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Gender-Related Reproductive (De) Effects after Paracetamol Use - Review of Pre-Clinical and Human Data

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Abstract

In this review the potential link between paracetamol use and reproductive health in animals and humans as well as the gender dependence of this effect is discussed.

Paracetamol is commonly used drug in a whole human population and the preferred analgesic among pregnant woman and infants. Many studies indicate that exposure in particular during prenatal period may pose a real risk to the reproductive health of offspring regardless of sex, doses and exposure timing. Mainly pre-clinical evidences confirm a connection between maternal intake of paracetamol and gonadal anomaly in both sexes but far larger abnormalities concerned males. Animal study suggests that maternal paracetamol consumption may favor genital malformation in male offspring, ovarian reprogramming in female and can be responsible for reproductive disorders in adulthood. The putative mechanism explaining the perturbed fertility, behavior and masculinization after paracetamol exposure in utero relates to hormonal disturbances e.g. lowered production of testicular testosterone.

Scientific data shows that paracetamol has a negative effect mainly in utero affecting gonadal development but also can influences fertility of adult males and females.

In this article we are trying to answer the question whether the use of paracetamol predicting poor reproductive potential and fertility is depending on gender.

Keywords: Paracetamol; Acetaminophen; Fecundity; Fertility; Reproduction; Gender

Introduction

Problem of pain medications use and reproductive dysfunctions is widely discussed and raises a lot of interest. Despite many studies conducted in this area, potential reproductive consequences of the use of paracetamol - mild analgesic classically intended for relieve pain and fever - are still poor recognized. Uncontrolled consumption of paracetamol during pregnancy may be associated with negative repercussions for the reproductive health of newborn. All indicates that the adverse effects of paracetamol are particularly marked in the male while the changes in females are less pronounced.

Modification of the gonadal architecture or function by various prenatal factors repeatedly causing long persistent effect on reproductive health. In this context common and unlimited access to paracetamol by pregnant woman triggered important concerns about its potential deleterious impact on reproductive competences in off spring. Both epidemiological data as well as experimental studies in vivo end ex vivo conducted in rodents or human testicular implants suggest that exposure to paracetamol can affect physiology and development of male reproductive tract [1-3].

Paracetamol can impairs reproductive competence by lowering testosterone production, strong pro-oxidative effect and inhibition of prostaglandin synthesis.

Endocrine-disrupting activity of paracetamol may be important also for the functioning of the reproductive system in adults. In mature individuals paracetamol can affecting fertility, semen quality and hormonal balance [4,5].

Pre-clinical and epidemiological data has reported conflicting results on reproductive safety of paracetamol and its gonadal toxicity, however all studies recommend caution and restraint in the use of this drug by pregnant women due to the potential health risk for offspring, especially male individuals. Considering the results of scientific research, this factor should also be taken into account in the event of difficulties in getting pregnant or problems with maintaining pregnancy.

Prenatal Exposure to Paracetamol and Urogenital Defects in Newborn

One of the most common birth problems in male are defects of the genitourinary system, which include cryptorchidism (undescended testis) and hypospadias characterized by incorrect placement of the urethra orifice, which is accompanied by bending the penis or narrowing of the outlet. The researcher emphasizes

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that cryptorchidism is a major risk factor for testicular cancer and also contributes to the deterioration of sperm quality later in life. Unfortunately, not much it is known about the predisposing agent the above mentioned drawbacks.

Some experimental data as well as epidemiological evidences indicate a likely connection between maternal use of analgesics like paracetamol and congenital reproductive abnormalities which may affect fertility in the future. According to some evidences, anomalies in the male genitals - hypospadias or cryptorchidism - manifested at birth can result from prolonged intrauterine exposure to paracetamol [1,6-13].

Jegou [1], indicates that the occurrence of congenital abnormal placement of the testicle in offspring may be associated with the use paracetamol alone or in combination with ibuprofen or aspirin during pregnancy. The negative effect of the drug on the process of testicular descent results from inhibition of fetal secretion of major factors involved in transabdominal testicular descent in particular testosterone, Insulin-Like Factor 3 (INSL3), prostaglandin E2, Calcitonin Gene-Related Peptide (CGRP) produced by genitofemoral nerve and Anti-Mullerian Hormone (AMH) from fetal Sertoli cells in human testes [6,14].

The relationship between time of exposure to paracetamol during pregnancy and the occurrence of birth defects has been described by Rebordosa et al. [15] in 2008. The questionnaire method was used in the study, followed by Computer-Assisted Telephone Interviews to verify the collected data. Information was obtained at 17 weeks and 32 week of pregnancy as well as at 6 and 18 month after delivery. A total of 88142 children took part in the study. Of these, 5847 participants were diagnosed with birth defects. As shown by the study, 80 male newborns were diagnosed with hypospadias, which was proven to be associated with prenatal exposure to paracetamol (HR: 1.01, 95% CI: 0.76 to 1.33). An increased risk of cryptorchidism has also been observed (HR: 1.24, 95% CI: 0.79 to 1.94). There was no correlation between prenatal exposure to paracetamol and occurrence of obstructive urinary congenital abnormalities in offspring (HR: 0.90, 95% CI: 0.63 to 1.28). The use of paracetamol both in II and III trimester increased the risk of cryptorchidism (HR: 1.33, 95% CI: 1.00 to 1.77) and orchidopexy in the newborn (HR: 1.26, 95% CI: 0.86 to 1.84). Exposure to paracetamol in all trimesters (HR: 1.17, 95% CI: 0.94 to 1.46) and only in the second trimester of pregnancy (HR: 1.17, 95% CI: 0.89 to 1.54) also increased the risk of this defect. The study also showed a relationship between the period of prenatal exposure as well as duration of paracetamol use and the occurrence of defects of the reproductive tract. Congenital malformations (HR: 1.32, 95% CI: 0.97 to 1.78) and orchidopexy (HR: 1.63, 95% CI: 1.13 to 2.34) were more common in offspring of mothers using paracetamol longer than 4 weeks during pregnancy. Summing up the above study, exposure to paracetamol, which lasts over 4 weeks, may favor the occurrence of cryptorchidism in the offspring.

Similarly, in another study it was confirmed that the key moment of exposure conditioning the occurrence of the abovementioned defects is the second trimester of pregnancy. A prospective birth cohort study in over two thousands pregnant woman has proved correlation between duration and timing of intrauterine exposure to mild analgesics like paracetamol and the occurrence of congenital cryptorchidism in children. Increased risk of impaired masculinization was reported after simultaneous use of several analgesic drugs notably during the second trimester [7].

Another study evaluating the potential relationship between the use of paracetamol during pregnancy and the occurrence of urogenital defects in children, there is a study published in 2010 by Jensen et al [6]. Its purpose was to clarify whether prenatal exposure to paracetamol, aspirin and ibuprofen has an effect on risk of cryptorchidism in offspring. Of the 47400 male newborns examined, 980 were diagnosed with cryptorchidism, of which 565 children underwent orchidopexy to remove this defect of the genitourinary system. Data from the Danish National Birth Cohort show that use of paracetamol both during the first and second trimesters predispose to the occurrence of cryptorchidism in boys (HR: 1.33, 95% CI: 1.00 to 1.77). Fetal exposure longer than 4 weeks during the postulated time-window of programming testicular descent (8 week to 14 week of pregnancy) was correlated with higher risk of cryptorchidism (HR: 1.38, 95% CI: 1.05 to 1.83).

In research presented in 2012 by Snijder et al. [8], it was also assessed whether the use of mild painkillers during pregnancy has an increased risk of cryptorchidism or hypospadias in offspring. The study was performed using the questionnaire method at 12, 20 and 30 week of pregnancy. Mothers were asked about the use of prescript and over-the-counter medicines. The study determined the use of the drug in four time intervals: in the period before and during the first trimester, in the first 14 weeks of pregnancy, between 14 and 22 week and between 20 and 32 week of pregnancy. The presence of malformations of the genitourinary system in 3184 boys was found during screening in primary care centers. As show the above study there is a small risk of cryptorchidism in the offspring of mothers using paracetamol before and during the first trimester (OR: 1.02, 95% CI: 0.44 to 2.36). Near twofold increase in the risk of cryptorchidism was observed in the offspring of women taking the drug between 14 week and 22 week of pregnancy (OR: 1.89,95% CI: 1.01 to 3.51) as well as the equally high risk in the offspring of mothers using paracetamol in the first 14 weeks of pregnancy and between 20 week and 32 week. There was more than a twofold increase in the number of cryptorchidism cases in children born to mothers using the drug during the first 14 weeks of pregnancy (OR: 2.24, 95% CI: 0.60 to 8.32). No increased risk was observed in the offspring of mothers receiving paracetamol from 14 week of pregnancy (OR: 0.32, 95% CI: 0.04 to 2.44). According to the study, there is also a relationship between timing of paracetamol use during pregnancy and the occurrence of cryptorchidism. As shown in the above study, the use of paracetamol during pregnancy may affect the occurrence of urogenital defects in the offspring however authors point to some limitations resulting from a small number of cases of cryptorchidism (n = 68) and hypospadias (n = 22) and the lack of some relevant data.

A systematic review and meta-analysis performed by Gurney et al. [10], show week association between pharmacological pain management during early to mid-pregnancy and the risk of cryptorchidism in human male offspring (pooled crude OR: 1.11, 95% CI: 1.00 to 1.23). Paracetamol exerts negative effects by a variety of mechanisms e.g. decreases androgen level, causes disruption of spermatogenesis, negatively influences the seminiferous tubule histology, reduces semen quality and increases time to pregnancy. The

key element conditioning these unfavorable changes in reproductive organs associated with maternal paracetamol use is the dose, duration and gestational age.

The assessment of regularity of development of the reproductive system and fetal androgen action can be made on the basis of such a predictive parameter as Anogenital Distance (AGD), which defines the distance between the anus and the external genitalia. It is an important marker indicating exposure to androgenic agents during critical window of embryonic testis development. Shortening of male AGD is the determinant of feminization, genital malformations at birth and reproductive problems in adulthood. Alteration in this parameter can be associated with prenatal exposure to several environmental factors including common drugs with potential endocrine disrupting activity. Shortening of the anogenital distance observed after prenatal paracetamol use is attributed to anti androgenic action [9,13,16].

In the one of the latest research published by Fisher et al. [9] connection between prenatal exposure to paracetamol and the occurrence of defects of the genitourinary system were evaluated in both sexes. The study included 116 boys and 114 girls whose mothers used paracetamol during pregnancy. In this study changes in the length of AGD were observed depending on gender. It was observed that prenatal exposure to paracetamol during 8 week to 14 week of pregnancy was primarily associated with shortening of AGD in boys while no changes were observed in newborn females. These problems were related with hormonal changes during pregnancy resulting from taking paracetamol - in particular with decrease in the level of sex hormones in this period. Despite that the above study assessed the sex-dependent effect of using paracetamol during pregnancy on AGD however, no significant association between drug exposure and other genital outcomes e.g. cryptorchidism was seen in the male offspring.

A similar study was published by Lind et al. [13] in 2017. A large study group, together - 470 girls and 557 boys, took part in it. Three months after delivery, a health balance was carried out in all children and AGD was also measured. Obtained results suggest that use of paracetamol during pregnancy may affect hormonal status in pregnant women causing tendency to reduction of AGD in male offspring. No changes in this parameter were observed in female newborns. As the authors emphasize, the above observations may be associated with reduced androgen secretion after paracetamol use. Hormonal disturbances resulting from early exposure to paracetamol can lead to lower testosterone levels, low sperm quality and disruption the development of the male reproductive tract.

In summary, both Fisher and Lind confirm the relationship between prenatal exposure to paracetamol and shortening male AGD. Due to the frequent use of painkillers during pregnancy, further research is needed to verify the hypothesis and detailed analyzes taking into account the impact of other environmental risk factors for the occurrence of urogenital defects in the offspring.

The Impact of Paracetamol on Hormonal Balance and Gonadal Structure

Testicular development and maturation are strongly controlled by neuroendocrine factors primarily by hormonal signals from hypothalamus. Secretion of male sex hormones is navigated *via* negative feedback control of the hypothalamic-pituitary-testicular axis. Paracetamol by its estrogenic activity may affect these pathways and induces testosterone deficits [17-19]. According to the some authors (Lind et al. [13]; Hurtado-Gonzalez et al. [20]; Rossitto et al. [21]) exposure to mild analgesics like paracetamol in utero can affect hypothalamic-pituitary-testicular axis as well as gonadal maturation and reproductive competences in the offspring. In the paper published in 2003, Selvage and Rivier. [22], reaffirmed existence of a descending, multi synaptic, pituitary-independent neural connection between hypothalamus and male gonads. Previously published studies support hypothesis that early paracetamol treatment can modify hypothalamic activity by changing the level of catecholamines and amino acids. In the hypothalamus, paracetamol causes increase of dopamine and metabolic products of dopamine as well as reduction of the noradrenaline metabolites and glutamic acid [23].

In turn, the occurrence of neuronal secretory and hormonal disturbances in the early stages of life can translate into a change in the structure of reproductive tissues. Male reproductive pathologies caused by early paracetamol treatment may be associated with inhibition of cyclooxygenase - enzymes catalyzing prostaglandin production. Prostaglandins are involved in many biological processes inter alia hormonal regulation, reproductive functions and gender specific behavior. Direct exposure to mild analgesics like paracetamol (10^{-5}M) inhibited testosterone production in the cultured adult human testis and steroid-producing human cell line (NCI-H295R) [18]. Endocrine disruption is probably related to the direct anti-prostaglandin activity of the drug. Mazaud-Guittot et al. [14], in 2013 based on the culture of human fetal testes from pregnant woman after induced abortion show that exposure to paracetamol and its active metabolite AM404 (N-(4-hydroxyphenyl)-arachidonoylethanolamide) causes endocrine disturbances e.g. decrease the level of insulin-like factor 3 required for transabdominal testicular descent and inhibit Production Of Prostaglandin 2 (PGE2). As demonstrated, in gonocytes of neonatal rats, paracetamol caused functional changes in the cells, affects genes of prostanoid pathway and inhibits production of PGE2 and PGF2a [24]. Drug does not affect gonocyte survival and apoptosis, however can stimulate gonocyte proliferation.

Most of the data on the adverse impact of paracetamol on the structure of the gonads comes from animal studies. In adult rats, 30-day long oral exposure to high doses of paracetamol (1000 mg/kg bw/day) causes reduction of testicular weight, interstitial volume and sperm count. Observed impairment of male fertility was associated with intensification of apoptotic processes in pachytene spermatocytes and in early spermatids [25].

Yano and Dolder. [26], showed that paracetamol treatment leading to the morphological modification of testicular structure in rats. After single dose 4.4 mmol/kg of the drug they find altered and degenerating seminiferous tubules. Studying the structure of the testicles, the researchers found fragmentation of Sertoli cells while spermatids showed changes in rough endoplasmic reticulum. Changes also concerned Golgi complexes where they observed irregularly compacted chromatin. Compared with untreated animals morphology of late spermatids of rats treated with paracetamol has changed significantly. Late spermatids have larger volume of residual cytoplasm.

It has been hypothesized that common reproductive disorders in

male offspring can be correlated with deficiencies in fetal testosterone biosynthesis but factors responsible for this effect remain unknown. Some of researchers suggest that paracetamol can be one of the environmental predisposing factors. Van den Driesche et al. [27], shows that the drug used in utero raises postnatal body weight but not affecting the frequency of cryptorchidism and hypospadias. Simultaneously he observed lack of significant differences in testicular weight and length of the penis. The research indicates that the use of human-equivalent therapeutic regimen of paracetamol significantly lowers plasma testosterone (45% reduction) and weight of seminal vesicle (18% reduction) in human fetal testicles in the validated xeno graft model. Changes induced by paracetamol, particularly the suppressing effect on fetal testosterone, are related to the lower expression of mRNA for steroidogenic enzymes - *CYP11A1* and *CYP17A1*. *CYP17A* is responsible for the conversion of the progesterone to the 17α-hydroxyprogesterone and androstenedione and determines the ability of testes to synthesize testosterone. Holm et al. [16,28] came to similar conclusions. Data received by his research team indicate that paracetamol impairs male reproductive development and decreases the level of androgens through an inhibition of *CYP17A1* and activation of CYP19 and CYP21.

Based on Human Estrogen Receptor Transcriptional Activation (hERTa) test and the H295R steroidogenesis assays it has been shown that paracetamol affected steroidogenesis *in vitro* causing strong inhibition of testosterone release and stimulation of estrogen secretion [29]. In male rats, the drug can also cause significant oxidative effect and change the glutathione status in the tissues of reproductive system. Acute administrations 1500 mg/kg of paracetamol generate perturbation of reproductive tract by decrease in the epididymal content of glutatione and pro-oxidative effect [30].

Simultaneously study on blue mussels Mytilus edulis has been shown that even short-term exposure to paracetamol alter the gonadal expression of several genes important for processes occurring in the reproductive system and may impair reproduction of mollusks [31].

Consistent with previously described data, Kristensen et al. [32], show that paracetamol (0.1 μM to 100 μM) inhibits testosterone production in rat fetal testes *in vitro* but has no effect on other hormones of Leydig cells. Using *ex-vivo* organotypic model they proved that anti-androgenic effect is probably uncoupled from inhibition of the prostaglandin synthesis by this drug.

Considering all the studies described above indicating a clear hormonal effect of paracetamol, it can be assumed that this drug can strongly affect the structure and functions of developing male gonads.

Effect of Paracetamol on Spermatogenesis and Semen Quality

Many preclinical data have confirmed that paracetamol may act as endocrine disruptor generating significant changes in testicular morphology, sperm morphometry and structure of sperm chromatin [17-21].

Excessive oxidative stress produced by paracetamol may be responsible for dysfunction and death of testicular cells finally leading to anomalies in the male reproductive tract [33]. Pro-oxidative properties can also affect the quality of sperm. In animal studies paracetamol indices reproductive problems causing decrease in sperm motility, increase the rate of dead or abnormal sperm cells by exacerbating oxidative stress [34].

In several studies in rodents, was confirmed that paracetamol at dose exceeding 400 mg/kg bw. Reduces semen quality causing anomalies in the morphology of sperm and chromatin structure. At the same time there were distorted proportions of various spermatid stages and followed reduction of sperm count, its viability and motility [17,26,34,35]. Based on hypothesis by Banihani. [36], impact of paracetamol on semen quality is the result of its structural similarity (presence of an acyl group and a phenol ring) to the steroid hormones. Chemical mimicry to the sex hormones, enhanced generation of reactive oxygen species, and reduction of nitric oxide formation and inhibition of prostaglandin synthesis in the presence of paracetamol is probably the reason of reduced testosterone synthesis and enhanced apoptosis of spermatocytes.

Wiger and coworkers. [17], demonstrated that high doses of paracetamol (400 mg/kg for 5 days i.p.) lead to testicular atrophy and reduction of relative testicular weight. They observed delay in spermiogenesis and important changes in populations of various spermatid stages e.g. reduction of early pachytene spermatocytes. In the time range from 27 day to 33 day after last injection they observed abnormal chromatin structure. Similar conclusion reach Smith et al. [37] who find that in mice liver high doses of paracetamol causes dramatic decrease in mRNA for histone important for structural organization of chromatin and DNA packing.

During embryogenesis exposure to common combination of paracetamol and ibuprofen leads to early differentiation and decreased proliferation of male embryonic germ cells in mouse. In the aftermath of these changes comes to retardation of Sertoli-cells maturation, reduction of the pool of spermatogonia A and reduction of sperm count [21].

Guiloski et al. [19] showed that environmental waterborne exposure to paracetamol (0.25, 2.5 μg/l for 21 days) causes disruption of hypothalamic-pituitary-gonadal axis (increase of dopamine and serotonine) and changes many important hematological parameters in male fish of the species Rhamdia quelen. Researchers find that paracetamol reduces testosterone level by over 58% but estradiol concentration was increased in 635.8%. During histological analysis of gonadal tissues they find that higher paracetamol concentrations inhibit spermatogenesis and causes predominance of spermatogonia.

Human studies in this area are scare. Prospective cohort study included 501 couples discontinuing contraception for the purposes of attempting conception confirms that higher urinary paracetamol concentrations among male were correlated with a longer time-to-pregnancy [4]. It was finding that high male urinary concentration of paracetamol is correlated with longer fecundability odds ratio (FOR: 0.67; 95%CI: 0.47 to 0.95). Moreover, higher concentrations of paracetamol in men urine was associated with decrease in beat cross-frequency and DNA fragmentation. The correlation between urinary drug metabolite - p-aminophenol and reduction in sperm head areas was also confirmed [5].

Hurtado-Gonzalez et al. [20], studied how exposure to paracetamol affects development of human fetal germ cell both sexes

in vitro and in xeno graft model. He discovered that in cultured first-trimester fetal testes in the presence of paracetamol in therapeutic concentrations the number of gonocytes is significantly reduced (-28%). It was similar in the case of xeno grafted second-trimester human fetal testes where after paracetamol treatment of host mice experimenters also confirmed the reduction in gonocyte population (-17% after one-day-long treatment and-30% after 7 days). In human line of germ tumor-derived cells - NTera2, cultured rat fetal testis and testes *in vivo* expression of epigenetic regulator TET1 and enhancer of zeste homolog 2 -Ezh2 was significantly increased after drug exposure [20].

Considering the cited studies, it can be stated that paracetamol can worsen semen quality mainly through the intensification the processes of oxidation that generate micro damage in male gametes.

Paracetamol and Female Fertility

Paracetamol use is the safest choice in pregnant and breast feeding woman and the best option for neonates with pain and fever [11]. As opposed to male offspring prenatally exposed to paracetamol, in newborn females consequences of drug exposure in utero on postnatal ovarian development and late-life reproductive health are not well known and scantily documented.

The recently published experimental studies show that the drug used during fetal life may cause reduction of primodial follicles, irregular menstrual cycle, premature ovarian insufficiency, acceleration of puberty and in consequence reduced fertility in adulthood [38,39].

It is assumed that in mammals already at the time of birth the set number of follicles that depletes through reproductive lifespan is strictly defined. The number of eggs in ovaries deepens on the total pull at birth and the speed with which they disappear throughout life. Therefore all factors that may interfere with the process of egg formation in utero may also affect the fertility and time of onset of menopause in the future. Paracetamol taken by the mother while pregnant is seen as one of the factors that can disrupt this process causing reproductive problems in female offspring. Preclinical data suggest that prenatal exposure to paracetamol may decrease follicle pool, causing premature menopause and fertility disorders in adulthood [40].

One of the few studies indicates that in mice exposure to paracetamol (50 mg/kg or 150 mg/kg bw.) or its precursor-aniline during gestational period can deteriorates reproductive capacity of the female offspring, causing reduction of follicles reserve and suppressing the primodial germ cell proliferation later in life [28]. *In vitro* paracetamol (10^{-8} to 10^{-5} M) suppress progesterone production to 81% of control by porcine granulosa cells. It may indicate the potential for clinical reproductive toxicity [41].

A possible mechanism of this adverse change it is related to the disruption of mitosis in early phases of germ cell development [28]. In mice, exposure to therapeutic doses of paracetamol and ibuprofen during prenatal sex determination has an important impact on development and maturation of germ cells in ovary leading to the accelerated ovarian senescence and sub-fertility in female offspring [42]. Drugs lead to the increased germ cell proliferation in embryonic ovaries as well as intensified formation of primordial follicle arising from altered activation of the AKT/FOXO3 cascade. AKT/FOXO3 signaling is important in control of follicle activation and survival in ovaries.

Evidence suggests that paracetamol can interfere with meiosis in gametes caused delay in meiotic entry or progression. In the study published by Hurtado-Gonzalez et al. [20], in 2018 was shown that paracetamol reduces gonocyte number in human fetal ovaries *in vitro* (-43%). Simultaneously they find many changes in expression of genes for gonocyte differentiation markers and epigenetic regulatory genes e.g. up-regulation of epigenetic regulator TET1 and enhancer of zeste homolog 2 - Ezh2.

In zebra fish (Danio rerio) exposed to paracetamol (1152 μg/l) for 21 days significant decrease in egg number and reduction in plasma vitellogenin was observed. Ayobahan et al. [43], pointed that this effect follows rather from impairment of liver function than from disruption of estrogen synthesis.

The harmful effect of this drug on female reproduction can be caused by disturbance of the signal transmission from the brain to the ovaries and, as a consequence, the earlier the age of onset of first menstruation (which can translates into an earlier onset of menopause), disruption of the proper menstrual cycle, which reduces the length of menstruation and the formation of fewer follicles in the ovaries.

Transplacental exposure to paracetamol may also influence the rate of sexual maturation. Data from over 15 thousands boys and girls in the Danish Nationwide Puberty Cohort suggest a tendency to earlier pubertal development in female exposed to the drug in utero. Females prenatally exposed to the paracetamol had signs of puberty such as pubic hair, axillary hair and acne development slightly earlier compared to the control, non-treated group [39]. Scientists associate this effect with paracetamol interferention with peripheral androgen production.

The problem may be significant because, according to Aagaard. [44], estimates the drug is being used by nearly 9% of pregnant woman. Dean et al. [45], suggest that disruption of female reproductive development generated by paracetamol can by also passed on to following generations which may be the cause of increasing fertility problems in the human population. The data in this regard are inconsistent.

Espey. [46], comparing the effect of different anti-inflammatory agents on prostaglandin level in rabbit follicles during ovulation proved that in this model paracetamol does not affect prostaglandin production but like other anti-inflammatory agents can slightly inhibited ovulation.

It should be noted that also studies investigating the effects of analgesics (including paracetamol) use around ovulation and implantation on conceiving a pregnancy in human are incoherent and has several limitations. According to the study by Matyas et al. [47], use of analgesics like paracetamol in normally cycling woman is not harmful to reproduction and can even improve ovulation.

The most recent data from a prospective cohort study involving 885 participants carried out in years 2008-2015 show that use of paracetamol by woman trying to conceive naturally during pre-

and peri-ovulation period as well as during implantation is not associated with fecundability [48]. The safety of pre-conceptive use of paracetamol has been also confirmed in other prospective cohort study [49]. Analyses of data from 2573 female pregnancy planners (aged 21 years to 45 years) show that paracetamol is not appreciably associated with fecundability problems.

It has been shown that paracetamol drug used alone or in combination with diclofenac to reduce pain and discomfort associated with Oocyte retrieval in infertile woman undergoing IVF treatment does not affect on implantation and pregnancy rates [50].

Simultaneously uses of paracetamol for menstrual pain significantly change basal hormone level in women. Researchers observed that analgesics medication affects reproductive hormones resulting in higher luteal level of progesterone, reduced estradiol and increased level of FSH in healthy premenopausal women. In observational study women who regularly used this drug have lower gonadotropin (especially LH) and estradiol level compared with women receiving other types of analgesics and persons not taking any medication [51]. This could potentially affect a women's reproductive capacity.

A scientist extensively discusses issues regarding the effect of paracetamol on fetus and pregnancy outcome [52,53]. Much information in this area is provided by preclinical studies, while data on the human population are scarcely represented. It has been demonstrated that prenatal use of a combination of paracetamol and caffeine in Wistar rats is not teratogenic but may cause intrauterine growth retardation. In dose-dependent manner the mixture decreases placental weight, length and body weight of fetus [54]. In mice, accidental use of paracetamol during early embryogenesis has no significant effect on the fertility end points such as: number of fetuses or a final liter size [35,55].

High doses of paracetamol using during pregnancy reduces birth weight, decreases pool of primodial follicle and consequently to accelerated reproductive senescence in rodents [35,56]. In parental breeding pairs of Swiss CD-1 mice exposure to paracetamol (1% in diet for 14 weeks) during cohabitation leads to reduction of the number of litters and adversely influences postnatal growth in offspring [35]. Similar effect was observed in study of Thiele et al. [57]. In mice injected intraperitoneally with paracetamol (50 mg/kg or 250 mg/kg) in 12.5 gestational day interferention with maternal endocrine as well as immune adaptation to pregnancy was observed. These changes included decrease in plasma concentration of progesterone, morphological alteration of placenta.

Nogueira et al. [58], reported that environmental exposure to paracetamol at the range 0.005 mg/L to 3.125 mg/L has a negative effect on locomotor behavior, causes increase of global DNA metylation and anzymatic activity of cholinesterses, glutathione preroxidase and glutathione-S-transferase as well as increase in lipid peroxidation level in zebrafish larvae and embryos.

Despite animal studies proved that paracetamol affect fetal activity, in human maternal administration of paracetamol usually not disturb fetal well-being and has no effect on such parameters like; body movement, numbers and time spent in fetal breathing [59]. Based on data derived from human cohort and case-control study's authors points out that the therapeutic use the of the drug during pregnancy does not increase the risk of congenital malformations to the fetus and other adverse pregnancy outcome.

Cohort analysis presented by Dathe et al. [60], included 604 pregnant women with third trimester exposure and 1192 woman received paracetamol in first and second trimester. This clinical finding do not confirm impact of paracetamol on increased health risk for fetus or neonates and support role of paracetamol as a first analgesic choice in pregnancy.

Other researchers indicated that use of paracetamol specifically after second trimester of pregnancy should be limited because of the potential link with premature constriction or closure of the fetal ductus arteriosus [61,62]. In addition, there is also a reasonable presumption that use of paracetamol increases the risk of preterm birth among woman with preeclampsia [63]. However the vast majority of researchers emphasizes that in human paracetamol use during pregnancy does not associated with relevant risk of fetotoxicity [60].

A separate issue is the paracetamol impact on the course of pregnancy and fetal development after maternal overdose. Nitsche et al. [64], confirm that pharmacokinetic parameters in the fetus are parallel with maternal parameters which testify to a good transplacental passage of paracetamol. Fetal and maternal parameters like T1/2 (respectively - T1/2: 82 min and 84 min), clearance (CI/F: 31.2 L/h and 28.8 L/h), volume of distribution (Vd/F: 61.2 L and 57.5 L) were similar. Based on single case reports, it has been shown that following maternal paracetamol overdose, placental damage and delayed fetal compromise may occurs [65]. Overdose may result in fetal fulminant liver failure and increase the likelihood of stillbirth, preterm delivery and neonatal death.

Some evidences suggest that many of reproductive disorders in woman can be manifestations of improper programming during fetal life. Paracetamol is considered rather as a factor with estrogenic activity but also opposite, anti-estrogenic effect of this drug was observed. Fortner et al. [66], show that in premenopausal woman use of paracetamol increases formation of entire estrogen metabolites as well as potentially genotoxic compounds like 2-hydroksyestrone-3-methyl ether and 16α-hydroxyestrone. Baandrup. [67], shows inverse correlation between prescription use of paracetamol and risk of epithelial ovarian cancer in female population in Denmark. The cancer risk was dependent on duration as well as dosage and was 50% lower in women who use large amounts of the drug chronically for a minimum of 10 years (OR:0.45; 95% CI:0.24 to 0.86).

Studies conducted *in vitro* demonstrate that in therapeutic doses paracetamol significantly stimulates proliferation of human estrogen-responsive breast cancer cell lines: T47D, MCF7, MDA-MB-231 and endometrial adenocarcinoma cells [68-70]. Proliferative activity of positional isomers of paracetamol was conditioned by location of hydroxyl group in the benzene ring and is the highest for the p isomers.

Dowdy et al. [71], in human endometrial adenocarcinoma cells stated faint anti-estrogenic effect of paracetamol and decline in basal and stimulated by estradiol enzymatic activity of alkaline phosphatase connected with higher lactate dehydrogenase release (Figure 1).

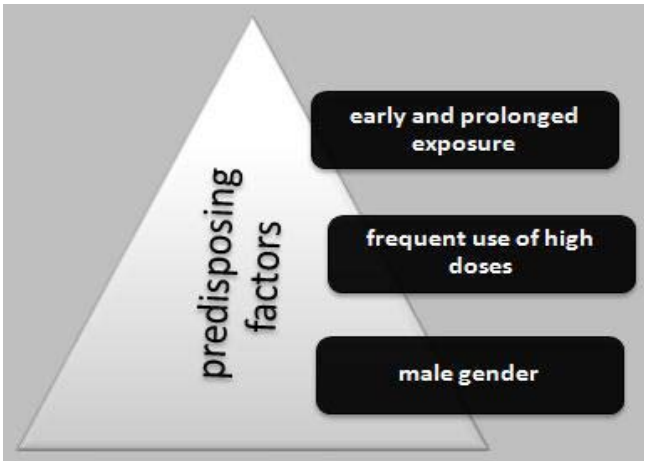

Figure 1: Paracetamol and the risk to the reproductive health.

Presented evidences support the hypothesis that paracetamol has hormonal activity and its use in utero may have long-lasting consequences for female reproductive health causing important changes in ovarian programming.

Impact of Paracetamol on the Occurrence of Gender-Specific Traits and Behavior

It is well known that paracetamol easily overcomes the blood-brain-barrier and is well distributed to the all brain structures [72]. Previously published studies support hypothesis that early exposure to paracetamol has a wide effect on neurotransmission in the central nervous system and changes behavior of male rats' offspring [73-75].

Adverse behavioral and cognitive outcomes occurring later in life applies to varying degrees both sexes exposed to paracetamol during pregnancy [76].

In human maternal paracetamol use may influence neurodevelopment and has been linked to some behavioral problems clearly related to the sex of the newborn child and are more expressed in male [77,78]. Paracetamol use in utero is associated with multiple neuro developmental end points like Attention-Deficit/Hyperactivity Disorder (ADHD).Cohort studies based on maternal reports confirms negative influence of prenatal paracetamol intake on psychomotor development in the field of gross motor skills, communication and emotionality mainly in male offspring [79-81].

It has been shown that exposure to the paracetamol during pregnancy causes not only developmental alterations in the reproductive tract but also impairs masculinization of brain and change sexual behavior in males. Anti androgenic properties of paracetamol induces important changes in the pattern of gene expression in the sexually dimorphic brain regions in the male offspring exposed to the drug during gestation and early postnatal period. Lichtensteiger et al. [82] proved that paracetamol administered to rat dams during gestational period changes pattern of gene expression in developing sexually dimorphic brain regions-preoptic area and ventromedial hypothalamus. Modifications particularly concerned the expression of genes encoding components important for excitatory glutamatergic synapses. Sex differences forming during gestation and early adolescence occurs in nearly all brain structures and are fundamental for sexually dimorphic behavior [83]. In mice, exposure to paracetamol during fetal development leading to the reduction of neurons in the sexually dimorphic nucleus of the preoptic area located in the anterior hypothalamus and consequently to the alterations in male sexual behavior. Male offspring exhibited reduced copulatory behavior e.g. poor intromission and ejaculations during mating, changes in urinary marking behavior and less inter-male aggression [83].

Reprogramming of the sexual neuro-behavioral features is probably triggered by reduction of testosterone biosynthesis in the Leydig cells or by the alterations in the level of neurotransmitters and prostaglandins in the brain.

Conclusion

The lack of consistent data on the effect on the endocrine system as well as insufficient proofs confirming estrogenic activity make difficult to clearly determine the consequences of using paracetamol both in the prenatal period and in the later stages of life on the reproductive system and fertility.

Considering all current data, exposure to paracetamol has a potency to impair reproductive development both male and female fetuses which may have a bearing on compromised fertility. Particularly prolonged duration of exposure and use of paracetamol in the second trimester - critical period for programming male and female reproductive development can be decisive. Studies indicate that male subjects are more likely to be adversely affected by the drug however to confirm or disprove definitively the association additional well-designed cohort studies are necessary. At the same time, many studies have not avoided substantial methodological constrains e.g. limited study size, self-reporting of paracetamol use which lids to the potential recall bias and misclassification. Therefore this problem requires further analysis based on well-conducted observational studies on large populations.

Pending the outcome of this dilemma paracetamol will be still considered safe first-line option for treatment of pain and fever during pregnancy and in neonates until new, hard clinical evidence or safer treatment options become available.

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