Paracetamol, aspirin and indomethacin display endocrine disrupting properties in the adult human testis in vitro

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STUDY QUESTION: Do mild analgesics affect the endocrine system of the human adult testis?

SUMMARY ANSWER: Mild analgesics induce multiple endocrine disturbances in the human adult testis in vitro.

WHAT IS KNOWN ALREADY: Mild analgesics have recently been incriminated as potential endocrine disruptors. Studies of the effects of these widely used molecules on the androgenic status of men are limited and somewhat contradictory. This prompted us to investigate whether these compounds could alter the adult human testicular function. We therefore assessed in parallel the effects of paracetamol, aspirin and indomethacin on organo-cultured adult human testis and on the NCI-H295R steroid-producing human cell line.

STUDY DESIGN, SIZE, DURATION: Adult human testis explants or NCI-H295R adrenocortical human cells were cultured with 10⁻⁴ or 10⁻⁵ M paracetamol, aspirin or indomethacin for 24–48 h. The effect of 10⁻⁵ M ketoconazole, used as an anti-androgenic reference molecule, was also assessed.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Testes were obtained from prostate cancer patients, who had not received any hormone therapy. The protocol was approved by the local ethics committee of Rennes, France and informed consent was given by the donors. Only testes displaying spermatogenesis, as assessed by transillumination, were used in this study. Hormone levels in the culture media were determined by radioimmunoassay (testosterone, insulin-like factor 3), Enzyme-Linked Immunosorbent Assay (inhibin B) or Enzyme Immunosorbent Assay [prostaglandin (PG) D2, and PGE2]. Tissues were observed and cells counted using classical immunohistochemical methods.

MAIN RESULTS AND THE ROLE OF CHANCE: The three mild analgesics caused multiple endocrine disturbances in the adult human testis. This was particularly apparent in the interstitial compartment. Effective doses were in the same range as those measured in blood plasma following standard analgesic treatment. The production of testosterone and insulin-like factor 3 by Leydig cells was altered by exposure to all these drugs. Inhibin B production by Sertoli cells was marginally affected by aspirin only. Our experiments also revealed that mild analgesics display direct anti-PG activity, which varied depending on the drug used, the dose and the duration of exposure. Nevertheless, associations between the alteration of the PG and testosterone profiles were not systematically observed, suggesting that a combination of mechanisms of endocrine disruption is at play.

LIMITATIONS, REASONS FOR CAUTION: Our studies were performed in vitro.

WIDER IMPLICATIONS OF THE FINDINGS: We provide the first evidence that direct exposure to mild analgesics can result in multiple endocrine disturbances in the human adult testis. Caution, concerning the consumption of mild analgesics by men, should be strengthened, particularly in high-risk population subgroups such as elite athletes.

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Introduction
Paracetamol and over-the-counter non-steroidal anti-inflammatory drugs (NSAIDs), herein referred to collectively as mild analgesics, are among the most sold pharmaceutical drugs worldwide. These molecules have excellent reputations within the general population and are widely used for self-medication and prophylaxis, and are also present in the environment (Verlicchi et al., 2012). However, they are often mis- or over-consumed, including in population subgroups, such as elite athletes, to prevent pain and treat injuries (Gors-...
for 1 h, and then for 15 min with the ABC complex. Bound antibody was visualized in red with 3-amin-9-ethyl carbazole (Beckman Coulter, Marseille, France). Sections were counterstained with hematoxylin and mounted in aqueous mount. CyP11A1-immunopositive cells and interstitial cells were counted in 500 fields using the Computer-Assisted Stereology Toolbox (CAST) Grid system (Olympus, Copenhagen, Denmark) with a light microscope (Olympus BX S1).

Culture of the NCI-H295R cell line and treatment
To reinforce the TEXAS approach as recently shown (Desdoits-Lethimonier et al., 2012), we also used the following procedure which is referred to as CELIAS for Cell Line Assay. The NCI-H295R human adrenocortical cell line, which expresses steroidogenic enzymes and produces steroids, has previously been used to study anti-androgenic compounds (Gracia et al., 2006) and was provided by Dr Feige (INSERM U878, Grenoble, France). As previously described by Desdoits et al. (2012), cells were grown for 1–10 passages in 75-cm² flasks with 10 ml of supplemented medium at 37°C under a 5% CO₂ atmosphere. The supplemented medium was RPMI medium 1640 (RPMI, Roswell Park Memorial Institute; Sigma-Aldrich) with 1% ITS plus (ITS: Insulin, human Tranferrin and Selenous acid; Becton Dickinson, Le Pont de Claix, France), 2% fetal bovine serum (Eurobio, Courtaboeuf, France) and antibiotics. The cells were then sub-cultured at a density of 10⁶ cells/well in a 12-well culture plate, and were incubated for 5 days. The medium was then replaced with 1 ml of serum-free RPMI 1640 containing 0.01% bovine serum albumin and 0.1% DMEM or 10⁻⁵ M ketocazol, or 10⁻⁴ M paracetamol, aspirin or indomethacin in 0.1% DMEM and the culture continued for 24 or 48 h. The media were collected and divided into three aliquots, which were stored at −80°C until hormone assays.

Measurement of testosterone levels
Testosterone in the culture media of both culture systems was assayed in duplicate using a commercial radioimmunoassay (RIA) based on competitive binding with 1³⁵S labeled testosterone (Immunotech, Beckman Coulter, Villepinte, France), according to the manufacturer’s recommendations. Standard curve interpolation was used and the results were expressed in nanograms per milliliter. The intra- and inter-assay coefficients of variation for serum samples were ≤8.6 and 11.9%, respectively. Testis explants produced an average ± SEM of 7.7 ± 1.15 ng/ml/explant testosterone after 24 h of culture, and 12.05 ± 1.39 ng/ml/explant after 48 h. Control NCI-H295R cells produced an average of 1.21 ± 0.08 ng/ml/well after 24 h of culture, and 2.08 ± 0.13 ng/ml/well after 48 h.

Measurement of INSL3 levels
INSL3 in the culture media of the TEXAS was assayed in duplicate using a commercial RIA (RK-035-27, Phoenix France, Strasbourg, France) according to the manufacturer’s instructions. Each sample was diluted 4-fold in RIA buffer prior to RIA reactions. The intra- and inter-assay coefficients of variation were ≤15 and 7%, respectively, and the lower limit of detection was 20.17 pg/ml. Control testis explants produced an average of 0.53 ± 0.66 ng/ml/explant INSL3 after 24 h of culture, and 0.39 ± 0.52 ng/ml/explant after 48 h.

Measurement of inhibin B levels
Inhibin B was assayed in the culture media of the TEXAS using a commercial Enzyme-linked immunosorbent Assay (ELISA) kit (DSL-10-84100 Active, Beckman Coulter, Villepinte, France) according to the manufacturer’s instructions. Each sample was diluted 2-fold in sample diluent solution prior to reactions. The intra- and inter-assay coefficients of variation for serum samples were ≤5.6 and 7.6%, respectively. Control testis explants produced an average of 0.18 ± 0.17 ng/ml/explant inhibin B after 24 h of culture, and 0.30 ± 0.36 ng/ml/explant after 48 h.

Measurement of PG levels
PGs D2 and E2 were assayed in the culture media of the TEXAS using commercial Enzyme Immunosorbsent Assay (EIA) kits (PGD2-MOX EIA Kit, no. S12011 and PGE2 EIA Kit Monoclonal, no. S14010, respectively, Cayman, MI, USA) according to the manufacturer’s instructions. Each sample was diluted 3 to 5-fold in sample diluent solution prior to reactions. The lower limits of detection were 3.1 and 15 pg/ml, respectively. Control testis explants produced an average of 0.024 ± 0.018 ng/ml/explant PGD2 and 1.33 ± 1.52 ng/ml/explant PGE2 after 24 h of culture.

Statistical analysis
All reported values are means ± SEM. For the two culture systems, values are expressed as percentages of the control value: each replicate in each condition was expressed as a percentage of the mean of control values, and then the mean for each condition was calculated for each donor. Finally, the mean ± SEM of all donors was calculated and were analyzed with the non-parametric signed-rank Mann–Whitney test. A P-value equal or inferior to 0.05 was considered statistically significant.

Results
Mild analgesics and human testis morphology
Exposure to mild analgesics had no obvious effect on the gross morphology of testis explants (Fig. 1). None of the treatments caused any global alteration of the seminiferous epithelium. Cell counts indicated that none of the treatments with mild analgesics significantly decreased the Leydig cell number (Fig. 2): after 24 h of exposure, the number of Leydig cells was significantly higher in samples treated with 10⁻³ M aspirin and 10⁻⁴ M indomethacin than in controls (by 29 and 22%, respectively; P < 0.05).

Mild analgesics and testosterone production by the human testis
When all the six independent experiments were taken into consideration, treatment with 10⁻³ M ketoconazole significantly decreased testosterone concentrations in the culture medium by 60% after 24 h, and by 69% after 48 h of exposure relative to control values (Fig. 3; P < 0.01). Exposure to 10⁻⁵ and 10⁻⁴ M paracetamol significantly decreased testosterone secretion after 24 h by 18 and 30%, respectively (Fig. 3; P < 0.01); 10⁻⁴ M indomethacin also significantly decreased testosterone production after 24 h by 14% (Fig. 3; P < 0.05). A 24-h exposure to 10⁻⁵ M aspirin and indomethacin induced decreasing trends of testosterone production by 19 and 12%, respectively, which did not reach statistical significance at P < 0.05 (Fig. 3). Following 48 h of exposure, none of the mild analgesics significantly decreased testosterone concentrations at the doses tested (Fig. 3).

When the experiments were considered individually, in each of the six independent experiments, the majority of the samples exposed for 24 h to the analgesics responded by a testosterone production that was below the control level (Supplementary data S1). After 48 h of exposure to the analgesics, if a majority of samples exposed to the
Figure 1 Light micrographs of areas of human testis explants after 48 h in culture in the presence of $10^{-5}$ M DMSO (Control; A), $10^{-5}$ M ketoconazole (B) or of $10^{-3}$ M or $10^{-4}$ M concentration of the test compound, namely paracetamol (C and D, respectively), aspirin (E and F, respectively) or indomethacin (G and H, respectively). Testis explants were fixed in Bouin fixative and stained with hematoxilin–eosin. No change in the gross morphology of the testis was apparent.

Figure 2 Effects of a 24-h exposure to $10^{-5}$ M ketoconazole (KT), or $10^{-5}$ or $10^{-4}$ M paracetamol, aspirin or indomethacin on the number of Leydig cells in human testicular explants. Each bar represents the mean ± SEM of three independent experiments with tissues from different donors. The number of Cyp11A1+ cells/1000 interstitial cells is expressed as a percentage of the control value; the difference from control (100%) is given in brackets where it is significant. *P < 0.05 by the non-parametric signed-rank Mann–Whitney test on unpaired data. NS, not significant.

Figure 3 Time- and dose-dependent effects of $10^{-5}$ M ketoconazole (KT), and $10^{-3}$ and $10^{-4}$ M paracetamol, aspirin or indomethacin on testosterone production by human testicular explants. Each bar represents the mean ± SEM of six independent experiments with samples from different donors. The difference from control (100%) is given in brackets where it is significant. *P < 0.05, **P < 0.01 by non-parametric signed-rank Mann–Whitney test on unpaired data. NS, not significant.
analgesics displayed a production of testosterone lower than the control level in a majority of experiments (4 out of 6), the patterns of responses were generally more contrasted than at 24 h, and larger intra- and inter-individual variations in testosterone production were seen (Supplementary data S1). This explained why when all the data of 48 h of exposure were globally analyzed, no significant differences were seen with the analgesics (Fig. 3).

**Mild analgesics and testosterone production by the NCI-H295R cell line**

Testosterone production was inhibited in a time-dependent manner by $10^{-5}$ M ketoconazole (Fig. 4). Exposure to $10^{-5}$ M paracetamol inhibited testosterone production by 13% after 48 h ($P < 0.01$), but testosterone levels after 24 h were not significantly different from those in controls. In contrast, following 24 h of treatment with $10^{-4}$ M paracetamol, the testosterone concentration was significantly lower than in controls ($-10\%$; $P < 0.05$). After 48 h, the difference was not significant anymore ($-12\%$; Fig. 4). Exposure to $10^{-5}$ and $10^{-4}$ M aspirin significantly reduced testosterone production after 24 h (by 18 and 17%, respectively; $P < 0.05$) and after 48 h (by 14%; $P < 0.001$ and 12%; $P < 0.05$, respectively). Testosterone production was markedly and significantly reduced by both $10^{-5}$ and $10^{-4}$ M indomethacin after 24 h (by 31 and 37%, respectively; $P < 0.05$) and after 48 h (by 41%; $P < 0.001$ and 43%; $P < 0.05$, respectively).

**Figure 4** Time- and dose-dependent effects of $10^{-5}$ M ketoconazole (KT), $10^{-5}$ and $10^{-4}$ M paracetamol, aspirin or indomethacin on testosterone production by NCI-H295R cells. The cells were treated for 24 or 48 h with $10^{-5}$ M ketoconazole (KT), or $10^{-5}$–$10^{-4}$ M paracetamol, aspirin or indomethacin. Values for testosterone are expressed as percentages of the control value. Each bar represents the mean ± SEM of 3 (24 h) to 6 (48 h) experiments. The difference from control (100%) is given in brackets where it is significant. $^aP < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ by non-parametric signed-rank Mann–Whitney test on unpaired data. NS, not significant; ND, not detected.

**Mild analgesics and INSL3 production by the human testis**

Neither ketoconazole nor paracetamol had a significant effect on INSL3 production (Fig. 5). INSL3 levels were 22 and 33% lower following 24 h of exposure to $10^{-5}$ and $10^{-4}$ M aspirin, respectively, than in controls (Fig. 5; $P < 0.05$). A 24-h exposure to $10^{-4}$ M indomethacin also significantly decreased INSL3 production by 34% (Fig. 5; $P < 0.05$). A 24-h exposure to $10^{-5}$ M indomethacin did not significantly reduce INSL3 production ($-16\%$, NS at $P < 0.05$). INSL3 concentrations after 48 h of exposure to any of the treatments were not significantly different from control values.

**Mild analgesics and inhibin B production by the human testis**

Exposure for 24 h to $10^{-5}$ M aspirin decreased inhibin B production by 11% ($P < 0.05$). None of the other treatments had a significant effect on inhibin B secretion by testis explants in culture, irrespective of the dose and the time of exposure tested (Fig. 6).

**Mild analgesics and PG synthesis by the human testis**

Following 24 h of exposure to $10^{-5}$ M ketoconazole, PGD$_2$ concentrations were more than doubled compared with the control values, and PGE$_2$ concentrations were 78% higher (Fig. 7; $P < 0.05$). After 24 h, all of the mild analgesic treatments, except for $10^{-5}$ M paracetamol, resulted in significantly lower than control concentrations of both PGD$_2$ (by 28% for $10^{-4}$ M paracetamol; by 28 and 57% for $10^{-5}$ and $10^{-4}$ M aspirin, respectively; by 79 and 75% for $10^{-5}$ and $10^{-4}$ M indomethacin, respectively; $P < 0.05$) and PGE$_2$ (by 38% for $10^{-4}$ M paracetamol; by 29 and 65% for $10^{-5}$ and $10^{-4}$ M aspirin, respectively; by 95 and 96% for $10^{-5}$ and $10^{-4}$ M indomethacin, respectively; $P < 0.05$).
Mild analgesics have recently been incriminated by several epidemiological and toxicological studies to act as endocrine disruptors during human fetal life (Berkowitz and Lapinski, 1996; Jensen et al., 2010; Kristensen et al., 2011b, 2012; Snijder et al., 2012).

We report that mild analgesics at pharmaceutical doses can directly cause multiple endocrine disturbances in the human adult testis, particularly in the interstitial compartment. The three molecules tested exerted both anti-androgenic and anti-prostaglandinic effects, somehow differing according to their nature, and to some extent, according to their dose. Paracetamol appeared to be the weaker inhibitor of PG secretion, and not to disrupt INSL3 production, whereas INSL3 production was inhibited by aspirin and 10^{-5} M indomethacin. Consistent with the anti-androgenic effect on cultured human testis, and as a validation for these observations, we found that mild analgesics also reduced steroidogenesis by 10–43% from the control values in human steroid-producing NCI-H295R cells.

According to the literature, a standard dose of 1 g paracetamol results in the serum concentration in adult men peaking 48 min after administration at 11 mg/ml, which corresponds to 7.3 \times 10^{-5} M (Prescott, 1980). A 600 mg dose intake of aspirin per os was found to lead to a maximal serum concentration of 2.10^{-4} M in adult men (Dalvi et al., 1985). Two hours after a single oral administration of 100 mg indomethacin, the plasma concentration in healthy adult men peaks at 6 mg/ml, which corresponds to 1.7 \times 10^{-5} M (Emori et al., 1976). As the testicular concentrations of paracetamol and NSAIDs are unknown in adult men, we used these pharmacokinetic data as an estimate of the human exposure in order to select the concentrations at which to test these mild analgesics: 10^{-5} and 10^{-4} M.

For all doses and drugs, the anti-androgenic effects on the testis explants declined between 24 and 48 h of exposure. This might be due to the increased variability in testosterone levels measured after 48 h of exposure. However, such a transient anti-androgenic effect has similarly been observed for phthalates using the same culture model (Desdoits-Lethimonier et al., 2012). The latter study revealed that human adult testis explants have the ability to biotransform some xenobiotics. Therefore, it is also possible that the mild analgesics added to the TEXAS system were at least partly metabolized between 24 and 48 h of incubation by the explants. This issue requires further investigation. Furthermore, it cannot be excluded that an hydrolysis of aspirin in water may also occur to explain the fading of its effects between 24 and 48 h, but this remains to be assessed.

In our experiments, the direct exposure of the human testis to mild analgesics had no effect on the gross morphology of the testis. As mild analgesics did not impact the number of Leydig cells, the hormonal disruptions observed cannot be attributed to their death. In contrast, 10^{-4} M aspirin and 10^{-5} M indomethacin induced an apparent increase in the relative proportion of Leydig cells in the interstitial tissue, the origin of which is unknown.

The well-documented anti-inflammatory properties of aspirin and indomethacin result from their specific ability to inhibit the COX site of Prostaglandin H2 synthetase (PGHS), an enzyme that transforms arachidonic acid into a number of PG inflammation mediators (Vane and Botting, 1996). Extractions and RIAs of PGE and PGF2α have demonstrated the expression of the PG system within the adult human testis (Carpenter et al., 1978), and both PG receptors...
and synthetases have been found in human Leydig cells (Tokugawa et al., 1998; Schell et al., 2007). PGs are also produced by the adult rat total testis (Carpenter et al., 1974) and Sertoli cells (Cooper and Carpenter, 1987), and Sertoli cells express PGE2 and PGF2 receptors (Ishikawa and Morris, 2006). PGs are also abundant in human semen, but are believed to originate mostly from the seminal vesicles (Ishikawa and Morris, 2006). PGs are also abundant in human semen, and Sertoli cells express PGE2 and PGF2 receptors (Gerozissis et al., 1982).

The implication of paracetamol in the PG biosynthesis pathway is less obvious than that of aspirin and indomethacin. Paracetamol may specifically inhibit the peroxidase site of PGHS (Anderson, 2008), but its mechanism of action is still controversial. It was recently suggested that paracetamol may act on the endocannabinoid system via the TRPA1 receptor pathway (Andersson et al., 2008), and this would explain why a higher concentration of paracetamol than of aspirin or indomethacin was needed to affect PG synthesis.

A few experiments have explored the existence of a relationship between PG production and steroidogenesis in both rats and primates. Several studies based on in vivo administration of PGE2 and/or PGF2α reported an inhibition of steroidogenesis in the adult rat (Saksena et al., 1973; Didolkar et al., 1981; Fuchs and Chantharaksri, 1981; Sawada et al., 1994; Gunnarsson et al., 2004) and more specifically of the androgenic response to LH (Fuchs and Chantharaksri, 1981; Marinero et al., 1996) or hCG (Romanelli et al., 1995). However, injection of PGF2α into male rhesus monkeys was followed by an abrupt and almost immediate increase in the production of both testosterone and LH (Kimball et al., 1979). To date, very little is known about the relationship between the PG system and steroidogenesis in men. Healthy male volunteers treated with 800 mg of aspirin daily for 7 days showed a decreased androgenic response to hCG stimulation (Conte et al., 1999). Oligospermic patients given 25–100 mg indomethacin for 30–90 days displayed increased plasma FSH and LH concentrations and decreased plasma testosterone concentrations (Barkey et al., 1984). In contrast, healthy young men treated with 25 mg of indomethacin for 14 days displayed unchanged testosterone, LH and FSH levels (Knuth et al., 1989). The relationships between the PG system and steroidogenesis, therefore, seem to be governed by complex hormonal balance mechanisms involving gonadotrophins, which remain to be elucidated. The poor or unresponsiveness of the human testis to LH or hCG stimulation in vitro (Huhtaniemi et al., 1982; Roulet et al., 2006)—attributed to the lower numbers of LH receptors present in the human compared with the rat testis (Huhtaniemi et al., 1982)—prevented us from exploring this aspect of testicular physiology in response to mild analgesics.

In the TEXAS system, the testosterone decrease induced by mild analgesics appeared to be uncoupled from PG production. Indeed, although aspirin and indomethacin treatments were associated with major anti-PG effects, only paracetamol and 10⁻⁵ M indomethacin significantly inhibited testosterone production. The opposite occurred with 10⁻³ M ketoconazole, for which a sharp inhibition of testosterone production and a substantial increase of PG synthesis were observed. Whether or not this increase in PG synthesis was a consequence of the germ cell loss induced by ketoconazole remains to be elucidated. In any case, it cannot be excluded that mild analgesics could act on steroidogenesis via both a PG-dependent and independent pathway.

INSL3, an hormone responsible for the first phase of testicular descent in mammals, is produced by both fetal and adult Leydig cells (Nef and Parada, 1999; Bay et al., 2011). Unlike testosterone and PGs, which were affected by all the treatments, INSL3 production was only significantly decreased by the two concentrations of aspirin tested and by the higher concentration of indomethacin. This is the first report of INSL3 disruption in adult human testis following exposure to chemical agents: in the same experimental setting, the effects of phthalates on Leydig cells remained restricted to their steroidogenic capabilities (Desdoits-Lethimonier et al., 2012). The role of INSL3 in normal adult testicular physiology is poorly understood, despite its constitutive expression (Mitchell et al., 2012). INSL3 may play a role in germ cell maintenance, affect other tissues such as prostate, kidney or thyroid (for review, Ivell and Anand-Ivell, 2009) and contribute to bone metabolism (Ferlin et al., 2008). Thus, the consequences of endocrine disruption that affects the ability of Leydig cells to produce INSL3 will remain obscure until the role(s) of this hormone is (are) better known.

The only hormone that remained almost totally unaffected by a direct exposure to mild analgesics was the Sertoli cell product inhibin B. Indeed, the inhibition of its production by a 24-h exposure to 10⁻³ M aspirin was weak (−11%; Fig. 6). In similar conditions, inhibin B production was unaffected by the exposure to phthalates (Desdoits-Lethimonier et al., 2012). Although PGE2 and PGF2α are synthesized by Sertoli cells in adult rats (Cooper and Carpenter, 1987), before this work their presence in adult human Sertoli cells has never been explored, and nothing is known about their link, if any, with Sertoli cell products. Inhibin B secretion by rat Sertoli cells is regulated by the inflammation mediator IL-1β (Okuma et al., 2005), and IL-1β induces COX-2 in various tissues, including Leydig cells in infertile men (Matzkin et al., 2010); therefore, the aspirin-specific inhibition of inhibin B production may be a consequence of a putative link between the PG system and inhibin B production by Sertoli cells. However, this suggestion is speculative at this stage.

We report the first evidence that direct exposure to mild analgesics can result in several endocrine disturbances, causing an imbalance in the hormonal activity of the adult testis in short-term culture. The active doses of analgesics were of the same order of magnitude as plasma concentrations during standard analgesic intake. Our study demonstrates that the testicular PG system is highly susceptible to these molecules. However, the interactions between different testicular hormones under COX-disruptive conditions, in particular the relationship between PGs and testosterone, remain unclear. Our findings are consistent with the concerns recently raised both by epidemiological studies on mild analgesic consumption and by in vitro experiments on the potential targeting of PGs by endocrine disruptors. The present in vitro results highlight the risks associated with the abusive use of mild analgesics for the testicular endocrine system in men.

**Supplementary data**

Supplementary data are available at http://humrep.oxfordjournals.org/

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Mild analgesics and the adult human testis

Authors’ roles

B.J. and N.D.-R. designed the research; O.A., C.D. and L.L. performed the research; F.G. and K.B. supervised and performed orchidectomies; A.L. contributed to critical discussion. All authors contributed to critically reviewing the draft manuscript. All authors saw and approved the final version of the report.

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Conflict of interest

The authors declare they have no competing interests, be it financial, personal or professional.

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