Review Article

Impaired reproduction after exposure to ADHD drugs: Systematic review of animal studies

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Abstract.

BACKGROUND: Few studies have reported on long-term harms caused by ADHD drugs but they are known to impair growth.

OBJECTIVE: To assess whether ADHD drugs impair reproduction in mammals.

METHODS: Systematic review of reproduction in studies of animals treated with ADHD drugs.

DATA SOURCES: PubMed, Biosis and EMBASE.

RESULTS: We included 17 studies. The studies were generally of poor quality or poorly reported. Two studies reported the use of one of three advised randomisation methods. Fifteen studies used placebo which suggested blinding. On clonidine, the ability to produce offspring was reduced for male rats, which approached two females each. In one study, 10 treated rats produced no offspring while all four controls did. In another study, 10 treated rats impregnated nine females while 10 controls impregnated 16. On methylphenidate, vaginal opening was delayed in two studies (in one, the mean difference was 4.0 days, 95% CI 2.5 to 5.6, and number of estrous cycles was halved; in the other, the minimum delay was 6 days), while in two other studies no difference occurred. Generally, the impairments improved after a drug-free period and were less pronounced when treatment started later in life.

CONCLUSION: ADHD drugs impair the reproduction in animals.

Keywords: Animals, ADHD, reproduction, methylphenidate, clonidine, fertility

1. Background

Little is known about long-term outcomes of ADHD drugs but they do not appear to be positive. The largest study, the Multimodal Treatment Study (MTA), randomised 579 children to methylphenidate, behavioural therapy, both, or routine community care [1, 2]. There were no clinically important effects of methylphenidate after 14 months, or after three and eight years of follow-up [3–5]. Similarly, a large cohort study that investigated overall functioning at six years of follow-up found that psychostimulants did not lower ADHD symptom severity [6].

The randomised trials included in a recent Cochrane review underreported the harms of methylphenidate in children [7]. Only 5% of the included trials reported on serious adverse events,

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while 14% reported non-serious adverse events; these were more common with methylphenidate than with placebo (risk ratio 1.29; 95% confidence interval 1.10 to 1.51).

Methylphenidate is a norepinephrine-dopamine reuptake inhibitor that acts predominantly through the dopamine system. There are concerns that treating children with stimulants may cause irreversible brain damage [8], as well as damages to the neuroendocrine system during development [9], because dopamine acts both as a neurotransmitter and a regulator of hormonal pathways [10].

Exposure to ADHD drugs stunts growth [11] and may possibly also impair the development of the reproductive system. A case has been described where a boy before being put on drugs experienced sexual hyperactivity and was diagnosed with precocious puberty [12]. He was treated off-label with methylphenidate from age five, which caused a decrease in both aggression and hypersexuality. Another case report is about a boy treated with methylphenidate for 17 years since childhood who experienced delayed puberty; at age 20 he had underdeveloped genitalia, lack of libido and also erectile dysfunction [13]. A study that investigated pubertal development in 65 boys on stimulant medication for at least three years found a slower rate of physical development of the genitals during puberty compared to 174 controls [14]. In contrast, some studies and case reports have shown increased sexual activity and priapism with methylphenidate [15, 16].

When methylphenidate was administered to young rats, corresponding in age to prepubertal children, it resulted in altered levels of dopamine transporters that persisted into adulthood, long after termination of treatment [9]. Another study in rats found that methylphenidate impaired spermatogenesis after eleven weeks of treatment, which indicated that the drug might reduce male fertility [17].

A large report from 2005 reviewed the reproductive and developmental effects of methylphenidate [18]. There were no data on humans and for animals, the working group felt it did not have sufficient data to give advice, although they mentioned two studies with rodents demonstrating reduced sperm motility and altered estrous cycling, respectively, and one study reporting no differences.

We decided to do a systematic review of animal studies to assess the impact of ADHD drugs on the development of the reproductive system.

2. Aim

To assess whether drugs approved for ADHD cause abnormal development of the reproductive system in animals.

3. Methods

3.1. Search strategy

Searches were performed in PubMed, Biosis and Embase, using MeSH terms or equivalents, for the eight most sold ADHD drugs in 2012 as reported by the US Food and Drug Administration: atomoxetine, clonidine, dexmethylphenidate, dextroamphetamine, guanfacine, lisdexamphetamine, methylphenidate and amphetamine [19]. The search strategies are available in Appendix A. We also checked the references in a previous review for eligible studies [18].

We contacted the authors when data were unclear or not reported for papers published in 2000 or later.

3.2. Eligibility criteria and data extraction

We included controlled studies of ADHD drugs given postnatally to healthy mammals, which were not primed by drugs or had been operated upon, if they had data on one or more of the following

outcomes: morphology, motility of spermatozoa, ejaculation, cell counts, cycles, pregnancies and births (impregnations, resorptions, number of stillborns, number of liveborns with malformations).

We did not assess the reproductive organs of offspring from dams treated during pregnancy.

One observer screened all records and a second observer screened all included records and a random sample of 15 excluded records (no disagreements). Potentially eligible articles were collected and read in full. References of eligible articles were scanned to locate further studies. Data were recorded in Excel. One investigator carried out data extraction and a second investigator checked the extracted data. Disagreements were resolved by consensus.

We extracted data according to published guidelines [20–23]: animals (species, strain, age, sex), study design (randomisation, blinding, drug intervention, group sizes, dose, route of administration, length of treatment and of follow-up), selective reporting within studies and relevant outcome measures.

3.3. Quantitative data synthesis

Meta-analyses were performed with Review Manager using data from the end of the intervention and, if available, the last time point after a drug free follow-up period. If the test had been carried out on several consecutive days, we only used the results from the first day.

If more than one drug had been tested in the same trial, we split the control groups accordingly to avoid double counting. For studies with several drug doses, dose groups were pooled [24]. Data from animals kept group-housed or isolated were pooled. Missing standard deviations were calculated or estimated by standard methods [24], e.g. imputation from SEs or *p*-values.

Risk of bias was assessed according to SYRCLE [20, 24]. For animal studies, the SYRCLE risk of bias tool recommends assessment of three categories of randomisation: randomisation of animals to intervention or control groups; random housing to prevent behavioural differences between groups introduced through differences in light intensity and temperature; and random outcome assessments to prevent influence on results from diurnal variation [20].

3.4. Statistical methods

We used a random effects model. For binary data, risk ratios were reported. For meta-analyses of continuous data and ranking scale data, the mean difference or the standardised mean difference were calculated.

The basic assumption was that the healthy control animals showed normal development and behaviour. As there were differences in the direction of the scales and outcomes, we multiplied some of the outcome values by –1 to ensure that values to the right in the graphs always meant an effect in favour of placebo or no treatment.

Proportions were compared with Fisher's exact test.

3.5. Amendment to the protocol

As our data extraction resulted in a large amount of outcomes, we decided to focus on the outcomes directly related to the ability to reproduce (motility of spermatozoa, ejaculations, cycles, pregnancies and labour) and not the indirect or surrogate ones (cell counts, morphology).

We had planned sensitivity analyses but there was too little data to allow these.

Our protocol is in Appendix B.

4. Results

We identified 518 unique records and excluded 461 based on title or abstract for one or more of these reasons: not an ADHD drug, primed animals, irrelevant outcomes, *in vitro* studies or otherwise not an animal study, or polytherapeutic interventions. We also excluded a study in Japanese [25]. Full text screening of the remaining 57 articles led to inclusion of 17 studies (see flow chart in Appendix C).

4.1. Study characteristics

All 17 included studies were controlled and they were published between 1975 and 2016 (Table 1). Thirteen studies were conducted in rats [17, 26–37]; two in mice [38, 39]; two in dogs [40, 41]; and one rat study also included rabbits [36]. Median number of animals per study was 30 for the intervention groups (range 4–100) and 17 for the control groups (range 4–41) for the 16 studies where information was given.

Methylphenidate was used in nine studies, clonidine in seven and amphetamine in one. The median intervention period was 28 days (range 1–90) and there were follow-up data for eight studies (median 18 days, range 1–63 days) [17, 27, 31, 33, 34, 36, 37, 41].

4.2. Risk of bias

The studies were generally of poor quality or poorly reported. Two studies did not use a placebo [17, 26]. Fifteen studies were placebo-controlled, which suggests that they were also blinded. Only one study [32], however, described in detail the blinding of the observers. According to the SYRCLE guidelines [21], we considered blinding of caregivers and personnel as unclear or at high risk of bias either because blinding was not mentioned or because the control groups did not receive placebo.

Randomisation to either intervention or control was mentioned in two trials [32, 41], but the method was not described. Randomisation of housing and outcome assessments was not described in any of the reports.

Three studies reported the outcomes selectively [26–28] whereas the remaining studies reported all described outcomes. Five studies reported on mortality or adverse events of the drugs [17, 26, 34–36].

Nine reports mentioned that funding was provided by public institutions [17, 28–33, 37, 39] and the authors of seven studies declared that they had no conflicts of interest.

In total, there were 12 studies eligible for meta-analysis; for the five remaining studies there were missing data we could not obtain [26, 28, 34, 40] or the data were not independent [32].

4.3. Amphetamine

One study tested amphetamine on 95 mice compared to 24 controls; there were no differences between the groups for number of ejaculations and ejaculation latency [39], see Fig. 1.

4.4. Clonidine

In one study [34], ten male clonidine-treated rats were tested for their ability to impregnate two female rats each, compared to four untreated rats. All control rats impregnated two dams each, but the treated rats failed to impregnate any of the female rats ($P = 0.001$ for difference between 4 of 4 and 0 of 10, our calculation). The rats were either unable to ejaculate (no sperm present in the vagina); or they were sterile (no embryos present in womb at day 12 after the encounters); or they did not approach

Table 1
Overview of included studies. PND – postnatal day, GD – gestational day

| ID: author year	Species, strain	Gender	First day of treatment	Treatment period, days	Post-treatment period, days	>one drug/dose compared to control	Total sample size	N (drug)	N (control)	Drug
Angelova 1975	Rats, Wistar	F	Adult	30	NA	Y	150	NA	NA	clonidine
Beckman 2008	Rats, Wistar Hannover	F	PND7	63	0, 35	Y	40	30	10	methylphenidate
Chatterjee-Chakrabarty 2005	Rats, Sprague-Dawley	F	PND28	28	0	N	20	10	10	methylphenidate
Clark 1991	Rats, Long-Evans	M	PND90	1	0	N	85	40	41	clonidine
da Silva Junior 2014	Rats, Wistar	M	PND90	7	0	N	34	17	17	clonidine
de Catanzaro 1996	Mice, C57BL/6J	M	PND70	1	0	Y	120	96	24	amphetamine
Fazelipour 2012	Mice, Balb/C	M	PND56	40	0	Y	27	6	3	methylphenidate
Greeley & Kizer 1980	Rats, Sprague-Dawley	F	PND5	21, 30	0, 18, 30	Y	66	35	31	methylphenidate
Kianifard 2013	Rats, Wistar	M	Adult	14, 77	0, 63	Y	31	23	8	methylphenidate
Lin 2015	Rat, Sprague-Dawley	M	PND90	90	0	N	20	10	10	clonidine
Montagnini 2014	Rat, Wistar	M	PND21	40	40	Y	78	52	26	methylphenidate
Montagnini 2016	Rat, Wistar	F	PND21	40	15	Y	70	42	28	methylphenidate
Ratnasooriya 1987	Rat, NA	M	Adult	7	0	Y	14	10	4	clonidine
Teo 2002	Rat, Sprague-Dawley	F	GD7	36	0	Y	125	100	25	methylphenidate
Teo 2003	Rat, Sprague-Dawley	F	GD7	11	1	Y	125	100	25	methylphenidate
Teo 2003	Rabbit, NA	F	GD6	13	1	Y	100	80	20	methylphenidate
Yonezawa 1986	Dog, Mongrel	M	Adult	1	0	N	8	4	4	clonidine
Yonezawa 1992	Dog, Mongrel	M	NA	28	0, 14	N	10	5	5	clonidine
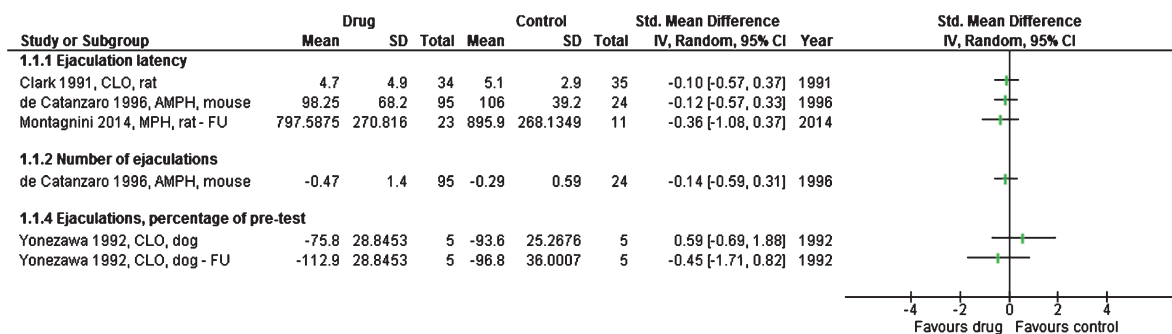
Fig. 1. Studies or subgroups – as listed by first author, publication year, short for the drug used, species, and FU if feasible – showing ejaculations as standardised mean difference and 95% confidence intervals. Total is number of animals per group. FU – follow-up, CLO – clonidine, AMPH – amphetamine, MPH – methylphenidate.
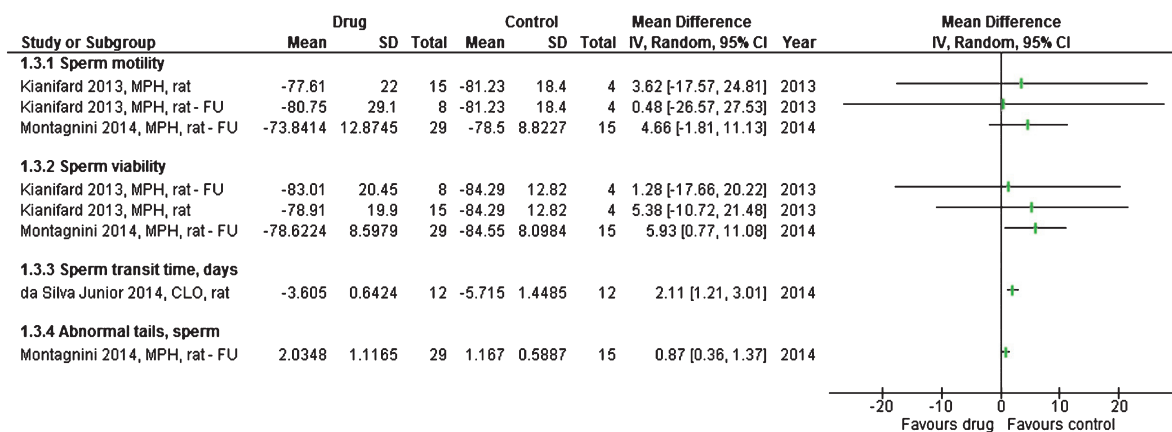

Fig. 2. Studies or subgroups – as listed by first author, publication year, short for the drug used, species, and FU if feasible – showing data for spermatozoa as mean difference with 95% confidence intervals. Total is number of animals per group. FU – follow-up, CLO – clonidine, MPH – methylphenidate.

the females (four cases). After a drug-free period of seven days, the treated male rats approached one female again; seven of 10 animals were able to impregnate one female rat each compared to all four control rats ($P = 0.51$, our calculation).

Another study found a similar effect [32]. Ten male rats treated with clonidine impregnated nine of 20 females, and ten control rats impregnated 16 of 20 females ($P = 0.05$ when disregarding that the observations were not independent, as each male rat approached two females).

For one study with four dogs in each group, the ejaculation score (from 0 to 3), elicited by manual stimulation by the experimenter one hour after a clonidine injection, was lower for treated dogs than for controls [40]. All control dogs scored the maximum of 3 (ejaculation happened), while the mean score for treated dogs was only 0.65 (SD 0.78) ($P = 0.004$, sign test of 0 vs 8, our calculation), see Fig. 1.

Sperm transit time through the epididymis, which is the time it takes the sperm to become mature, was significantly shorter in clonidine-treated rats than in controls (mean difference 2.1 days; 1.2 to 3.0; 6 treated vs 6 controls) [30], see Fig. 2.

We could not extract any meaningful data from another clonidine study because the number of rats was not mentioned in the results section [26]. Half of 40 female rats treated with clonidine before and during pregnancy were sterile but the results for the control group were not mentioned [26].

4.5. Methylphenidate

A rat study found that vaginal patency was delayed when treatment started five days after birth (mean difference 4.0 days; 2.5 to 5.6; 13 treated vs 11 controls) [31]. When treatment started 21 days after birth, there was an insignificant delay (mean difference 3.1; –1.6 to 7.8; 9 treated animals vs 9 controls). Another study with ten rats in each group found vaginal opening to be delayed for a minimum of six days; vaginal opening remained poor throughout the entire 30-day study for six of the treated rats [28]. In contrast, two other studies, with 42 vs 28 [37] and 30 vs 10 rats [27], found no difference between groups for age at vaginal opening.

Female rats showed abnormal estrous cycling (counted as number of days per cycle) when the treatment started at postnatal day 21; the number of cycles was halved for treated rats (mean difference 3.1 days; 2.4 to 3.8; 9 treated rats vs 9 controls) [31]. After a 30-day drug-free period, estrous cycling did not normalise for rats starting treatment early, at postnatal day 5 (mean difference 2.9; 1.7 to 4.2; 13 treated vs 11 controls), but it did normalise for rats that started treatment at postnatal day 21 (9 rats per group).

Another study, in which treatment started at postnatal day 21, found no differences in estrous cycling patterns (42 vs 28 rats) after 40 days of treatment and a 15-day drug-free follow-up [37], whereas a study, in which treatment started at postnatal day 28 and continued for four weeks, with 10 rats per group, reported that all the treated rats showed impaired estrous cycling [28].

Fertility rates were 42% for six methylphenidate-treated mice approaching 3 female mice each and 95% for three untreated animals (counted as number of embryos per corpora lutea) [38]. On average, the gestation period was significantly longer in 100 treated rats compared to 24 control rats, but the difference was only half a day [35]. The results are shown in Figs. 3 and 4.

For the pregnancies that led to successful deliveries, no differences were seen in number of stillborns, offspring or exterior malformations [27, 35, 36], see Fig. 5.

Abnormal tail development occurred in 2.0% of sperms in 29 methylphenidate-treated rats and in 1.2% of 15 controls (mean difference 0.9%; 0.4 to 1.4) [33]. Forty days after end of treatment, 79% vs 85% of the sperms were viable (mean difference 6%; 1 to 11), see Fig. 2. There was no difference in number of ejaculations [33], see Fig. 1.

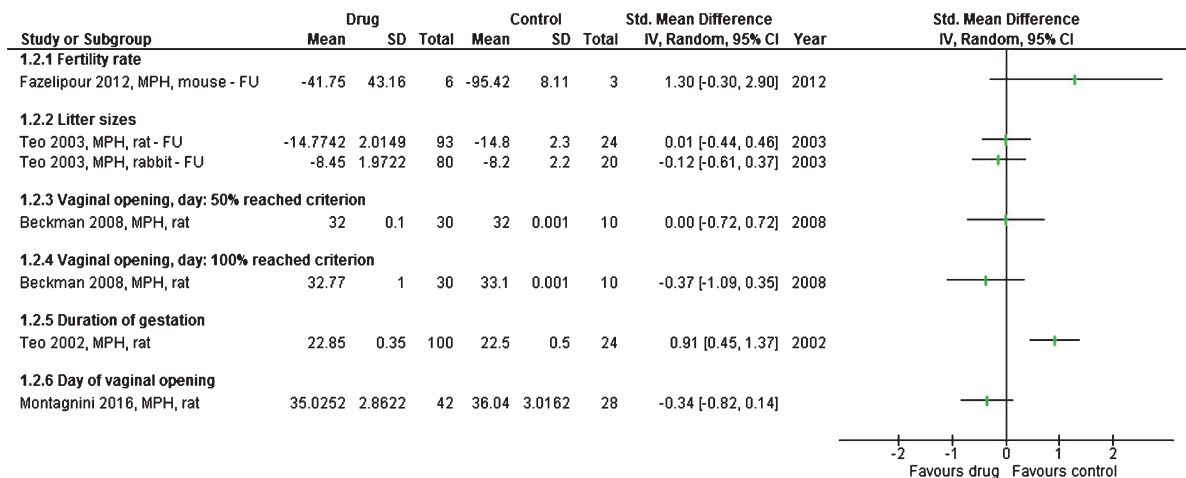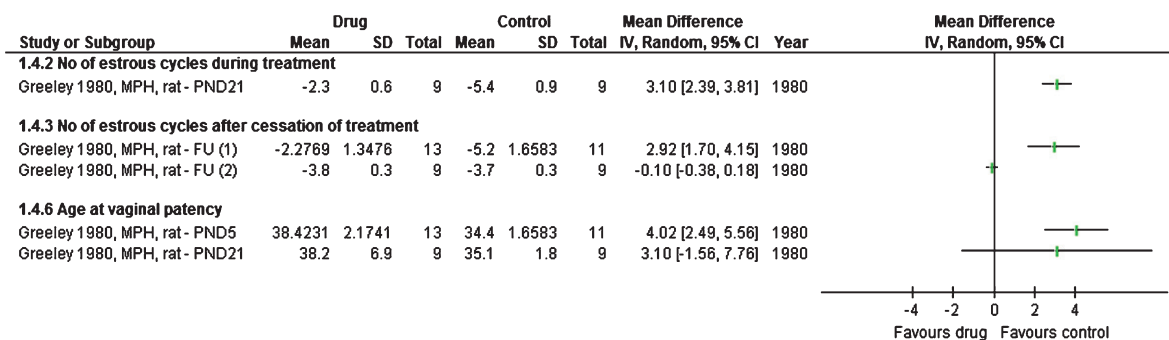| Study or Subgroup	Drug Mean	SD	Total	Control Mean	SD	Total	Std. Mean Difference IV, Random, 95% CI	Year
1.2.1 Fertility rate								
Fazelipour 2012, MPH, mouse - FU	-41.75	43.16	6	-95.42	8.11	3	1.30 [-0.30, 2.90]	2012
1.2.2 Litter sizes								
Teo 2003, MPH, rat - FU	-14.7742	2.0149	93	-14.8	2.3	24	0.01 [-0.44, 0.46]	2003
Teo 2003, MPH, rabbit - FU	-8.45	1.9722	80	-8.2	2.2	20	-0.12 [-0.61, 0.37]	2003
1.2.3 Vaginal opening, day: 50% reached criterion								
Beckman 2008, MPH, rat	32	0.1	30	32	0.001	10	0.00 [-0.72, 0.72]	2008
1.2.4 Vaginal opening, day: 100% reached criterion								
Beckman 2008, MPH, rat	32.77	1	30	33.1	0.001	10	-0.37 [-1.09, 0.35]	2008
1.2.5 Duration of gestation								
Teo 2002, MPH, rat	22.85	0.35	100	22.5	0.5	24	0.91 [0.45, 1.37]	2002
1.2.6 Day of vaginal opening								
Montagnini 2016, MPH, rat	35.0252	2.8622	42	36.04	3.0162	28	-0.34 [-0.82, 0.14]	

Fig. 3. Studies or subgroups – as listed by first author, publication year, short for the drug used, species, and FU if feasible – showing pregnancy and labour outcomes as standardised mean difference with 95% confidence intervals. Total is number of animals per group. FU – follow-up, MPH – methylphenidate.

Study or Subgroup	Drug Mean	SD	Total	Control Mean	SD	Total	Mean Difference IV, Random, 95% CI	Year
1.4.2 No of estrous cycles during treatment								
Greeley 1980, MPH, rat - PND21	-2.3	0.6	9	-5.4	0.9	9	3.10 [2.39, 3.81]	1980
1.4.3 No of estrous cycles after cessation of treatment								
Greeley 1980, MPH, rat - FU (1)	-2.2769	1.3476	13	-5.2	1.6583	11	2.92 [1.70, 4.15]	1980
Greeley 1980, MPH, rat - FU (2)	-3.8	0.3	9	-3.7	0.3	9	-0.10 [-0.38, 0.18]	1980
1.4.6 Age at vaginal patency								
Greeley 1980, MPH, rat - PND5	38.4231	2.1741	13	34.4	1.6583	11	4.02 [2.49, 5.56]	1980
Greeley 1980, MPH, rat - PND21	38.2	6.9	9	35.1	1.8	9	3.10 [-1.56, 7.76]	1980

Footnotes
(1) PND5
(2) PND21

Fig. 4. Studies or subgroups – as listed by first author, publication year, short for the drug used, species, and FU if feasible – showing reproductive ability as mean difference with 95% confidence intervals. Total is number of animals per group. FU – follow-up, MPH – methylphenidate.

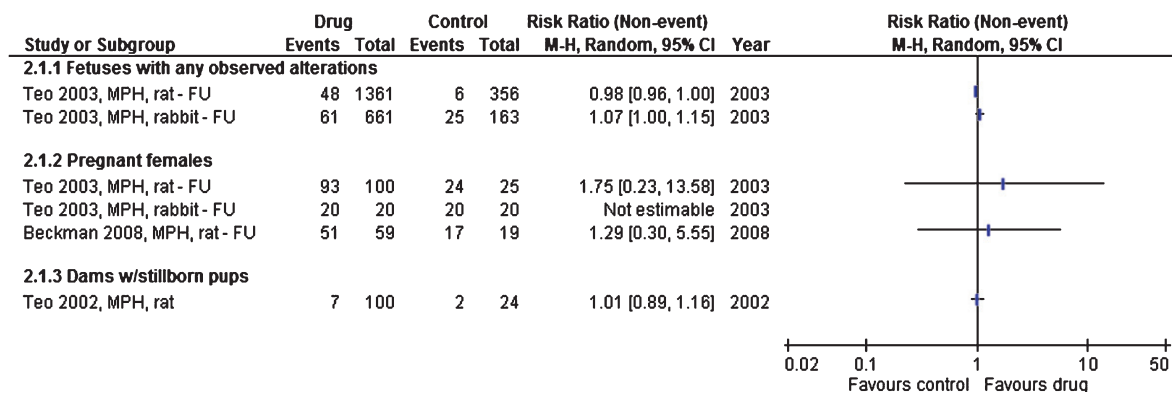| Study or Subgroup	Drug Events	Total	Control Events	Total	Risk Ratio (Non-event) M-H, Random, 95% CI	Year
2.1.1 Fetuses with any observed alterations						
Teo 2003, MPH, rat - FU	48	1361	6	356	0.98 [0.96, 1.00]	2003
Teo 2003, MPH, rabbit - FU	61	661	25	163	1.07 [1.00, 1.15]	2003
2.1.2 Pregnant females						
Teo 2003, MPH, rat - FU	93	100	24	25	1.75 [0.23, 13.58]	2003
Teo 2003, MPH, rabbit - FU	20	20	20	20	Not estimable	2003
Beckman 2008, MPH, rat - FU	51	59	17	19	1.29 [0.30, 5.55]	2008
2.1.3 Dams w/stillborn pups						
Teo 2002, MPH, rat	7	100	2	24	1.01 [0.89, 1.16]	2002

Fig. 5. Studies or subgroups – as listed by first author, publication year, short for the drug used, species, and FU if feasible – showing binary outcomes as the risk ratio with 95% confidence intervals. Total is number of animals per group. FU – follow-up, MPH – methylphenidate.

In a study of 15 vs 8 rats receiving methylphenidate or placebo for 11 weeks, there were only trivial differences between motility and viability of the sperms [17], see Fig. 2.

5. Discussion

We found that clonidine and methylphenidate impaired reproduction in animals. These harms might be reversible if the treatment is not started early in life. We did not find an effect on number of stillborns, number of offspring or visible malformations. However, one of our included studies assessed the newborns' skeletons and found that the pelvis, pubis and sternum were incompletely ossified compared to controls [36].

5.1. Strengths and limitations

It was a strength that nine of the 17 studies were publicly funded and that we only included studies where the animals had not been primed in any way, as priming might make the results difficult to interpret.

Fifteen of the 17 studies were placebo-controlled. This usually implies blinding of the observers, but this information was only given in detail for one study. No studies mentioned whether the caregivers and other personnel involved in the study were blinded, but in animal studies, the caregiver and the observer are sometimes the same person. This may mean that use of a placebo has not necessarily led to blinding since there could be differences in, for example, the colour of the food.

No studies adequately described that the animals were randomised in the three ways advised by SYRCLE [20]. For our study, the possible lack of random outcome assessments may have had little influence, as few of our outcomes were subjective or addressed behavior, which could vary over the day. The possible lack of random housing could have influenced the neuroendocrine system and thereby the results.

Eleven studies were published before the introduction of the ARRIVE guidelines in 2010 [42]. The guidelines have not yet been sufficiently implemented, as researchers, journal editors and ethical committees do not focus enough on the importance of blinding and randomisation for minimizing bias [43]. Animal research is often the basis for studies in humans and they therefore need to be performed with the same rigour as randomised trials in patients in order to reduce research waste because of false leads.

The studies were rather small and highly heterogeneous, using different species, strains, age at start of intervention, treatment periods, route of administration, test settings and outcome measures. In the two studies of methylphenidate that did not find differences between treated groups for age at vaginal opening, the drug was given as oral gavage [27, 37], whereas it was administered subcutaneously in the two studies that did find a difference [28, 31]. The significant findings might be due to the animals receiving a higher dose. Delayed vaginal opening is similar to a late entering into puberty in humans [44].

It can be discussed to which extent the studies reflected clinical use of ADHD drugs [45]. In some studies, the treatment was started shortly after birth, which does not happen in humans. On the other hand, the length of treatment was short compared to the clinical setting. The results we found might therefore have both over- and underestimated the problems with these drugs.

5.2. Comparison with data from humans

Data on reproductive parameters in humans when treated with ADHD drugs is sparse [12–16] and we have not found any long-term data that could elucidate whether ADHD drugs irreversibly impair the reproductive system. Children are being treated with ADHD drugs at an ever earlier age and sometimes continue with the drugs for decades although these long-term treatments are based on short-term studies. In a large Cochrane review of methylphenidate in children, the median length of treatment for parallel group trials was only 49 days [7]. This review found that all trials were of high risk of bias [7], and only 5% of trials reported on serious adverse events while 14% reported on non-serious adverse events. In addition to harms being seriously underreported, there is a lack of long-term trials with a drug-free follow-up in both animal and human research.

It is very worrying that the large Multimodal Treatment Study (MTA) in children after 16 years of follow-up reported that people who continued to take methylphenidate lost 5 cm in height compared to those who only took a negligible amount of drug after the 14-months randomised phase was over [46].

Clonidine is an antiadrenergic agent approved for treatment of hypertension in 1974 [47]. It is well known that hypotensive agents can cause sexual dysfunction [48, 49]; to prescribe clonidine to children and adolescents may have a similar negative impact.

5.3. Implications

We consider it likely that the harms to the reproductive system seen in animals can also occur in humans. This possibility should be studied in observational studies and also in the large MTA study.

Acknowledgments

Conflict of interest

None to report.

Funding

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Appendix A: Searches

Searches were performed in PubMed, Biosis and Embase, using MeSH terms or the equivalent, for drugs approved for ADHD symptoms (the eight most sold ADHD drugs as reported by the Food and Drug Administration for 2012 [19] (atomoxetine, clonidine, dexmethylphenidate, dextroamphetamine, guanfacine, lisdexamphetamine, methylphenidate and amphetamine)). We also went through the NTP-CERHR report for eligible studies [18].

PubMed

Search **(("Reproductive Physiological Processes"[Mesh] OR "Reproduction"[Mesh]) AND (lisdexamfetamine OR "lisdexamfetamine dimesylate"[Supplementary Concept] OR "Methylphenidate"[Mesh] OR "atomoxetine"[Supplementary Concept] OR "Guanfacine"[Mesh] OR "Clonidine"[Mesh]))** Filters: **Other Animals**

Result 140316 : 173

Embase

1	(dexamphetamine or atomoxetine or clonidine or guanfacine or methylphenidate or lisdexamphetamine).mp. [mp = title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	69304
2	reproduct*.mp. [mp = title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	282123
3	("non-human primate" or "nonhuman primate" or baboon or macaque or chimpanzee or orangutan or "rhesus monkey" or "rhesus macaque" or marmoset or "cynomolgus monkey" or gibbon or grivet or "squirrel monkey" or cat or dog or rodent or rat or mouse or rabbit or guinea pig or hamster or gerbil).mp. [mp = title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	4087030
4	1 and 2 and 3	62

Search result 29 March 2016: 62 records

BIOSIS:

(from BIOSIS Previews)

You searched for: ((**TOPIC:** ((((((((((((((((((("non-human primate" OR "nonhuman primate") OR baboon) OR macaque) OR chimpanzee) OR orangutan) OR "rhesus monkey") OR "rhesus macaque") OR marmoset) OR "cynomolgus monkey") OR gibbon) OR grivet) OR "squirrel monkey") OR cat) OR dog) OR rodent) OR rat) OR mouse) OR rabbit) OR guinea pig) OR hamster) OR gerbil) *AND* **TOPIC:** (reproduct*)) *AND* **TITLE:** (((((((((dexamphetamine OR atomoxetine) OR clonidine) OR guanfacine) OR methylphenidate) OR lisdexamfetamine) OR dexmethylphenidate) OR dextroamphetamine) OR amphetamine))

Timespan: All years. **Indexes:** BIOSIS Previews.

Results 29 March 2016: 335 records

Appendix B: Protocol

Background

The MTA study was the first and largest controlled trial aiming at assessing long-term outcomes from use of ADHD drugs (1). However, the trial was not placebo-controlled or double-blinded (2). There were no meaningful differences in outcomes after 14 months, three and eight years between methylphenidate and patients receiving behavioural or community alternatives (3)(4)(5). Another prospective study investigated overall functioning at six years follow-up and found that psychostimulants had no beneficial impact on ADHD symptom severity (6). In contrast, sustained effects were seen with behavioural therapy (7).

Exposure to ADHD drugs can impair sexual function (8) and may possibly also impair the development of the reproductive system. A case has been described where a boy experienced precocious puberty with sexual hyperactivity and was diagnosed with familial male precocious puberty (9). He was treated with methylphenidate from age five, which rapidly caused a decrease in aggression and hypersexuality whereas other studies and case reports have shown increased sexual activity with methylphenidate (9)(10), i.e. priapism from use of methylphenidate (11). Another case story is about a boy treated with methylphenidate for 17 years since childhood and who experienced delayed puberty; at age 20 he had underdeveloped genitalia, lack of libido and also erectile dysfunction (12).

A longitudinal study that investigated pubertal development in 65 boys on stimulant medication for at least three years compared to 174 controls (13) found a slower rate of physical development during puberty for medicated boys.

In pregnant mice, methylphenidate has been shown to cross the placenta and the drug was measurable in the fetal brain at levels comparable to maternal plasma (14), which may affect the offspring's development. The percentage of offspring with cleft palate increased with increasing dose of clonidine given to murine dams at day 11 of pregnancy (15).

It has been shown that methylphenidate administration to young rats, corresponding in age to prepubertal children, resulted in altered levels of dopamine transporters that persisted into adulthood, long after termination of treatment (16).

A large report from 2005 investigated the reproductive and developmental effects of methylphenidate (17). There were no data on humans and for animals, the working group did not have sufficient data to give advice on reproduction, although they mentioned two studies demonstrating reduced sperm motility and altered estrous cycling, respectively, and one study reporting no differences. A study in rats found that methylphenidate impaired normal spermatogenesis after eleven weeks of treatment, which indicated that the drug might reduce male reproductivity (18).

A review from 2006 of sexual maturation in children and adolescents included only two studies and had no clear conclusion (19). We decided to do a systematic review of animal studies to study the impact of ADHD drugs on the development of the reproductive organs.

Aim

To assess whether drugs approved for ADHD symptoms cause abnormal development of reproductive organs in animals.

Methods

Search strategy

Searches will be performed in PubMed, Biosis and Embase, using MeSH terms or the equivalent, for drugs approved for ADHD symptoms (the eight most sold ADHD drugs as reported by the Food and Drug Administration for 2012 (20) *(atomoxetine, clonidine, dexmethylphenidate, dextroamphetamine, guanfacine, lisdexamphetamine, methylphenidate and amphetamine)). We will also go through the NTP-CERHR report for eligible studies* (17).

We will contact authors for outcome data that are unclear or not reported in the papers published in 2006 or later, going back ten years.

Eligibility criteria and data extraction

We will include monotherapeutic, controlled studies on drugs for ADHD symptoms on healthy mammals, i.e. they will not be castrated or ovariectomized or otherwise primed for behaviour resembling ADHD symptoms. We will only investigate the changes in the reproductive organs of the animals; we will include studies on outcomes related to the pregnancy period (miscarriages) and labour (animals not able to give birth or number of stillborns).

We will extract data on outcomes like sizes of reproductive organs, motility, cell counts, morphology and morphometrics, ability of ejaculation, estrous cycle, pregnancy, live births. Outcomes will be categorized as morphology incl. metrics, sperm motility, ejaculation, cell counts, cycles, pregnancy/labour.

We will not be investigating if the drugs cause changes in the offspring or whether the animals are normally lactating, therefore only postnatal interventions are eligible. Interventions must be carried out in live animals, thus we exclude in vitro studies. We will exclude human studies, methodological papers and studies not having the reproductive system as an outcome category as well as pharmacological or biochemical surrogate outcomes. Gender-specific data will not be lumped due to differences in outcomes. Non-English articles will be excluded due to amount of resources.

If there are at least two eligible studies for an outcome, meta-analysis will be performed using the last time point at the end of intervention. If there are several active groups (different drugs related to the same control group), we will split the control group accordingly to avoid double counting.

One observer will screen all records and a second observer will screen a random sample of 15 excluded records and all included records to check for inter-rater variability.

Potentially eligible articles will be collected and read in full. References of eligible articles will be scanned to locate further studies. Data will be entered in Excel and in ReviewManager for analysis.

One investigator will be carrying out data extraction independently and a second investigator will be controlling for validity of the extracted data. If disagreements, consensus will be reached, using a third assessor if disagreement arises.

Quantitative data synthesis

We will extract data according to published guidelines (21)(22)(23)(24): *animals (species, strain, age, sex), study design (drug intervention, group size, dose, route of administration, housing condition, treatment length and follow-up length), risk of bias (blinding, randomisation, selective reporting) and relevant outcome measures.*

If several doses are included per study, they will be pooled per mean and standard deviation (25). *If in doubt, we will ask an arbiter who has not seen the data set. Animals kept group-housed or isolated will be pooled. The total number of intervention groups after combining them will determine the splitting of the control group. If similar tests are performed on consecutive days, results for the first test day will be reported.*

As we do not expect medication for ADHD symptoms to cause better outcomes related to reproductive parameters, outcomes will be transformed by multiplying with -1 when relevant.

We will perform sensitivity analyses with respect to choice of drug. Exploratory subgroup analyses will be performed for rats only, blinded observers, age at intervention (young/adolescent, intervention ended <PND56 vs adults, intervention ended >PND56), and a simple comparison of assessments at end of intervention vs after a drug-free follow-up period.

Risk of bias is assessed according to the methods by Syrcle (21)(25). *For animal studies, the Syrcle risk of bias tool recommended assessment of three categories of randomisation, namely randomisation of animals to intervention or control groups; random housing to prevent behavioural differences between groups introduced through differences in light intensity and temperature; and random outcome assessments to prevent influence on results from diurnal variation* (21).

All results will be reported in relevant tables and text.

Statistical methods

We will use a random effects model. For binary data, risk ratios (RR) will be reported. For the meta-analyses of continuous data and ranking scale data, the standardised mean difference is calculated or the mean differences will be used.

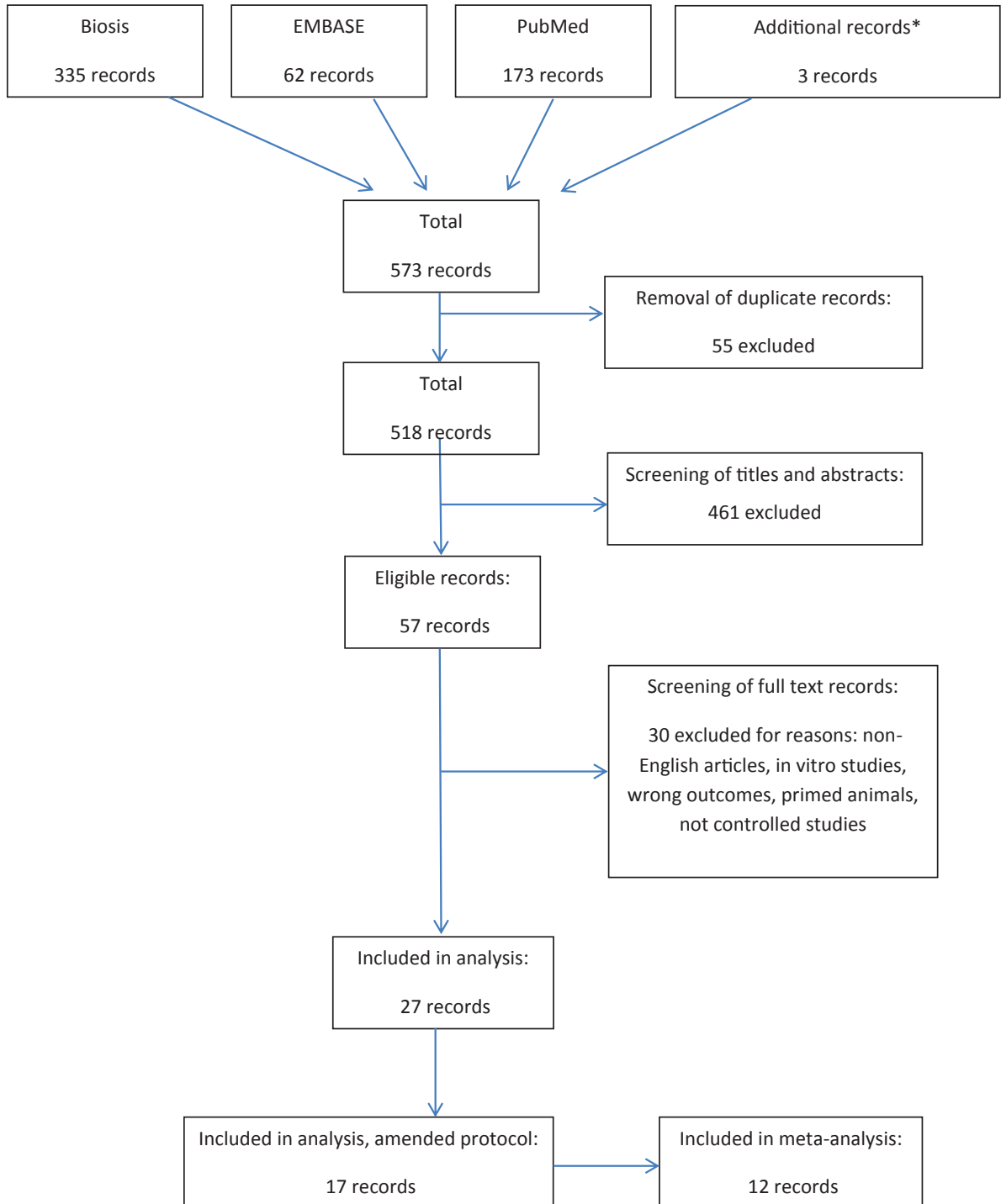*Pooling of means and standard deviations is done as advised by the Cochrane Handbook* (25).

Heterogeneity is assessed as I-square and further explored by subgroup and sensitivity analyses to determine the impact of explanatory factors (23)(25)(26).

Missing standard deviations are calculated or estimated by standard methods (25), *e.g. imputing from SEs or p-values. If SD values are not estimable, results will not be used for meta-analysis.*

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Appendix C: Literature search – flow chart

```
┌──────────────┐  ┌──────────────┐  ┌──────────────┐  ┌──────────────────┐
│    Biosis    │  │    EMBASE    │  │    PubMed    │  │ Additional records* │
│              │  │              │  │              │  │                  │
│  335 records │  │  62 records  │  │  173 records │  │    3 records     │
└──────────────┘  └──────────────┘  └──────────────┘  └──────────────────┘
```

Total 573 records	Removal of duplicate records: 55 excluded
Total 518 records	Screening of titles and abstracts: 461 excluded
Eligible records: 57 records	Screening of full text records: 30 excluded for reasons: non-English articles, in vitro studies, wrong outcomes, primed animals, not controlled studies
Included in analysis: 27 records	
Included in analysis, amended protocol: 17 records	Included in meta-analysis: 12 records

*Additional records identified through "related papers" function and references of screened papers.