this time-window on AGD at birth, although there was an effect of exposure to a chemical “mixture” that included paracetamol [33]. In vitro

Table 3. Leydig cell function following exposure to NSAIDs

	Drug	Species	Model	Duration (days)	Dose ^a	Age (start of treatment)	Result	Publication
Testosterone	Aspirin	Rat	In vivo	7	200	GD13	↓ (~60%) ^b	Kristensen <i>et al.</i> 2011 [21]
			In vitro	1	10 μmol/L	e14.5	↓ (~70%)	Kristensen <i>et al.</i> 2011,2012 [21,31]
		Human	In vitro	3	100 μmol/L	8–10 GW	↔↑	Mazaud-Guittot, 2013 [34]
			In vitro	3	1 μmol/L	10–12 GW	↔	Mazaud-Guittot <i>et al.</i> 2013 [34]
	Indomethacin	Rat	In vivo	3	0.8	e15.5	↔	Dean <i>et al.</i> 2013 [36]
			In vitro	1	10 μmol/L	e14.5	↓ (~30%)	Kristensen <i>et al.</i> 2012 [31]
		Human	In vitro	2	10 μmol/L	8–12 GW	↑ (~20%)	Mazaud-Guittot <i>et al.</i> 2013 [34]
AGD	Aspirin	Rat	In vivo	7	250	GD13	↔	Kristensen <i>et al.</i> 2011 [21]
			In vivo	3	150	GD11	↓ (~20%)	Gupta and Goldman, 1986 [35]
	Indomethacin		In vivo	3	1	GD11	↓ (~20%)	Gupta and Goldman, 1986 [35]
			In vivo	3	0.8	e15.5	↔	Dean <i>et al.</i> 2013 [36]
Insl3	Aspirin	Human	In vitro	3	10 μmol/L	8–12 GW	↔	Mazaud-Guittot <i>et al.</i> 2013 [34]
	Indomethacin		In vitro	3	10 μmol/L	8–12 GW	↔	Mazaud-Guittot <i>et al.</i> 2013 [34]

For significant effects (↑/↓), the result shown represents the minimum dose and shortest duration showing significance. For nonsignificant effects (↔), the highest dose and longest duration is shown. e, embryonic day; GD, gestational day; GW, gestational weeks.

^aDoses are given by mg/kg/day unless otherwise stated.

^bOnly significant after 3 h incubation (150 mg/kg/d—no significant effect).

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Androgens

studies have also demonstrated analgesic effects during the MPW. Exposure of e14.5 rat fetal testis to 1 $\mu\text{mol/L}$ paracetamol for 48 h resulted in a 15–50% reduction in testosterone with significant reductions described across a range of doses (0.5–10 $\mu\text{mol/L}$) at 72 h, whereas a similar approach using culture of human fetal testis (8–12 weeks gestation) did not demonstrate any effect on testosterone production following exposure to 10 $\mu\text{mol/L}$ for 24, 48, or 72 h [34]. One study has investigated the effect of exposure to a therapeutic regimen (60 mg/kg/d) of paracetamol (60 mg/kg/d) on human fetal testis (14–20 weeks gestation) xenografts. In this study 7 day exposure to paracetamol significantly reduced seminal vesicle (androgen dependent organ) weight (18% reduction) and serum testosterone (45% reduction) in the castrate nude mice hosts compared to vehicle controls, whilst a similar effect did not occur following a shorter (1 day) exposure [30²²]. Differences between the results of the two studies using human fetal testis may relate to the different gestational ages or alternatively may reflect differences in model system.

Similar studies have been performed using NSAIDs. In vivo studies have demonstrated a significant reduction in AGD (~20% reduction) in males exposed to aspirin (150 mg/kg/day) or indomethacin (1 mg/kg/day) from GD11 to 14 [35], whereas no reduction in AGD was found in similar studies involving exposure during the MPW in rats [21,35], although in one of these studies testosterone production was reduced in the case of aspirin [21] but not indomethacin [36]. However, in vitro rat fetal testis studies have demonstrated a reduction in testosterone production following 24 h exposure to aspirin (70% reduction; 10 $\mu\text{mol/L}$) and indomethacin (30% reduction; 10 $\mu\text{mol/L}$) from e14.5 [21,31]. These findings contrast with studies using in vitro culture of human testis [34]. Exposure of human fetal testis (8–12 GW) to indomethacin for 48 h resulted in a significant increase in testosterone (~20%; 10 $\mu\text{mol/L}$). A similar increase was demonstrated for aspirin exposure, although this was restricted to 8–10GW, with no effect at 10–12GW [34]. The reason for the discrepancy between the effect of NSAID exposure on testosterone production in the fetal rat and human testis is unclear and may relate to the model systems or to genuine species differences; however, this clearly illustrates potential limitations of extrapolating effects in rodent model systems directly into the human.

Analgesic exposure and *Ins13* production

INSL3 is responsible for the first phase of testicular descent and mutations in INSL3 gene may lead to

cryptorchidism in mice [37]. Paracetamol exposure (350 mg/kg/d) did not result in a change in *Ins13* mRNA in rat fetal testis following in-utero exposure from e13.5 to e16.5 [30²²]. This was also the case for *Ins13* measured in the media following in vitro culture of rat fetal testis (e14.5) for 72 h [31]. However, in human fetal (8–12GW) testis cultures paracetamol (10 $\mu\text{mol/L}$; 72 h) exposure resulted in a significant reduction in *Ins13* production, whilst no effect was observed following exposure to the same concentrations of aspirin or indomethacin [34].

Overall, the experimental studies suggest that exposure to analgesics can result in effects on Leydig cells in the fetal testis which may have the potential to result in male reproductive disorders. Whilst this conclusion is supported by the results of studies utilizing human fetal testis tissue there remain some important questions relating to the dose and duration of exposure and the degree of hormonal suppression that might be required to induce male reproductive disorders in humans. In addition, the mechanism by which analgesics might affect Leydig cell function requires further elucidation.

Effect of analgesics on prostaglandins

Prostaglandins have been proposed to play a role in mediating the effects of paracetamol exposure on Leydig cell function. Culture of e14.5 fetal rat testis showed a significant decrease in prostaglandin D2 (PGD2) after 24 h exposure to 1 $\mu\text{mol/L}$ paracetamol [21]. However, there were no significant reductions across a range of doses (1–100 $\mu\text{mol/L}$) for 24, 48, and 72 h in a subsequent study by the same authors [31]. For human fetal testis (7–12 weeks) in vitro exposure to paracetamol (10 $\mu\text{mol/L}$) for 72 h did not reduce prostaglandin D2 (PGD2) production, but it did significantly reduce prostaglandin E2 (PGE2) [34].

For NSAIDs, the effect of exposure on prostaglandins appears to depend on the specific agent. A dose dependent reduction in PGD2 after exposure to aspirin was demonstrated in culture of e14.5 fetal rat testis after 48 and 72 h [21]; however, this was not confirmed in a subsequent study, with nonsignificant reductions only occurring at 100 $\mu\text{mol/L}$ [31]. Similarly, for human fetal testis culture (7–12 weeks), no effect on PGD2 was observed following aspirin (10 $\mu\text{mol/L}$) exposure. However, similar to results for paracetamol there was a significant reduction in PGE2 following aspirin exposure [34]. For indomethacin, PGD2 was reduced following in vivo and in vitro exposure of the fetal rat testis during the MPW [21,36,38²²], which again was not demonstrated in human fetal testis cultures

[34]. As with paracetamol and aspirin, exposure to indomethacin did result in a significant reduction in PGE₂, albeit this was restricted to 7–10 weeks of gestation. Taken together, the present data relating to the effect of analgesics on prostaglandins appear to indicate variable effects on prostaglandins dependent, at least in part to the specific agents and the model species.

Effect of analgesics on germ cell development and fertility

Recent studies have begun to focus on the potential for analgesics to affect germ cell development and fertility including inter-generational effects [38^{***},39^{***}]. Dean *et al.* investigated the effects of exposure of pregnant rats to 350 mg/kg/day paracetamol or 0.8 mg/kg/day indomethacin, during a period of gestation that includes the MPW. Pups exposed to indomethacin (male and female) showed ~50% decreased GC number and a decreased gonadal weight at e21.5 [38^{***}]. For females, this resulted in reduced fertility, as indicated by a reduced number of pups per litter, whereas in males no effect on fertility was seen. For paracetamol exposure, there was a similar effect on females with significant reduction in germ cell number, gonadal weight, and pups per litter [38^{***}], with effects on fertility also described for female mice exposed to paracetamol in-utero [39^{***}]. However, for males, despite an overall reduction in germ cell number and gonadal weight at e21.5, there was no significant effect on fertility [38^{***}]. Investigation of the reduced germ cell numbers in males revealed premature loss of gonocytes following exposure to both paracetamol and indomethacin. The loss of this proliferative population of germ cells is likely to result in the reduced germ cell number; however, this is compensated for by early puberty [38^{***}]. Another study involving paracetamol (50 mg/kg/d) exposure in mice (e7–e13.5) showed no effect on male germ cells at e13.5 or on germ cells or testicular weight in adulthood [32^{*}]. The differences between the findings in terms of the gonocyte population in fetal life may relate to differences in species, paracetamol dose, or timing of exposure.

Interestingly, recent studies have demonstrated effects of analgesic exposure on the F2 generation of rats exposed to paracetamol in utero. The F2 females exhibited a significant reduction in ovarian weight and in primordial follicle number at pnd25. Remarkably, this was seen independent of whether the F1 parent was male or female, raising the intriguing possibility that this may be as a result of epigenetic modification of the germline in both sexes [38^{***}].

These recent rodent studies demonstrate that analgesic exposure can affect germ cell development in the fetal testis; however, these findings need to be reproduced in human studies, including epidemiological and experimental approaches.

CONCLUSION

Over the past 5 years, several studies have investigated the potential effect of analgesic exposure to (paracetamol and NSAIDs) on the development of male reproductive disorders. Epidemiological evidence exists for associations between exposures to several analgesics and the development of cryptorchidism. Experimental studies in rodents have also demonstrated effects during fetal life on Leydig cell function (including testosterone production) and fertility. Recent in vitro and ex vivo (xenograft) studies using human fetal testicular tissue have lent support to the concept that analgesic exposure may interfere with Leydig cell function in the fetal testis. However, differences remain between the findings of these studies that are likely to reflect variations in species, model system, dosing schedule, and timing of exposure. Further work is required to determine the potential risk that analgesics may pose to human reproductive health at human-relevant exposures. Whilst the current evidence does not support a definitive answer to this question, avoiding pain or pyrexia is important for fetal health. With this in mind, a pragmatic approach would be to use analgesics only when necessary and for the shortest possible duration.

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Conflicts of interest

None.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Gabrielsen JS, Tanrikut C. Chronic exposures and male fertility: the impacts of environment, diet, and drug use on spermatogenesis. *Andrology* 2016; 4:648–661.
 2. Bonde JP, Flachs EM, Rimborg S, *et al.* The epidemiologic evidence linking prenatal and postnatal exposure to endocrine disrupting chemicals with male reproductive disorders: a systematic review and meta-analysis. *Human Reproduction Update* 2017; 23:104–125.
- Meta-analysis describing effects of prenatal and postnatal exposure to endocrine disruptors on male reproduction

Androgens

3. Radmayr C, Dogan HS, Hoebeke P, *et al.* Management of undescended testes: European Association of Urology/European Society for Paediatric Urology Guidelines. *J Pediatr Urol* 2016; 12:335–343.
 4. Sepúlveda X, Egaña P-JL. Current management of nonpalpable testes: a literature review and clinical results. *Transl Pediatr* 2016; 5:233–239.
 5. Mitchell RT, Camacho-Moll ME, Macdonald J, *et al.* Intratubular germ cell neoplasia of the human testis: heterogeneous protein expression and relation to invasive potential. *Modern Pathology* 2014; 27:1255–1266.
 6. Niedzielski JK, Oszkowska E, Słowikowska-Hilczek J. Undescended testis – current trends and guidelines: a review of the literature. *Arch Med Sci* 2016; 12:667–677.
 7. Springer A, van den Heijkant M, Baumann S. Worldwide prevalence of hypospadias. *J Pediatr Urol* 2016; 12:152.e1–152.e7.
 8. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Human Reproduction* 2001; 16:972–978.
 9. Welsh M, Saunders PTK, Fiskin M, *et al.* Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. *J Clin Invest* 2008; 118:1479–1490.
- First study identifying the MPW in rats, demonstrating a link with cryptorchidism and hypospadias
10. Fowler PA, Bhattacharya S, Flannigan S, *et al.* Maternal cigarette smoking and effects on androgen action in male offspring: unexpected effects on second-trimester anogenital distance. *J Clin Endocrinol Metab* 2011; 96:E1502–E1506.
 11. Skakkebaek NE, Rajpert-De Meyts E, Buck Louis GM, *et al.* Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. *Physiol Rev* 2016; 96:55–97.
- Publication focused on how environmental and genetic factors can affect male reproductive disorders
12. Werler M, Mitchell A, Hernandez-Diaz S, *et al.* Use of over-the-counter medications during pregnancy. *Am J Obstet Gynecol* 2005; 193:771–777.
 13. Jensen MS, Rebordosa C, Thulstrup AM, *et al.* Maternal use of acetaminophen, ibuprofen, and acetylsalicylic acid during pregnancy and risk of cryptorchidism. *Epidemiology* 2010; 21:779–785.
 14. Philippat C, Giorgis-Allemand L, Chevrier C, *et al.* Analgesics during pregnancy and undescended testis. *Epidemiology* 2011; 22:747–749.
 15. Kristensen DM, Mazaud-Guittot S, Gaudriault P, *et al.* Analgesic use – prevalence, biomonitoring and endocrine and reproductive effects. *Nat Rev Endocrinol* 2016; 12:381–393.
- Review article focused on analgesics during pregnancy. It also shows consumption trends across Europe
16. Nitsche JF, Patil AS, Langman LJ, *et al.* Transplacental passage of acetaminophen in term pregnancy. *Am J Perinatol* 2016; 13:1223–1225.
 17. Weigand U, Chou RC, Maulik D, Levy G. Assessment of biotransformation during transfer of propoxyphene and acetaminophen across the isolated perfused human placenta. *Pediatr Pharmacol (New York)* 1984; 4:145–153; 1-1.
 18. Naga Rani MA, Joseph T, Narayanan R. Placental transfer of paracetamol. *J Indian Med Assoc* 1989; 87:182–183.
 19. Berkowitz GS, Lapinski RH. Risk factors for cryptorchidism: a nested case-control study. *Paediatr Perinat Epidemiol* 1996; 10:39–51.
- First epidemiology study able to show a correlation between paracetamol intake during pregnancy and cryptorchidism
20. Snijder CA, Kortenkamp A, Steegers EAP, *et al.* Intrauterine exposure to mild analgesics during pregnancy and the occurrence of cryptorchidism and hypospadias in the offspring: the Generation R Study. *Hum Reprod* 2012; 27:1191–1201.
 21. Kristensen DM, Hass U, Lesne L, *et al.* Intrauterine exposure to mild analgesics is a risk factor for development of male reproductive disorders in human and rat. *Hum Reprod* 2011; 26:235–244.
- Study showing epidemiological and experimental relation between common analgesic exposure during pregnancy and male reproductive disorders. Epidemiological studies show an increase of cryptorchidism and experimental study in rat, demonstrating a decrease in testosterone and prostaglandins.
22. Hernandez RK, Werler MM, Romitti P, *et al.* National Birth Defects Prevention Study: nonsteroidal antiinflammatory drug use among women and the risk of birth defects. *Am J Obstet Gynecol* 2012; 206: 228.e1-8.
 23. Slone D, Siskind V, Heinonen OP, *et al.* Aspirin and congenital malformations. *Lancet* 1976; 1:1373–1375.
- First epidemiology studies of the possible link of aspirin with congenital malformations
24. Lind JN, Tinker SC, Broussard CS, *et al.* National birth defects prevention study: maternal medication and herbal use and risk for hypospadias: data from the National Birth Defects Prevention Study, 1997-2007. *Pharmacoepidemiol Drug Saf* 2013; 22:783–793.
 25. Correy JF, Newman NM, Collins JA, *et al.* Use of prescription drugs in the first trimester and congenital malformations. *Aust N Z J Obstet Gynaecol* 1991; 31:340–344.
 26. Hsieh CL, Fei T, Chen Y, *et al.* Enhancer RNAs participate in androgen receptor-driven looping that selectively enhances gene activation. *Proc Natl Acad Sci U S A* 2014; 111:7319–7324.
 27. Thankamony A, Lek N, Carroll D, *et al.* Anogenital distance and penile length in infants with hypospadias or cryptorchidism: comparison with normative data. *Environ Health Perspect* 2014; 122:207–211.
 28. Lind DV, Main KM, Kyhl HB, *et al.* Maternal use of mild analgesics during pregnancy associated with reduced anogenital distance in sons: a cohort study of 1027 mother-child pairs. *Hum Reprod* 2017; 32: 223–231.
- First publication to show a direct relation between paracetamol intake during pregnancy and human AGD
29. Hughes IA, Acerini CL. Factors controlling testis descent. *Eur J Endocrinol* 2008; 159 (Suppl 1):S75–82.
- Review explaining the different factors controlling testis descent, including testosterone and INSL3
30. van den Driesche S, Macdonald J, Anderson RA, *et al.* Prolonged exposure to acetaminophen reduces testosterone production by the human fetal testis in a xenograft model. *Sci Transl Med* 2015; 7:288ra80–ra1288.
- First study using the human fetal testis xenograft model to investigate effects of analgesic exposure. The study demonstrates a reduction in testosterone following exposure to paracetamol. This study also demonstrates a decrease of testosterone and AGI in-vivo in rat fetal testis
31. Kristensen DM, Lesne L, Le Fol V, *et al.* Paracetamol (acetaminophen), aspirin (acetylsalicylic acid) and indomethacin are antiandrogenic in the rat foetal testis. *Int J Androl* 2012; 35:377–384.
 32. Holm JB, Chalmey C, Modick H, *et al.* Aniline is rapidly converted into paracetamol impairing male reproductive development. *Toxicol Sci* 2015; 148:288–298.
- Article showing a common industrial component, aniline, can be metabolised to paracetamol in humans. They also study how paracetamol can affect male reproductive development
33. Axelstad M, Christiansen S, Boberg J, *et al.* Mixtures of endocrine-disrupting contaminants induce adverse developmental effects in preweaning rats. *Reproduction* 2014; 147:489–501.
 34. Mazaud-Guittot S, Nicolas Nicolaz C, Desdoits-Lethimonier C, *et al.* Paracetamol, aspirin, and indomethacin induce endocrine disturbances in the human fetal testis capable of interfering with testicular descent. *J Clin Endocrinol Metab* 2013; 98:E1757–E1767.
- First paper to use human fetal testis in-vitro to determine effects of exposure to common analgesics. Also, first paper to show a possible mechanism of action of paracetamol exposure during pregnancy.
35. Gupta C, Goldman AS. The arachidonic acid cascade is involved in the masculinizing action of testosterone on embryonic external genitalia in mice. *Proc Natl Acad Sci U S A* 1986; 83:4346–4349.
 36. Dean A, Mungall W, McKinnell C, Sharpe RM. Prostaglandins, masculinization and its disorders: effects of fetal exposure of the rat to the cyclooxygenase inhibitor indomethacin. *PLoS One* 2013; 8:e62556.
 37. Nef S, Parada LF. Cryptorchidism in mice mutant for *Insl3*. *Nat Genet* 1999; 22:295–299.
 38. Dean A, van den Driesche S, Wang Y, *et al.* Analgesic exposure in pregnant rats affects fetal germ cell development with inter-generational reproductive consequences. *Sci Rep* 2016; 6:19789.
- First article showing an effect of mild analgesics on fetal germ cells in testis and ovary. Using a rat model, they showed a decrease in germ cell number and effects on ovary/testis weight. Also, first article suggesting possible inter-generational consequences of analgesic exposure during pregnancy.
39. Holm JB, Mazaud-Guittot S, Danneskiold-Samsøe NB, *et al.* Intrauterine exposure to paracetamol and aniline impairs female reproductive development by reducing follicle reserves and fertility. *Toxicol Sci* 2016; 1:178–189.
- Article using an experimental in-vivo approach to show the effect of paracetamol on follicle reserve in the ovary

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