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Impact of trenbolone on selected organs

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Abstract

Trenbolone is a synthetic analogue of testosterone, belonging to the nandrolone group. It has both a strong anabolic effect and a limited androgenic effect (i.e. an androgen and anabolic steroid — AAS). It is used illegally by professional or amateur athletes, who want to improve their athletic performance and appearance by increasing their muscle mass. Trenbolone, like other AASs, are harmful, with 90% of users experiencing injurious side effects. It acts systemically on the body, and as such, its side effects can manifest as symptoms from different systems. Nevertheless, its popularity is increasing. This paper reviews the current state of knowledge regarding the adverse effects of trenbolone on the nervous, reproductive, immune systems and breast, muscular and adipose tissues. However, various other adverse consequences of trenbolone utilization are observed, with severe acne and gynaecomastia affecting approximately one-third of all users, as well as excessive body hair, stretch marks, hypertension and cardiac arrhythmia. The drugs are also subject to contamination, with use frequently resulting in local inflammation at the injection site, muscle adhesions and fibrosis, nerve damage or, in extreme cases, necrosis of the injection site. Additionally, due to the lack of available knowledge on the subject, many of the effects of trenbolone use remain unknown. Moreover, the fact that multiple AASs may be used simultaneously presents a significant problem in their study. Therefore, further research is necessary to better understand the effects of AAS on the body, and to expand our currently incomplete knowledge of their functional pathways. **(Endokrynol Pol 2024; 75 (3): 267–278)**

Key words: *androgens; anabolic steroids; steroid cycle; doping*

Introduction

Anabolic-androgenic steroids (AAS) are among the most dangerous substances commonly used in Western societies. The global lifetime prevalence rate obtained in 2014 was 3.3%, with the rate being four times higher for men than for women [1]. In most countries, access to AASs is unrestricted, either through numerous websites or illegal sales by private individuals, often in sports centres.

Under Polish law, limited possession of such substances in undetermined quantities for personal use is not illegal [2]. As many as 70–80% of AAS users are amateur level athletes, who primarily want to improve their appearance by increasing their muscle mass, or their athletic performance [3]. Worryingly, many AAS users lack adequate medical knowledge and use the substances without any medical supervision. Indeed, more than 90% of users have been found to experience injurious side effects [3].

One of the most potent [4] and commonly-used substances [3] is trenbolone, a steroidal analogue of testosterone. Trenbolone was introduced to the market in the 1970s, when it was used in the agri-food industry as a growth promoter for livestock in the USA [5]. The cattle farmers used it to obtain defatted animal meat containing more muscle tissue. In such cases, trenbolone was administered to livestock as a subcutaneous implant with a slow release time.

Since the 1980s, the use of AASs, and thus trenbolone, has become more popular in among athletes in Western societies. A few decades ago, most users were believed to be professional athletes; however, this has since changed dramatically, with as many as 70–80% of AAS users now being amateur athletes [1, 3, 6]. Furthermore, studies indicate a growing tendency in the use of these substances in developed countries, with most of users commencing "steroid cycles" at a median age of around 20 years [3].

Structure and chemical characteristics

Trenbolone (Fig. 1) is a synthetic analogue of testosterone, belonging to the nandrolone group [7, 8]. Compared to testosterone, this group is characterised by the demethylation of the carbon at the C19 position. This augments the anabolic effect of nandrolones, while also simultaneously limiting their androgenic effect on the body [3, 9].

After entering the mammalian body, trenbolone is rapidly hydrolysed to 17-β-trenbolone (17β-hydroxyestra-4,9,11-trien-3-one) [10]. This metabolite is officially

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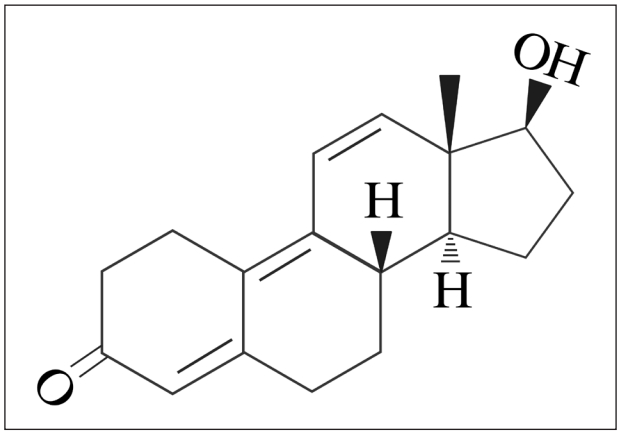

Figure 1. *Trenbolone (C11H22O2) — structural formula*

banned in sports and figures on the World Anti-Doping Association (WADA) list [11]. 17β-trenbolone is a potent mammalian androgen receptor (AR) agonist comparable to 5α-dihydrotestosterone (DHT), which is considered the most biologically active metabolite of testosterone [4, 12]. However, due to the presence of a 3-oxotriene structure in its formation [10], it is not a substrate for the enzyme 5-alpha-reductase or aromatase [4]. Due to its lack of aromatisation to estrogens, trenbolone is more androgenic than nandrolone, another potent testosterone analogue [7].

Side effects

Studies indicate that as many as 90% of users experience a wide array of side effects on various organs, which creates a significant issue for public health institutions on a global scale. Its perceived side effects are so serious that it has been placed on the USA register of dangerous substances [13].

Effects on nervous tissue

An important biochemical characteristic of trenbolone is its structure. It contains a sterane core, and is thus classified as a lipophilic compound [7]. It is thus able to penetrate the blood-brain barrier [7, 14] and exert a potential effect on the central nervous system. A number of studies have found AAS [15–17], and hence trenbolone, to influence numerous neurochemical systems and receptors in rodent brains, resulting in a range of somatic symptoms and changes in the behavioural patterns of the studied animals. While it is possible that similar effects might be observed on human neural tissue, these have yet to be confirmed.

One of the most severe impacts of trenbolone is its influence on the cerebral cortex: long-term use of this agent has been shown to reduce its volume in rats, which implies that brain tissue atrophy may take place [18]. It is suspected that there is a link between the induction of pro-apoptotic processes caused by this agent [18-22] and a reduction in viability, as well as an increase in toxicity to central nervous system cells. The induction of apoptosis is believed to be triggered the activation of both caspase 3 (CASP3) and caspase 7 (CASP7) by trenbolone, which can occur even at low doses [18]. Both caspases belong to a group of 12 serine proteases whose function is intrinsically linked to programmed cell death, and are among those known as *apoptosis executors* [23, 24]. Hence, it is believed that the observed decrease in neuronal abundance in the brain and thus its volume, may be due to the activation of these caspases by trenbolone.

Furthermore, trenbolone use has been associated with decreased mitochondrial activity and elevated blood lactate dehydrogenase (LDH) levels, indicating general damage to cells throughout the body. This was found to be accompanied by chromatin condensation and nuclear fragmentation in the structure of brain cells, which may reflect the damage occurring in the cells and the occurrence of apoptosis [14]. The effects in question depended on the dose and duration of trenbolone use, with even nano-molar concentrations increasing the risk of excitotoxic cell death in mouse cortical cultures [24].

These cytotoxic effects occurring in both animals and cell cultures could possibly be responsible for the behavioural changes observed in users of AASs, such as trenbolone. These included disturbances in parts of the central nervous system, particularly those concerning cognitive function, learning efficiency and visuospatial memory [3]. Furthermore, specific changes were noted in the medial prefrontal cortex (mPFC) region, which plays a key role in the biochemical stress axis in the brain [25, 26]. The region was found to manifest changes in oligodendrocyte differentiation, translating into alterations in the formation of mouse myelin basic protein [26]. These changes have been correlated with characteristic behavioural patterns in mice, such as disorders in establishing social relationships and high levels of anxiety [26]. Similar disturbances are also observed in trenbolone users [27, 28].

A separate issue is the effect of trenbolone on amyloid plaque formation processes. Studies on a rat model found trenbolone use to reduce the amount of the protein presenilin 1 (PS1), involved in the activity of the enzyme γ-secretase, which is connected to the formation of abnormal protein-Aβ42. The formation of this abnormal protein is directly proportional to the trenbolone dose taken, and the highest amount was observed in the hippocampus [14]. This protein is responsible for the formation of senile plaques and shows strong neurotoxic effects, both from the larger agglomerations of

the protein and the smaller oligomers and protofibrils surrounding them. The structures modify the curvature of neuronal cells and distort them, which can affect neurotransmission along their length and disrupt the transmission of neural signals through neuronal synapses [29]. A similar polypeptide has been found to accumulate in other neurodegenerative diseases such as Alzheimer's disease [29]. The formation of protein deposits can be correlated with symptoms from the hippocampus, which include impaired multiple memory function [30]. Such memory deficits are also observed in trenbolone users, and mainly take the form of deficits in everyday memory [31].

Another observed activity of trenbolone in the central nervous system is its effect on ionotropic N-methyl-D-aspartate receptors (NMDARs), which are heterotetramers consisting of two obligatory GluN1 subunits, and two GluN2 or GluN3 [32, 33]. All subunits can be encoded by different genes and can be formed by alternative splicing. It has been found that these components can combine in as many as 60 combinations that can fit NMDARs [32]. The fact that changes in the composition of the subunits in the receptor can affect the biochemical activity of NMDARs [34, 35] and that the expression of specific subunits is dynamic and can fluctuate with time [36, 37] may be of importance.

Taking testosterone analogues such as trenbolone has been shown to modulate subunit composition and thus decrease the formation of mRNA encoding the GluN2 subunit in the hippocampus and hypothalamus. Moreover, studies on rat models have found usage to be associated with a reduction in the overall amount of NMDARs, particularly in the hypothalamus and hippocampus [15]. In both the hypothalamus and hippocampus, NMDARs have important roles in brain function. In the hypothalamus, for instance, they are responsible for aggression [38], whereas in the hippocampus they are responsible for personal memory, and their disruption has been implicated as a possible cause of epileptic disorders [39].

As mentioned earlier, changing the proportion of individual subunits can affect the function of NMDARs. In the case of the hypothalamus, such alterations may be responsible for the aggressive behaviours reported abundantly in animal research [40]. In human studies, trenbolone users also reported aggression as one of the most perceived side effects [41]. A decrease in the number of receptors in the hippocampus [15] is also likely to affect memory deficits in users; however, these may arise through a different pathway [31].

Gamma-amino butyric acid (GABA) receptors are present in the central nervous system and their function is significantly affected by AASs such as trenbolone. Studies suggest that GABA receptors may be sensitive to natural sex steroids. Their influence may manifest as specific behavioural changes during sexual maturation in users who are experiencing hormonal dysregulation [42, 43]. These hormones lead to changes in the expression and function of GABA receptors, such as modification of neuronal transmission, its allosteric modulation and the activation and sensitisation of receptors [43]. GABA type A (GABAA) receptors in the forebrain, specifically in regions such as the medial preoptic area (mPOA), ventromedial nucleus accumbens (VMN) and medial amygdala (MeA), are thought to influence feelings of anxiety, aggression and sexual desire in mammals [43]; all have been reported to be altered in AAS users [3, 25–28, 41], which may also result from the modification of their receptors by trenbolone.

Effects on muscle tissue

The most commonly-reported motivation for beginning AAS use is the desire to obtain a muscular, proportional and aesthetic physique in the shortest possible time [3]. However, some athletes training for a specific sport may turn to AASs to improve their own performance or enhance their appearance (e.g. professional bodybuilders) [3].

Trenbolone exhibits one of the highest anabolic-androgenic ratios, and is therefore one of the most potent agents for stimulating muscular development [44]. Such an effect is called anabolism, a state in which nitrogen is retained in lean body mass by stimulating the synthesis of new proteins and/or inhibiting the degradation of proteins already present. It promotes an increase in muscle protein synthesis and collagen fibres, and an upsurge in bone metabolism [45].

Muscle hypertrophy, i.e. an increase in the size and thus volume of muscle fibres, is the leading desired effect of trenbolone use. Unfortunately, the exact mechanisms of this effect have not been fully characterised. Studies have observed that hypertrophy occurs through several pathways induced by androgens such as trenbolone. The first of these, known for many years as the genomic, or classical, pathway (Fig. 2), occurs through the anabolic agent binding to the cytosolic AR; in such cases, trenbolone has three times higher affinity than testosterone. This results in the translocation of the agent and the bound AR into the nucleus of the muscle cell, with movement being dependent on the dose of the attached agent. Finally, in the cell nucleus, the androgen and receptor combine with chromosomal DNA, at sites called androgen response elements (AREs), in the form of homodimers. These promote the transcription of specific genes and subsequent translation of proanabolic proteins [4]. This pathway is slower, and its effects can manifest over a longer period of time.

In contrast, the second biochemical/molecular pathway also involves the AR, but the anabolic effects that occur are mediated by the Wnt/ β-catenin and Notch pathways (Fig. 2). Wnt signalling is an extremely important pathway, associated with a set of glycoproteins. Its purpose is to regulate myogenesis, both during embryogenesis and at maturity. It promotes the differentiation of stem cells into myogenic cells and, if required, the repair of damaged muscle tissues [46–48]. First, β-catenin acts as a transcriptional effector. The molecule binds to the transcriptional repressor Tcf2/Lef in the nucleus of the muscle cell, converting it into transcriptional activators that induce proanabolic genes acting as targets of the Wnt pathway. Several studies indicate that the administration of trenbolone increases β-catenin levels and thus Wnt pathway levels [49–51]. This is likely due to the negative effects of trenbolone on glycogen synthase kinase 3 (GSK3), another component of this molecular system. The kinase is related to the Notch pathway, which inhibits the Wnt pathway, and marks β-catenin for intracellular degradation by phosphorylation; in the absence of GSK3, the amount of β-catenin will increase and perform its role as a translation factor [4].

Both the Notch pathway and the Wnt-related pathway share very similar cellular functions in regulating myogenesis; however, Notch pathway is Wnt-related pathway antagonist and additionally promotes satellite cell activation and differentiation [52–55]. Both pathways constitute an integrated molecular system in which each component interacts with the other. The exact interactions between the pathways are not fully understood, but the Numb molecule, which inhibits the Notch pathway and participates in myocyte cell gene recombination, is thought to act as a signalling molecule [55–61].

Nandrolone or trenbolone administration results in increased levels of β-catenin, and thus its greater attachment to the regulatory sites of the Numb gene promoter (at the Tcf site); this causes an increase in the expression of the gene encoding Numb, and thus greater Numb mRNA/protein levels in the nuclear

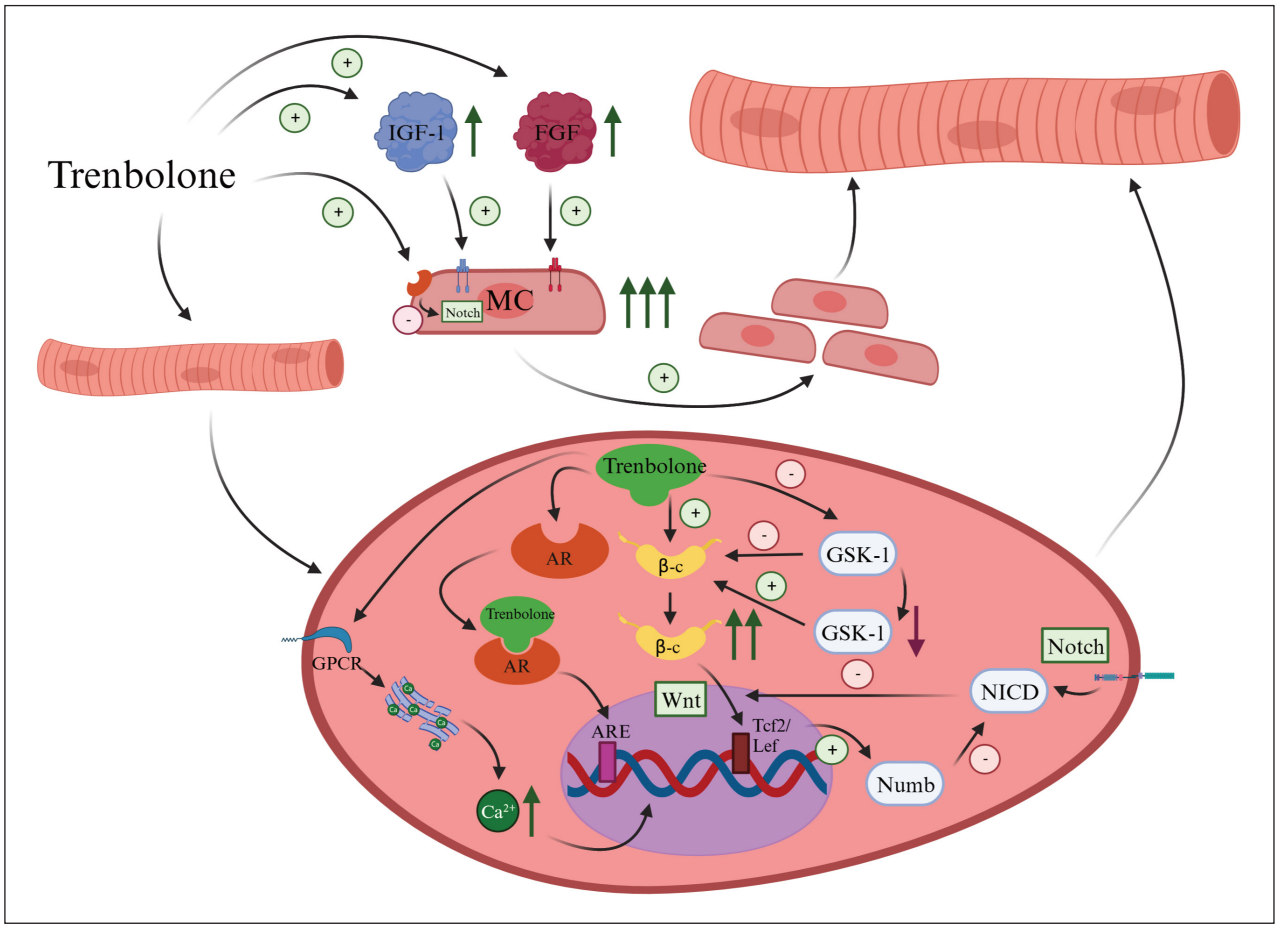

Figure 2. *Skeletal muscle hypertrophy. AR — androgen receptor; ARE — androgen response elements; β-c — β-catenin; FGF — fibroblast growth factor; GPCR — G protein-related receptor; GSK-1 — glycogen synthase kinase 1; IGF-1 — insulin-like growth factor 1; MC — myosatellite cell; NICD — Notch intracellular domain; Notch — Notch signalling pathway; Tcf2/Lef — transcriptional repressors/activators; Wnt — Wnt signalling pathway*

and cytosolic fractions [62–64]. This probably occurs through conformational stabilisation of the Numb protein; this is made possible by reducing the concentration of the MDM2 molecule, related to the p53 protein, and altering the level of the Musashi protein, which is involved in organismal development and the fate determination of individual cells [65]. In summary, trenbolone administration increases the levels of the Numb molecule, thus enhancing the pro-anabolic action of the Wnt pathway by further inhibiting the Notch pathway, an antagonist of the Wnt pathway.

However, recent studies indicate the presence of another pathway through which androgens can potentially act, *viz.* the non-genomic pathway (Fig. 2), also referred to as the *fast pathway*, because its action involves a surface receptor associated with the G protein. The pathway is activated by a sudden increase in calcium (Ca2+) levels from intracellular stores. Unfortunately, the pathway is believed to act through testosterone, not trenbolone, and has been described in IC-21 macrophage cells [66, 67]. Even so, it is still possible that this pathway could occur in cells other than macrophages, such as muscle cells, and that they could influence the action of anabolic substances: they may stimulate the binding androgens to AR or influence gene expression [63, 68, 69]. Furthermore, it is possible that by acting on similar structures in the cell, trenbolone could have similar non-genomic activity as testosterone on various cell types. However, more research on this topic is required to confirm this.

Finally, trenbolone can also influence skeletal muscle tissue growth by changes associated with muscle satellite cells (Fig. 2). This is a group of cells located in the niche between the muscle fibre membrane and the surrounding basement membrane, which have the ability to differentiate into myoblasts and form subsequent generations of muscle cells. They are categorised as tissue-targeted unipotent stem cells [70–72]. During laboratory studies, their activation was recorded as early as the first hour after trenbolone application, and this continued for 12 hours. They then entered a resting state, from which they could re-activate under the influence of trenbolone after a further 24-hour period [53]. Their activation is probably driven by ARs, which increase in number after trenbolone treatment, reflected in an increase in their mRNA levels [73, 74]. AR stimulation may induce the expression of mind bomb 1 (Mib1) — ubiquitin ligase and the subsequent trans-endocytosis of the Notch pathway ligands Delta-like 1 (DLL1) and Jagged 1 (JAG1) in the myofibers [75, 76]. These actions inhibit Notch pathway signalling, which is responsible for maintaining satellite cells in a quiescent state. This leads to the activation of satellite cells, during which, the satellite cells become direct precursors of skeletal muscle, i.e. myoblasts. The newly-formed myoblasts then fuse with each other and integrate into adjacent mature muscle cells by fusion, resulting in significant changes in the muscle tissue: an increase in the number of cell nuclei with a corresponding increase in the amount of DNA by up to 60% in some of the muscles studied, an expansion in the total amount of cytoplasm and contractile proteins (actin and myosin). The increase in the amount of actin and myosin results in the muscle cell generating more total force [44].

These changes may potentially be responsible for user reports of significant increases in total muscle strength following use of the drug. The exact molecular mechanisms behind the activation of satellite cells by trenbolone have not been fully explained. However, studies have shown that direct addition of trenbolone to the *in vitro* cell culture containing satellite cells did not increase their proliferation, differentiation or fusion. Instead, it was found that trenbolone increased the sensitivity of satellite cells to two growth factors, IGF-1 and FGF, which acted as mediators indirectly stimulating the activation and further function of satellite cells [77].

In addition to satellite cell sensitivity, trenbolone is also believed to be involved in the potentiation of various mediators. Notably, various studies report a build-up of IGF-1 mRNA in many tissues after trenbolone treatment, resulting in an increase in blood IGF-1 level [78, 79].

Such an increase may significantly affect sensitised satellite cells and induce their activation and proliferation, potentially resulting in skeletal muscle growth.

Effects on adipose tissue

It was observed that hypogonadal rats, characterised by low levels of testosterone, demonstrated a statistically-significant decrease in muscle mass and increase in adipose tissue, which is analogous to observations of humans [4, 80]. In this study, moderate doses of exogenous testosterone halted the decrease in muscle mass and reduced fat gain, while higher doses resulted in a noticeable increase in muscle mass and a concomitant reduction in body fat compared to control rats without induced hypogonadism [4]. The study yielded extremely satisfactory results regarding the marked inhibition of catabolic effects in the body, and confirmed the validity of hormone replacement therapy in humans with low testosterone levels.

Unfortunately, the mechanisms by which trenbolone affects adipose tissue remain unknown. However, trenbolone is known that trenbolone demonstrates a high affinity for ARs commonly found in the cells that make up adipose tissue e.g. adipocytes, pre-

adipocytes and mesenchymal stem cells [81], and that androgens inhibit the differentiation of preadipocytes and mesenchymal stem cells into mature adipocytes through AR [82]. Such effects may occur through reduced expression of adipogenic genes, such as PPARγ and C/EBPα, as well as through increased nuclear translocation of β-catenin and activation of Wnt signalling, promoting the myogenic lineage [76, 83]. All these activities may lead to lipolysis of adipose tissue. In various laboratory studies, rats have been observed to demonstrate a marked loss of subcutaneous adipose tissue, intramuscular fat, retroperitoneal fat and perirenal fat after trenbolone treatment [84–86]. It has also been shown that trenbolone can directly induce lipolytic effects by affecting the liver; presumably, this may occur by increasing the expression of the enoylo-CoA hydratase (ECH) and acyl-CoA dehydrogenase involved in fat metabolism in this organ [87].

Hence, it can be concluded that trenbolone appears to have a lipolytic effect on adipose tissue. These findings could potentially confirm the beneficial fat-burning effects of androgens reported in human cell studies [82]. However, studies are needed to confirm this effect in human groups, particularly with regard to trenbolone, and identify the mechanism responsible.

REVIEW

Effects on male reproductive system

The World Health Organisation (WHO) classifies infertility as the state of not being able to get pregnant, despite regular sexual intercourse (three or four times a week), maintained for more than 12 months, without any preventive measures [88]. Infertility is a serious problem in developed countries, where it is estimated to affect about 10–12% of couples; this would equate to nearly one million couples in Poland. It is highly probable that the number of infertile men and women will increase over time, as a result of delayed decisions about parenthood. The increasing use of AASs by men, and its associated infertility, adds to the problems facing the public health institutions of developed countries in the realities of the modern world.

Infertility in users of AAS, including trenbolone, most commonly manifests as oligo- and azoospermia, as well as abnormalities in sperm mobility and morphology [89]. In addition, significant hormonal deviations can be observed, but decreased libido levels or erectile dysfunction, are typically only reported after ceasing the use of exogenous hormones. Such changes include decreased levels of endogenous testosterone and the pituitary hormones LH and FSH. The symptoms can be defined by the term "functional hypogonadism" [44, 90], which manifests as a significant decrease in endogenous testosterone, testicular atrophy and impaired spermatogenesis [91]. The endocrine abnormalities associated with the condition derive from the negative effects of exogenous androgens on the hypothalamic-pituitary-gonadal axis, as well as their direct action on Leydig cells, which produce testosterone under physiological conditions [91].

A number of studies have also report AAS-induced morphological changes in various cells of the male reproductive system and the male gametes. One such change observed in male rat testes involves structural changes in Leydig cells, and an overall reduction in their number, resulting in a decrease in endogenous testosterone; in contrast, physiological concentration determines optimal spermatogenesis [92, 93]. The impaired spermatogenesis has also been observed as a lack of advanced forms of spermatids (spermatogenesis arrest) and various changes in spermatozoa. One of the most noticeable changes was a decrease in sperm cell motility. Fluorescent in-situ hybridization (FISH) studies have noted changes in cellular ultrastructure in some spermatozoa, as well as an increased frequency of sex chromosome (XY) disomy, indicating a segregation anomaly at the first meiotic division and of chromosome 9 disomy [94, 95]. Last but not least, elevated apoptosis has been noted in sperm cells; when research subjects are administered agents from the nandrolone group, such as trenbolone, the degree of apoptosis increases above level as noted during intensive physical exercise under physiological conditions, which is already elevated from proper intensity of apoptosis, anyway [96].

Some studies were carried out in rats with nandrolone, a compound with a similar mechanism of action and belonging to the same AAS group, so it can be speculated that trenbolone will induce similar changes. In these experiments a decrease in the diameter of seminiferous tubules was noticed, what is associated with the lower height of the seminiferous epithelium and hypospermatogenesis [97]. Moreover, changes in the blood-testis barrier were found, such as the degradation of the tight junction protein 1 (TJP1), deregulation of metalloproteinase 9, metalloproteinase 2 (MMP-2), the tissue inhibitor of MMP-2 and mislocalization of mucin 1 [98]. All these changes can lead to impairment of blood-testis barrier and, as a consequence, disturb the immune integrity of testicles.

In most men, a spontaneous return of semen quality occurs four to twelve months after discontinuation of exogenous anabolic agents [44, 92]. However, in those who do not recover, treatment may be applied as in other forms of hypogonadotropic hypogonadism. Gonadotropins or their analogues are then used. It has been found that the greatest chance of restoring fertility is associated with the use of an LH/hCG-acting preparation, alone or in combination with an FSH-act-

ing preparation. Antiestrogens (aromatase inhibitors and selective estrogen receptor modulators — SERMs) are used to unblock pituitary, irrespective of blood estradiol levels. Reduced inhibiting influence of estrogen on hypothalamus and pituitary gland leads to an increase in both gonadotropins secretion and thus spermatogenesis recovery [99].

With these methods, it has been shown that even prolonged infertility, manifested by oligozoospermia, can be cured up to five years after AAS withdrawal [100]. However, the use of these agents permanently affects semen quality, as indicated in studies of previous AAS users; the treatment results in a higher than normal amount of hypokinetic and abnormal sperm in the semen [44, 100, 101].

Effects on the breast gland

Gynecomastia, which is characterized by development and swelling of breast tissue, is frequent side effect in trenbolone users. Although trenbolone does not convert to estrogen it binds with high affinity to the progesterone receptor [102]. Progesterone appears to be required to form true glandular acini acting in synergy with insulin growth factor 1 (IGF-1) [103]. AAS cause also a reduction in the level of thyreoglobulin (TBG), which binds thyroid hormones in the blood. This implies a reduction in the serum levels of free triiodothyronine (fT3) and thyroxine (fT4) [104]. As a result of a negative return loop this causes an increase in TRH secretion, which stimulates prolactin secretion [105]. Prolactin receptors have been demonstrated in the breast tissue. Moreover, hyperprolactinemia probably plays an indirect role in gynaecomastia, since it causes central hypogonadism and alters the androgen/estrogen ratio [106].

Effects on the immune system

Another interesting issue regarding the use of trenbolone, is its effect on the immune system. So far, relatively few studies have explored the effects of high concentrations of AAS in this regard; however, a relationship has been noted between the immune and endocrine systems in vertebrate organisms [107–109], and it seems certain that AAS use can cause disturbances in hormone levels and general endocrine disruption. As mentioned, these disorders are not isolated from the immune system, and it is important to note that androgens can influence innate and specific immunity. Furthermore, it is known that endogenous steroid sex hormones reduce immunity [110], and the potential harm of the synthetic steroid trenbolone on immunity merits further interest. Men using AAS report decreased immunity during and immediately after a trenbolone "cycle", such as an increase in the incidence of various colds, viral and bacterial infections and hemiplegia, with recurrences noted after treatment [111, 112].

It was revealed that endogenous testosterone has a suppressive effect on the immune system [113]. This action affected T lymphocytes in particular and included a general reduction in their activity levels. However, the pathway by which this effect occurs was not explained. Instead, it was found that it could occur directly by acting on the AR present on the lymphocytes or by converting testosterone to estrogen and acting through a pathway related to it. In another study, castrated rats demonstrated a 90% increased immune response [114] compared to controls, and this value decreased exponentially after external testosterone was administered.

While these findings provide a general picture of the immunosuppressive role of endogenous, steroidal sex hormones, more detailed information has been obtained regarding their influence on T lymphocytes. T lymphocytes can be divided into three subpopulations based on their function: T cytotoxic (Tc), T helper (Th) and T regulatory (Treg) lymphocytes. Accordingly, the first population is responsible for destroying cells infected by microorganisms (including viruses) and tumor cells and the second supports the immune response through various cytokinins, while the final one regulates the immune response process and, if necessary, inhibits it. It has been presented, that the immune response exponent was the phytohemagglutinin PHA skin test, which yields a classical type IV delayed response: being a myotogen, PHA activates T lymphocytes without the antigen induction necessary in a type IV response [115]. This results in the activation of tissue macrophages by substances secreted by the lymphocytes, and the development of local inflammation on the skin, i.e. delayed cutaneous hypersensitivity (DCH). This reaction is dependent on Th lymphocytes. The study found all applied AASs, including trenbolone, to cause a marked reduction in DCH levels, indicating a reduction in Th lymphocyte activity [116]. The exact metabolic pathways of trenbolone are not known, but it is thought to act both directly through AR, which has been shown to be present on various T-lymphocyte subpopulations [116–118], as well as by modulating hormone secretion by the adrenal cortex or reproductive organs [116].

Trenbolone may also act on T lymphocytes via the activation of membrane receptors caused by increased Ca^{2+} levels. This would initiate a sequence of molecular events in the cell [119, 120] resulting in the successive phosphorylation of individual tyrosine-based substrates, the membrane linker of T cell activation and Src domains [121, 122] and thus the activation of transcription factors, including nuclear factor of activated T cells (NFAT). The final effect of this

pathway is increased expression of transcription factors associated with T cells: T-bet, Gata3, RORt and Foxp3 [123]. These are necessary for the generation of a subpopulation of effector T cells: CD4+ helper T cells. In addition, the activation of NFAT results in increased secretion of various cytokines, e.g. IL-2 and various chemokines, together with the suppression of Treg lymphocytes [120, 124], which may affect the overall immune response in the body.

The use of high levels of trenbolone was found to decrease lysozyme levels significantly in a tissue-specific manner [125]: a significant decrease was observed in trout liver and plasma, while a statistically insignificant decrease was found in the kidney. Interestingly, no significant changes in the expression of the genes encoding this protein were recorded in the two tested organs. However, it is important to note that mRNA and protein expression are not always directly proportional to each other, and in this case, there may have been a simple delay in the expression of this gene connected to a decrease in the level of the enzyme; the timing of the study may not have allowed this to be visualised [126]. Lysozyme is part of the innate immune system and can have a destructive effect on the bacterial cell wall by hydrolysing the glycosidic bond between N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) [127]. In conclusion, reduced levels of lysozyme may have clinical implications, in terms of more frequent or clinically-intense bacterial infections, but more research is needed to confirm this.

Another component of the innate immune system that is ambiguously affected by trenbolone is the complement system. Its levels fluctuated significantly under the influence of different concentrations of an administered anabolic androgen hormone in trout. As with lysozyme, such changes depended very much on the specific area of the body: a significant decrease in activity was demonstrated in plasma, while the opposite effect occurred in kidneys [125]. As a component of the innate immune system, the complement plays various roles during the immune response, following the sequential activation of a protein cascade. Among other things, it is responsible for initiating the inflammatory process, opsonisation and facilitating phagocytosis, and bacterial cell lysis. While it has been proposed that disturbances in its plasma activity can, like lysozyme, impair the immune response against bacteria, more research is needed to confirm this.

A final aspect of the action of trenbolone that should be mentioned is its effect on genes involved in the development and maturation of different lymphoid cell populations. Administration was associated with a marked decrease in the expression of the RAG-1 and RAG-2 genes, which are essential for the proper functioning of lymphocytes [125]. However, no changes were noted in the total number of lymphocytes and the immunoglobulins produced by them. This may be due to the same reasons as described above for lysozyme.

Summary

The effects of trenbolone are not limited to a single tissue. It acts systemically on the body, and as such, its side effects can manifest as symptoms from different systems (Tab. 1).

Table 1. *Effects of trenbolone — summary*

Tissue type	Effects of trenbolone
Nervous	Pro-apoptotic effect on neurons
	Reduction in brain tissue volume
	Accumulation of the toxic protein Aβ-42
	Modulation of the structure and function of GABA and NDMA receptors
Muscle	Stimulation of skeletal muscle for supra-physiological growth through direct effects on muscle fibres and indirect effects on myosatellite cells and endogenous growth factors
Adipose	Lipolytic effects resulting in a reduction of fat deposits in various areas of the body
Reproductive	Induction of functional hypogonadism
	Disturbances in the structure and number of Leydig cells
	Disturbances of the structure and number of sperm cells
	Acceleration of sperm apoptosis processes
Immune	Decrease in T lymphocyte activity
	Decrease in plasma lysozyme levels
	Decrease in plasma complement system activity

GABA — gamma-aminobutyric acid; NDMA — N-nitrosodimethylamine

This article focuses only on the most significant consequences of trenbolone use with regard to selected tissue structures, and the morphological and functional changes observed in them. However, various other adverse consequences of trenbolone utilization are included in the literature, with severe acne and gynaecomastia affecting approximately one-third of all users [3], as well as excessive body hair, stretch marks, hypertension and cardiac arrhythmia [4]. The drugs are also subject to contamination, with use frequently resulting in local inflammation at the injection site [128], muscle adhesions and fibrosis, nerve damage or, in extreme cases, necrosis of the injection site (Nicolau syndrome) [129].

In addition, the fact that multiple AASs may be used simultaneously presents a significant problem in their study. The users may report that they use smaller doses of different agents to minimise the negative side-effects that are more likely to occur when one agent is taken at an increased dose. Bodybuilders usually take trenbolone together with various testosterone esters (cypionate, enanthate or propionate) and nandrolone phenylpropionate [3, 4, 44]. This makes it very difficult to precisely determine the AAS responsible for the negative side effects, and the results cannot always be related to the actual conditions under which trenbolone acts in the body.

Additionally, due to the lack of available knowledge on the subject, many of the effects of trenbolone use remain unknown. Therefore, given its prevalence and potential danger, further research is necessary to better understand the effects of trenbolone use on the body, and to expand our currently incomplete knowledge of its functional pathways.

Author contributions

R.B. — conception, drafting the article, preparation of figures, final approval; P.B. — drafting the article, revising, final approval; J.S.-H. — Conception, drafting the article, preparation of figures, final approval.

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Conflict of interest

Authors declare no conflict of interest.

References

1. Sagoe D, Molde H, Andreassen CS, et al. The global epidemiology of anabolic-androgenic steroid use: a meta-analysis and meta-regression analysis. Ann Epidemiol. 2014; 24(5): 383–398, doi: 10.1016/j.annepidem.2014.01.009, indexed in Pubmed: 24582699.
2. Art. 124 ustawy z dnia 6 września 2001 r. Prawo farmaceutyczne (Dz.U.2022.2301) 2022.
3. Parkinson AB, Evans NA. Anabolic androgenic steroids: a survey of 500 users. Med Sci Sports Exerc. 2006; 38(4): 644–651, doi: 10.1249/01.mss.0000210194.56834.5d, indexed in Pubmed: 16679978.
4. Yarrow JF, McCoy SC, Borst SE. Tissue selectivity and potential clinical applications of trenbolone (17beta-hydroxyestra-4,9,11-trien-3-one): A potent anabolic steroid with reduced androgenic and estrogenic activity. Steroids. 2010; 75(6): 377–389, doi: 10.1016/j.steroids.2010.01.019, indexed in Pubmed: 20138077.
5. Kicman AT. Pharmacology of anabolic steroids. Br J Pharmacol. 2008; 154(3): 502–521, doi: 10.1038/bjp.2008.165, indexed in Pubmed: 18500378.
6. Elks J, Ganellin CR. The Dictionary of Drugs: Chemical Data: Chemical Data, Structures and Bibliographies. Springer, New York 2014: 472.
7. Shahidi NT. A review of the chemistry, biological action, and clinical applications of anabolic-androgenic steroids. Clin Ther. 2001; 23(9): 1355–1390, doi: 10.1016/s0149-2918(01)80114-4, indexed in Pubmed: 11589254.
8. Pottier J, Cousty C, Heitzman RJ, et al. Differences in the biotransformation of a 17 beta-hydroxylated steroid, trenbolone acetate, in rat and cow. Xenobiotica. 1981; 11(7): 489–500, doi: 10.3109/00498258109045859, indexed in Pubmed: 7293236.
9. World Anti-Doping Code. International Standard Prohibited List 2023. WADA, Montreal 2023: 6.
10. Bauer ER, Daxenberger A, Petri T, et al. Characterisation of the affinity of different anabolics and synthetic hormones to the human androgen receptor, human sex hormone binding globulin and to the bovine progestin receptor. APMIS. 2000; 108(12): 838–846, doi: 10.1111/j.1600-0463.2000.tb00007.x, indexed in Pubmed: 11252818.
11. Smith Z, Johnson B. Mechanisms of steroidal implants to improve beef cattle growth: a review. J Appl Anim Res. 2020; 48(1): 133–141, doi: 10.1080/09712119.2020.1751642.
12. Selk-Ghaffari M, Shab-Bidar S, Halabchi F. The Prevalence of Anabolic-Androgenic Steroid Misuse in Iranian Athletes: A Systematic Review and Meta-Analysis. Iran J Public Health. 2021; 50(6): 1120–1134, doi: 10.18502/ijph.v50i6.6411, indexed in Pubmed: 34540733.
13. Geraline CL, Erinoff L. Anabolic steroid abuse. NIDA Res Monogr. 1990; 102: 1–241.
14. Ma F, Liu D. 17β-trenbolone, an anabolic-androgenic steroid as well as an environmental hormone, contributes to neurodegeneration. Toxicol Appl Pharmacol. 2015; 282(1): 68–76, doi: 10.1016/j.taap.2014.11.007, indexed in Pubmed: 25461682.
15. Le Grevès P, Huang W, Johansson P, et al. Effects of an anabolic-androgenic steroid on the regulation of the NMDA receptor NR1, NR2A and NR2B subunit mRNAs in brain regions of the male rat. Neurosci Lett. 1997; 226(1): 61–64, doi: 10.1016/s0304-3940(97)00244-9, indexed in Pubmed: 9153642.
16. Hallberg M, Johansson P, Kindlundh AM, et al. Anabolic-androgenic steroids affect the content of substance P and substance P(1-7) in the rat brain. Peptides. 2000; 21(6): 845–852, doi: 10.1016/s0196-9781(00)00218-7, indexed in Pubmed: 10959007.
17. Hallberg M. Impact of anabolic androgenic steroids on neuropeptide systems. Mini Rev Med Chem. 2011; 11(5): 399–408, doi: 10.2174/1389557511795445961, indexed in Pubmed: 21443512.
18. Zelleroth S, Nylander E, Nyberg F, et al. Toxic Impact of Anabolic Androgenic Steroids in Primary Rat Cortical Cell Cultures. Neuroscience. 2019; 397: 172–183, doi: 10.1016/j.neuroscience.2018.11.035, indexed in Pubmed: 30500611.
19. Basile JR, Binmadi NO, Zhou H, et al. Supraphysiological doses of performance enhancing anabolic-androgenic steroids exert direct toxic effects on neuron-like cells. Front Cell Neurosci. 2013; 7: 69, doi: 10.3389/fncel.2013.00069, indexed in Pubmed: 23675320.
20. Bjørnebekk A, Westlye LT, Walhovd KB, et al. Structural Brain Imaging of Long-Term Anabolic-Androgenic Steroid Users and Nonusing Weightlifters. Biol Psychiatry. 2017; 82(4): 294–302, doi: 10.1016/j.biopsych.2016.06.017, indexed in Pubmed: 27616036.
21. Caraci F, Pistarà V, Corsaro A, et al. Neurotoxic properties of the anabolic androgenic steroids nandrolone and methandrostenolone in primary neuronal cultures. J Neurosci Res. 2011; 89(4): 592–600, doi: 10.1002/jnr.22578, indexed in Pubmed: 21290409.
22. Cunningham RL, Giuffrida A, Roberts JL. Androgens induce dopaminergic neurotoxicity via caspase-3-dependent activation of protein kinase Cdelta. Endocrinology. 2009; 150(12): 5539–5548, doi: 10.1210/en.2009-0640, indexed in Pubmed: 19837873.
23. Julien O, Wells JA. Caspases and their substrates. Cell Death Differ. 2017; 24(8): 1380–1389, doi: 10.1038/cdd.2017.44, indexed in Pubmed: 28498362.
24. Orlando R, Caruso A, Molinaro G, et al. Nanomolar concentrations of anabolic-androgenic steroids amplify excitotoxic neuronal death in mixed mouse cortical cultures. Brain Res. 2007; 1165: 21–29, doi: 10.1016/j.brainres.2007.06.047, indexed in Pubmed: 17662261.
25. Lehmann ML, Weigel TK, Elkahloun AG, et al. Chronic social defeat reduces myelination in the mouse medial prefrontal cortex. Sci Rep. 2017; 7: 46548, doi: 10.1038/srep46548, indexed in Pubmed: 28418035.

26. Zhang S, Zhang S, Zhu D, et al. Effects of 17β-trenbolone exposure on sex hormone synthesis and social behaviours in adolescent mice. Chemosphere. 2020; 245: 125679, doi: 10.1016/j.chemosphere.2019.125679, indexed in Pubmed: 31869672.
27. Khoodoruth MA, Khan AA. Anabolic steroids-induced delirium: A case report. Medicine (Baltimore). 2020; 99(33): e21639, doi: 10.1097/MD.0000000000021639, indexed in Pubmed: 32872027.
28. Trenton AJ, Currier GW. Behavioural manifestations of anabolic steroid use. CNS Drugs. 2005; 19(7): 571–595, doi: 10.2165/00023210-200519070-00002, indexed in Pubmed: 15984895.
29. Soria Lopez JA, González HM, Léger GC. Alzheimer's disease. Handb Clin Neurol. 2019; 167: 231–255, doi: 10.1016/B978-0-12-804766-8.00013-3, indexed in Pubmed: 31753135.
30. Bondi MW, Edmonds EC, Salmon DP. Alzheimer's Disease: Past, Present, and Future. J Int Neuropsychol Soc. 2017; 23(9-10): 818–831, doi: 10.1017/S135561771700100X, indexed in Pubmed: 29198280.
31. Heffernan T, Battersby L, Bishop P, et al. Everyday memory deficits associated with anabolic-androgenic steroid use in regular gymnasium users. Open Psychiatr J. 2015; 9: 1–6.
32. Baez MV, Cercato MC, Jerusalinsky DA. NMDA Receptor Subunits Change after Synaptic Plasticity Induction and Learning and Memory Acquisition. Neural Plast. 2018; 2018: 5093048, doi: 10.1155/2018/5093048, indexed in Pubmed: 29706992.
33. Paoletti P, Bellone C, Zhou Q. NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. Nat Rev Neurosci. 2013; 14(6): 383–400, doi: 10.1038/nrn3504, indexed in Pubmed: 23686171.
34. Sucher NJ, Awobuluyi M, Choi YB, et al. NMDA receptors: from genes to channels. Trends Pharmacol Sci. 1996; 17(10): 348–355, indexed in Pubmed: 8979769.
35. Binvignat O, Olloquequi J. Excitotoxicity as a Target Against Neurodegenerative Processes. Curr Pharm Des. 2020; 26(12): 1251–1262, doi: 10.2174/1381612826666200113162641, indexed in Pubmed: 31931694.
36. Yashiro K, Philpot BD. Regulation of NMDA receptor subunit expression and its implications for LTD, LTP, and metaplasticity. Neuropharmacology. 2008; 55(7): 1081–1094, doi: 10.1016/j.neuropharm.2008.07.046, indexed in Pubmed: 18755202.
37. Sanz-Clemente A, Nicoll RA, Roche KW. Diversity in NMDA receptor composition: many regulators, many consequences. Neuroscientist. 2013; 19(1): 62–75, doi: 10.1177/1073858411435129, indexed in Pubmed: 22343826.
38. Lu CL, Shaikh MB, Siegel A. Role of NMDA receptors in hypothalamic facilitation of feline defensive rage elicited from the midbrain periaqueductal gray. Brain Res. 1992; 581(1): 123–132, doi: 10.1016/0006-8993(92)90351-9, indexed in Pubmed: 1354005.
39. Flood JF, Morley JE, Roberts E. Memory-enhancing effects in male mice of pregnenolone and steroids metabolically derived from it. Proc Natl Acad Sci U S A. 1992; 89(5): 1567–1571, doi: 10.1073/pnas.89.5.1567, indexed in Pubmed: 1531874.
40. McGinnis MY. Anabolic androgenic steroids and aggression: studies using animal models. Ann N Y Acad Sci. 2004; 1036: 399–415, doi: 10.1196/annals.1330.024, indexed in Pubmed: 15817752.
41. Piatkowski TM, Neumann DL, Dunn M. 'My mind pretty much went to mush': A qualitative exploration of trenbolone in the performance and image enhancing drug community. Drug Alcohol Rev. 2023; 42(6): 1566–1576, doi: 10.1111/dar.13656, indexed in Pubmed: 36992616.
42. Penatti CAA, Costine BA, Porter DM, et al. Anabolic androgenic steroids and forebrain GABAergic transmission. Neuroscience. 2006; 138(3): 793–799, doi: 10.1016/j.neuroscience.2005.08.039, indexed in Pubmed: 16310317.
43. Henderson L, Jorge J. Steroid modulation of GABAA receptors:from molecular mechanisms to CNS roles in reproduction, dysfunction and drug abuse. Adv Mol Cell Biol. 2004: 219–250, doi: 10.1016/s1569-2558(03)32010-7.
44. de Souza GL, Hallak J. Anabolic steroids and male infertility: a comprehensive review. BJU Int. 2011; 108(11): 1860–1865, doi: 10.1111/j.1464-410X.2011.10131.x, indexed in Pubmed: 21682835.
45. Sjöqvist F, Garle M, Rane A. Use of doping agents, particularly anabolic steroids, in sports and society. Lancet. 2008; 371(9627): 1872–1882, doi: 10.1016/S0140-6736(08)60801-6, indexed in Pubmed: 18514731.
46. Cossu G, Borello U. Wnt signaling and the activation of myogenesis in mammals. EMBO J. 1999; 18(24): 6867–6872, doi: 10.1093/emboj/18.24.6867, indexed in Pubmed: 10601008.
47. Pelton LM, Maris SA, Loseke J. The Effects of Anabolic-Androgenic Steroids on Gene Expression in Skeletal Muscle: A Systematic Review. Int J Exerc Sci. 2023; 16(3): 53–82, indexed in Pubmed: 37113659.
48. Brack AS, Conboy IM, Conboy MJ, et al. A temporal switch from notch to Wnt signaling in muscle stem cells is necessary for normal adult myogenesis. Cell Stem Cell. 2008; 2(1): 50–59, doi: 10.1016/j.stem.2007.10.006, indexed in Pubmed: 18371421.
49. Zhao JX, Hu J, Zhu MJ, et al. Trenbolone enhances myogenic differentiation by enhancing β-catenin signaling in muscle-derived stem cells of cattle. Domest Anim Endocrinol. 2011; 40(4): 222–229, doi: 10.1016/j.domaniend.2011.01.004, indexed in Pubmed: 21402455.
50. Yan Xu, Zhu MJ, Xu W, et al. Up-regulation of Toll-like receptor 4/nuclear factor-kappaB signaling is associated with enhanced adipogenesis and insulin resistance in fetal skeletal muscle of obese sheep at late gestation. Endocrinology. 2010; 151(1): 380–387, doi: 10.1210/en.2009-0849, indexed in Pubmed: 19887565.
51. Taurin S, Sandbo N, Qin Y, et al. Phosphorylation of beta-catenin by cyclic AMP-dependent protein kinase. J Biol Chem. 2006; 281(15): 9971–9976, doi: 10.1074/jbc.M508778200, indexed in Pubmed: 16476742.
52. Altuwaijri S, Lee DK, Chuang KH, et al. Correction to: Androgen receptor regulates expression of skeletal muscle-specific proteins and muscle cell types. Endocrine. 2020; 69(2): 474–475, doi: 10.1007/s12020-020-02350-0, indexed in Pubmed: 32617754.
53. Wilson VS, Lambright C, Ostby J, et al. In vitro and in vivo effects of 17beta-trenbolone: a feedlot effluent contaminant. Toxicol Sci. 2002; 70(2): 202–211, doi: 10.1093/toxsci/70.2.202, indexed in Pubmed: 12441365.
54. Johnson BJ, Anderson PT, Meiske JC, et al. Effect of a combined trenbolone acetate and estradiol implant on feedlot performance, carcass characteristics, and carcass composition of feedlot steers. J Anim Sci. 1996; 74(2): 363–371, doi: 10.2527/1996.742363x, indexed in Pubmed: 8690672.
55. Samber JA, Tatum JD, Wray MI, et al. Implant program effects on performance and carcass quality of steer calves finished for 212 days. J Anim Sci. 1996; 74(7): 1470–1476, doi: 10.2527/1996.7471470x, indexed in Pubmed: 8818790.
56. Liu XH, Wu Y, Yao S, et al. Androgens up-regulate transcription of the Notch inhibitor Numb in C2C12 myoblasts via Wnt/β-catenin signaling to T cell factor elements in the Numb promoter. J Biol Chem. 2013; 288(25): 17990–17998, doi: 10.1074/jbc.M113.478487, indexed in Pubmed: 23649620.
57. Katoh M, Katoh M. NUMB is a break of WNT-Notch signaling cycle. Int J Mol Med. 2006; 18(3): 517–521, indexed in Pubmed: 16865239.
58. Cheng X, Huber TL, Chen VC, et al. Numb mediates the interaction between Wnt and Notch to modulate primitive erythropoietic specification from the hemangioblast. Development. 2008; 135(20): 3447–3458, doi: 10.1242/dev.025916, indexed in Pubmed: 18799543.
59. Gulino A, Di Marcotullio L, Screpanti I. The multiple functions of Numb. Exp Cell Res. 2010; 316(6): 900–906, doi: 10.1016/j.yexcr.2009.11.017, indexed in Pubmed: 19944684.
60. Conboy IM, Rando TA. The regulation of Notch signaling controls satellite cell activation and cell fate determination in postnatal myogenesis. Dev Cell. 2002; 3(3): 397–409, doi: 10.1016/s1534-5807(02)00254-x, indexed in Pubmed: 12361602.
61. Jory A, Le Roux I, Gayraud-Morel B, et al. Numb promotes an increase in skeletal muscle progenitor cells in the embryonic somite. Stem Cells. 2009; 27(11): 2769–2780, doi: 10.1002/stem.220, indexed in Pubmed: 19785007.
62. Liu XH, Yao S, Levine AC, et al. Nandrolone, an anabolic steroid, stabilizes Numb protein through inhibition of mdm2 in C2C12 myoblasts. J Androl. 2012; 33(6): 1216–1223, doi: 10.2164/jandrol.112.016428, indexed in Pubmed: 22700758.
63. Foradori CD, Weiser MJ, Handa RJ. Non-genomic actions of androgens. Front Neuroendocrinol. 2008; 29(2): 169–181, doi: 10.1016/j.yfrne.2007.10.005, indexed in Pubmed: 18093638.
64. Benten WP, Lieberherr M, Stamm O, et al. Testosterone signaling through internalizable surface receptors in androgen receptor-free macrophages. Mol Biol Cell. 1999; 10(10): 3113–3123, doi: 10.1091/mbc.10.10.3113, indexed in Pubmed: 10512854.
65. Feldman JL, Stockdale FE. Skeletal muscle satellite cell diversity: satellite cells form fibers of different types in cell culture. Dev Biol. 1991; 143(2): 320–334, doi: 10.1016/0012-1606(91)90083-f, indexed in Pubmed: 1991555.
66. Archacka K, Kowalski K, Brzóska E. [Are satellite cells stem cells?]. Postepy Biochem. 2013; 59(2): 205–218, indexed in Pubmed: 24044285.
67. Kamanga-Sollo E, Pampusch MS, Xi G, et al. IGF-I mRNA levels in bovine satellite cell cultures: effects of fusion and anabolic steroid treatment. J Cell Physiol. 2004; 201(2): 181–189, doi: 10.1002/jcp.20000, indexed in Pubmed: 15334653.
68. Fu R, Liu J, Fan J, et al. Novel evidence that testosterone promotes cell proliferation and differentiation via G protein-coupled receptors in the rat L6 skeletal muscle myoblast cell line. J Cell Physiol. 2012; 227(1): 98–107, doi: 10.1002/jcp.22710, indexed in Pubmed: 21374593.
69. Chin ER. The role of calcium and calcium/calmodulin-dependent kinases in skeletal muscle plasticity and mitochondrial biogenesis. Proc Nutr Soc. 2004; 63(2): 279–286, doi: 10.1079/PNS2004335, indexed in Pubmed: 15294044.
70. Rahman F, Christian HC. Non-classical actions of testosterone: an update. Trends Endocrinol Metab. 2007; 18(10): 371–378, doi: 10.1016/j.tem.2007.09.004, indexed in Pubmed: 17997105.
71. Mader TL, Kreikemeier WM. Effects of growth-promoting agents and season on blood metabolites and body temperature in heifers. J Anim Sci. 2006; 84(4): 1030–1037, doi: 10.2527/2006.8441030x, indexed in Pubmed: 16543582.
72. Dunn JD, Johnson BJ, Kayser JP, et al. Effects of flax supplementation and a combined trenbolone acetate and estradiol implant on circulating

insulin-like growth factor-I and muscle insulin-like growth factor-I messenger RNA levels in beef cattle. J Anim Sci. 2003; 81(12): 3028–3034, doi: 10.2527/2003.81123028x, indexed in Pubmed: 14677858.
73. Lee CY, Henricks DM, Skelley GC, et al. Growth and hormonal response of intact and castrate male cattle to trenbolone acetate and estradiol. J Anim Sci. 1990; 68(9): 2682–2689, doi: 10.2527/1990.6892682x, indexed in Pubmed: 2211398.
74. Reichhardt CC, Ahmadpour A, Christensen RG, et al. Understanding the influence of trenbolone acetate and polyamines on proliferation of bovine satellite cells. Domest Anim Endocrinol. 2021; 74: 106479, doi: 10.1016/j.domaniend.2020.106479, indexed in Pubmed: 32615508.
75. Kim JH, Han GC, Seo JY, et al. Sex hormones establish a reserve pool of adult muscle stem cells. Nat Cell Biol. 2016; 18(9): 930–940, doi: 10.1038/ncb3401, indexed in Pubmed: 27548913.
76. Rizk J, Sahu R, Duteil D. An overview on androgen-mediated actions in skeletal muscle and adipose tissue. Steroids. 2023; 199: 109306, doi: 10.1016/j.steroids.2023.109306, indexed in Pubmed: 37634653.
77. Johnson BJ, White ME, Hathaway MR, et al. Effect of a combined trenbolone acetate and estradiol implant on steady-state IGF-I mRNA concentrations in the liver of wethers and the longissimus muscle of steers. J Anim Sci. 1998; 76(2): 491–497, doi: 10.2527/1998.762491x, indexed in Pubmed: 9498357.
78. Lee CY, Lee HP, Jeong JH, et al. Effects of restricted feeding, low-energy diet, and implantation of trenbolone acetate plus estradiol on growth, carcass traits, and circulating concentrations of insulin-like growth factor (IGF)-I and IGF-binding protein-3 in finishing barrows. J Anim Sci. 2002; 80(1): 84–93, doi: 10.2527/2002.80184x, indexed in Pubmed: 11831532.
79. Johnson BJ, Hathaway MR, Anderson PT, et al. Stimulation of circulating insulin-like growth factor I (IGF-I) and insulin-like growth factor binding proteins (IGFBP) due to administration of a combined trenbolone acetate and estradiol implant in feedlot cattle. J Anim Sci. 1996; 74(2): 372–379, doi: 10.2527/1996.742372x, indexed in Pubmed: 8690673.
80. Barbonetti A, D'Andrea S, Francavilla S. Testosterone replacement therapy. Andrology. 2020; 8(6): 1551–1566, doi: 10.1111/andr.12774, indexed in Pubmed: 32068334.
81. Esteve Ràfols M. Adipose tissue: cell heterogeneity and functional diversity. Endocrinol Nutr. 2014; 61(2): 100–112, doi: 10.1016/j.endonu.2013.03.011, indexed in Pubmed: 23834768.
82. Blouin K, Nadeau M, Perreault M, et al. Effects of androgens on adipocyte differentiation and adipose tissue explant metabolism in men and women. Clin Endocrinol (Oxf). 2010; 72(2): 176–188, doi: 10.1111/j.1365-2265.2009.03645.x, indexed in Pubmed: 19500113.
83. Gentile MA, Nantermet PV, Vogel RL, et al. Androgen-mediated improvement of body composition and muscle function involves a novel early transcriptional program including IGF1, mechano growth factor, and induction of {beta}-catenin. J Mol Endocrinol. 2010; 44(1): 55–73, doi: 10.1677/JME-09-0048, indexed in Pubmed: 19726620.
84. Bruns KW, Pritchard RH, Boggs DL. The effect of stage of growth and implant exposure on performance and carcass composition in steers. J Anim Sci. 2005; 83(1): 108–116, doi: 10.2527/2005.831108x, indexed in Pubmed: 15583049.
85. Donner DG, Beck BR, Bulmer AC, et al. Improvements in body composition, cardiometabolic risk factors and insulin sensitivity with trenbolone in normogonadic rats. Steroids. 2015; 94: 60–69, doi: 10.1016/j.steroids.2014.12.017, indexed in Pubmed: 25554582.
86. Foutz CP, Dolezal HG, Gardner TL, et al. Anabolic implant effects on steer performance, carcass traits, subprimal yields, and longissimus muscle properties. J Anim Sci. 1997; 75(5): 1256–1265, doi: 10.2527/1997.7551256x, indexed in Pubmed: 9159272.
87. Reiter M, Walf VM, Christians A, et al. Modification of mRNA expression after treatment with anabolic agents and the usefulness for gene expression-biomarkers. Anal Chim Acta. 2007; 586(1-2): 73–81, doi: 10.1016/j.aca.2006.10.049, indexed in Pubmed: 17386698.
88. Rowe PJ, Comhaire FH, Hargreave TB. et al.. WHO Manual for the Standardized Investigation and Diagnosis of the Infertile Male. Cambridge University Press, Cambridge 2000.
89. Dohle GR, Smit M, Weber RFA. Androgens and male fertility. World J Urol. 2003; 21(5): 341–345, doi: 10.1007/s00345-003-0365-9, indexed in Pubmed: 14566423.
90. Corona G, Goulis DG, Huhtaniemi I, et al. European Academy of Andrology (EAA) guidelines on investigation, treatment and monitoring of functional hypogonadism in males: Endorsing organization: European Society of Endocrinology. Andrology. 2020; 8(5): 970–987, doi: 10.1111/andr.12770, indexed in Pubmed: 32026626.
91. Gazvani MR, Buckett W, Luckas MJ, et al. Conservative management of azoospermia following steroid abuse. Hum Reprod. 1997; 12(8): 1706–1708, doi: 10.1093/humrep/12.8.1706, indexed in Pubmed: 9308797.
92. Ledesma BR, Weber A, Venigalla G, et al. Fertility outcomes in men with prior history of anabolic steroid use. Fertil Steril. 2023; 120(6): 1203–1209, doi: 10.1016/j.fertnstert.2023.09.016, indexed in Pubmed: 37769866.
93. Grokett BH, Ahmad N, Warren DW. The effects of an anabolic steroid (oxandrolone) on reproductive development in the male rat. Acta Endocrinol (Copenh). 1992; 126(2): 173–178, doi: 10.1530/acta.0.1260173, indexed in Pubmed: 1543024.
94. Moretti E, Collodel G, La Marca A, et al. Structural sperm and aneuploidies studies in a case of spermatogenesis recovery after the use of androgenic anabolic steroids. J Assist Reprod Genet. 2007; 24(5): 195–198, doi: 10.1007/s10815-005-9002-4, indexed in Pubmed: 17342427.
95. Shokri S, Aitken RJ, Abdolvahhabi M, et al. Exercise and supraphysiological dose of nandrolone decanoate increase apoptosis in spermatogenic cells. Basic Clin Pharmacol Toxicol. 2010; 106(4): 324–330, doi: 10.1111/j.1742-7843.2009.00495.x, indexed in Pubmed: 20002066.
96. Turek P, Williams R, Gilbaugh J, et al. The Reversibility of Anabolic Steroid-Induced Azoospermia. J Urology. 1995: 1628–1630, doi: 10.1097/00005392-199505000-00079, indexed in Pubmed: 7714991.
97. Sretenovic J, Joksimovic Jovic J, Srejovic I, et al. Morphometric analysis and redox state of the testicles in nandrolone decanoate and swimming treated adult male rats. Basic Clin Androl. 2021; 31(1): 17, doi: 10.1186/s12610-021-00134-8, indexed in Pubmed: 34261436.
98. Barone R, Pitruzzella A, Marino Gammazza A, et al. Nandrolone decanoate interferes with testosterone biosynthesis altering blood-testis barrier components. J Cell Mol Med. 2017; 21(8): 1636–1647, doi: 10.1111/jcmm.13092, indexed in Pubmed: 28244681.
99. Whitaker DL, Geyer-Kim G, Kim ED. Anabolic steroid misuse and male infertility: management and strategies to improve patient awareness. Expert Rev Endocrinol Metab. 2021; 16(3): 109–122, doi: 10.1080/17446651.2021.1921574, indexed in Pubmed: 33973822.
100. Menon DK. Successful treatment of anabolic steroid-induced azoospermia with human chorionic gonadotropin and human menopausal gonadotropin. Fertil Steril. 2003; 79 Suppl 3: 1659–1661, doi: 10.1016/s0015-0282(03)00365-0, indexed in Pubmed: 12801577.
101. Rizzuti A, Alvarenga C, Stocker G, et al. Early Pharmacologic Approaches to Avert Anabolic Steroid-induced Male Infertility: A Narrative Review. Clin Ther. 2023; 45(11): e234–e241, doi: 10.1016/j.clinthera.2023.09.003, indexed in Pubmed: 37806813.
102. Guddat S, Thevis M, Kapron J, et al. Metabolism of anabolic androgenic steroids. Clin Chem. 1996; 42(7): 1001–1020, indexed in Pubmed: 8674183.
103. Ruan W, Monaco ME, Kleinberg DL. Progesterone stimulates mammary gland ductal morphogenesis by synergizing with and enhancing insulin-like growth factor-I action. Endocrinology. 2005; 146(3): 1170–1178, doi: 10.1210/en.2004-1360, indexed in Pubmed: 15604210.
104. Fortunato RS, Rosenthal D, Carvalho DP. [Abuse of anabolic steroids and its impact on thyroid function]. Arq Bras Endocrinol Metabol. 2007; 51(9): 1417–1424, doi: 10.1590/s0004-27302007000900003, indexed in Pubmed: 18209883.
105. Sharma S, Meena K, Tabiyad A, et al. Correlation between prolactin, thyroid, LH, FSH, estradiol and progesterone in the infertile women. Int J Reprod Contracept Obstet Gynecol. 2023; 12(4): 1017–1022, doi: 10.18203/2320-1770.ijrcog20230805.
106. Gill S, Peston D, Vonderhaar BK, et al. Expression of prolactin receptors in normal, benign, and malignant breast tissue: an immunohistological study. J Clin Pathol. 2001; 54(12): 956–960, doi: 10.1136/jcp.54.12.956, indexed in Pubmed: 11729217.
107. Yada T, Nakanishi T. Interaction between endocrine and immune systems in fish. Int Rev Cytol. 2002; 220: 35–92, doi: 10.1016/s0074-7696(02)20003-0, indexed in Pubmed: 12224552.
108. Verburg-van Ke, Stolte HH, Metz JR, et al. Neuroendocrine-immune interactions in teleost fish. Fish Neuroendocrinol. 2009; 28: 313–364.
109. Chaves-Pozo E, García-Ayala A, Cabas I. Effects of Sex Steroids on Fish Leukocytes. Biology (Basel). 2018; 7(1), doi: 10.3390/biology7010009, indexed in Pubmed: 29315244.
110. Sakiani S, Olsen NJ, Kovacs WJ. Gonadal steroids and humoral immunity. Nat Rev Endocrinol. 2013; 9(1): 56–62, doi: 10.1038/nrendo.2012.206, indexed in Pubmed: 23183675.
111. LoBue SA, Goldman A, Giovane RA, et al. Recurrent Herpes Zoster Ophthalmicus Preceded by Anabolic Steroids and High-Dose L-Arginine. Case Rep Ophthalmol Med. 2020; 2020: 8861892, doi: 10.1155/2020/8861892, indexed in Pubmed: 33457029.
112. Althobaiti YS, Alzahrani MS, Alhumayani SM, et al. Potential Association between the Use of Anabolic Steroids and COVID-19 Infection. Healthcare (Basel). 2022; 10(2), doi: 10.3390/healthcare10020196, indexed in Pubmed: 35206811.
113. Hoffmann JP, Liu JA, Seddu K, et al. Sex hormone signaling and regulation of immune function. Immunity. 2023; 56(11): 2472–2491, doi: 10.1016/j.immuni.2023.10.008, indexed in Pubmed: 37967530.
114. Grossman CJ, Sholiton LJ, Roselle GA. Estradiol regulation of thymic lymphocyte function in the rat: mediation by serum thymic factors. J Steroid Biochem. 1982; 16(5): 683–690, doi: 10.1016/0022-4731(82)90105-4, indexed in Pubmed: 6980328.
115. Mendenhall CL, Grossman CJ, Roselle GA, et al. Phytohemagglutinin skin test responses to evaluate in vivo cellular immune function in rats. Proc Soc Exp Biol Med. 1989; 190(1): 117–120, doi: 10.3181/00379727-190-42838, indexed in Pubmed: 2521392.

116. Mendenhall CL, Grossman CJ, Roselle GA, et al. Anabolic steroid effects on immune function: differences between analogues. J Steroid Biochem Mol Biol. 1990; 37(1): 71–76, doi: 10.1016/0960-0760(90)90374-t, indexed in Pubmed: 2242353.
117. Sthoeger ZM, Chiorazzi N, Lahita RG. Regulation of the immune response by sex hormones. I. In vitro effects of estradiol and testosterone on pokeweed mitogen-induced human B cell differentiation. J Immunol. 1988; 141(1): 91–98, doi: 10.4049/jimmunol.141.1.91, indexed in Pubmed: 3288699.
118. Ansar Ahmed S, Dauphinee MJ, Talal N. Effects of short-term administration of sex hormones on normal and autoimmune mice. J Immunol. 1985; 134(1): 204–210, indexed in Pubmed: 3964814.
119. D'Ascenzo S, Millimaggi D, Di Massimo C, et al. Detrimental effects of anabolic steroids on human endothelial cells. Toxicol Lett. 2007; 169(2): 129–136, doi: 10.1016/j.toxlet.2006.12.008, indexed in Pubmed: 17267145.
120. Oh-hora M. Calcium signaling in the development and function of T-lineage cells. Immunol Rev. 2009; 231(1): 210–224, doi: 10.1111/j.1600-065X.2009.00819.x, indexed in Pubmed: 19754899.
121. Li QJ, Dinner AR, Qi S, et al. CD4 enhances T cell sensitivity to antigen by coordinating Lck accumulation at the immunological synapse. Nat Immunol. 2004; 5(8): 791–799, doi: 10.1038/ni1095, indexed in Pubmed: 15247914.
122. Kuhns MS, Davis MM, Garcia KC. Deconstructing the form and function of the TCR/CD3 complex. Immunity. 2006; 24(2): 133–139, doi: 10.1016/j.immuni.2006.01.006, indexed in Pubmed: 16473826.
123. Hermann-Kleiter N, Baier G. NFAT pulls the strings during CD4+ T helper cell effector functions. Blood. 2010; 115(15): 2989–2997, doi: 10.1182/blood-2009-10-233585, indexed in Pubmed: 20103781.
124. Brenu EW, McNaughton L, Marshall-Gradisnik SM. Is there a potential immune dysfunction with anabolic androgenic steroid use?: A review. Mini Rev Med Chem. 2011; 11(5): 438–445, doi: 10.2174/138955711795445907, indexed in Pubmed: 21443507.
125. Massart S, Redivo B, Flamion E, et al. The trenbolone acetate affects the immune system in rainbow trout, Oncorhynchus mykiss. Aquat Toxicol. 2015; 163: 109–120, doi: 10.1016/j.aquatox.2015.04.007, indexed in Pubmed: 25889087.
126. Scholz S, Kordes C, Hamann J, et al. Induction of vitellogenin in vivo and in vitro in the model teleost medaka (Oryzias latipes): comparison of gene expression and protein levels. Mar Environ Res. 2004; 57(3): 235–244, doi: 10.1016/S0141-1136(03)00082-5, indexed in Pubmed: 14580810.
127. Fastrez J. Phage lysozymes. EXS. 1996; 75: 35–64, doi: 10.1007/978-3-0348-9225-4_3, indexed in Pubmed: 8765293.
128. Rich JD, Dickinson BP, Feller A, et al. Abscess related to anabolic-androgenic steroid injection. Med Sci Sports Exerc. 1999; 31(2): 207–209, doi: 10.1097/00005768-199902000-00001, indexed in Pubmed: 10063807.
129. Friedman Or, Arad E, Ben Amotz O. Body Builder's Nightmare: Black Market Steroid Injection Gone Wrong: a Case Report. Plast Reconstr Surg Glob Open. 2016; 4(9): e1040, doi: 10.1097/GOX.0000000000001040, indexed in Pubmed: 27757350.