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Mesterolone (Proviron) induces low sperm quality with reduction in sex hormone profile in adult male Sprague Dawley rats testis

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Anabolic-androgenic steroid compounds are one of the most widely abused drugs by athletes and muscle builders with the goal of improving performance/muscle mass. However, increasing concern has been expressed because these compounds not only offer unappreciable benefits to infertile and subfertile males, but also might have deleterious effects on both human and animal physiology including sperm quality. In addition, there is the conflicting outcome of AAS usage in the clinical settings with its attendant reduced spermatogenesis and hypopituitarism in patient management. Hence, we aim to evaluate the effects of mesterolone, an anabolic-androgenic steroid, on the histomorphometry of seminiferous tubules with serum hormonal and seminal analyses in adult male Sprague-Dawley rat. Twenty adult male Sprague dawley rats divided into two groups of 10 each. The treated group received 0.06 mg/g body weight/ day of mesterolone (proviron) by oral gavage for six weeks while the control group received equal volume of 0.9% normal saline per day. SPSS analysis of data generated with P< 0.05 considered statistically significant. The result showed significant (P< 0.05) body weight gain in all the animals. However, both the raw testicular weight and relative testicular weight per 100 g bwt was significantly (P< 0.05) higher in control than treated. The mean sperm count significantly decreased by 28% (P< 0.05) and the motility reduced significantly by 56% (P< 0.05) in the treated compared to control. In addition, both FSH (follicle stimulating hormone) and T (testosterone) of the treated were significantly lowered by 73% (P< 0.05) and 63% (P< 0.05) respectively compared to the control. The use of mesterolone is with caution and short intermittent therapy is desirous for better semen quality and improved overall fertility.

Key words: Proviron, sperm parameters, germ cells, stereology, hormonal profiles, Sprague dawley rats.

INTRODUCTION

Increasing concern has been expressed recently about the use and role of anabolic- androgenic steroid (AAS) compounds, which are one of the most widely, abused drugs by athletes and muscle builders with the sole purpose of improving performance /ability, appearance or muscle mass (Robert, 2000; Shittu et al., 2006).

However, anabolic steroids usage though not legal in many countries is on the rise worldwide. It can result in many different types of side effects, which can be deleterious to ones health. Although, these side effects may at times be reversible after drugs have been discontinued in some cases.

Figure from the Canadian Centre for Drug Free Sport estimated that in 1993, about 83,000 adolescent school children between the ages of 11 - 18 had used androgenic anabolic steroids in the preceding one year (Melia, 1994)

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and this is bound to be on the increase.

Moreover, synthetic (exogenous) androgens have been used in the treatment of male infertility or subfertility. This is however based on the fact that the process of spermatogenesis is both androgenic and follicle stimulating hormone (FSH) -dependent (Abel et al., 2008).

In addition, androgens stimulation as a whole is responsible for the maintenance of spermatogenesis and secondary sexual characteristics especially in the male. Thus, the major androgen in the circulation of men and adult males of most mammalian species including the rat based on extensive literature search is testosterone (T) that is produced by Leydig cells of the testis (Ren-Shan and Mathew, 2006).

Nevertheless, the mechanism of actions of most androgens are either through their direct stimulatory increase in intratesticular testosterone level which in turn enhances spermatogenesis and positively influences sperm transport and maturation through its action on epididymis, ductus deferens and seminal vesicles (Van-dekerckhove et al., 2000). However, the other androgenic pathway of action is via rebound effect (e.g. testosterone enanthate) through the suppression of both spermatoge-nesis and gonadotrophins (follicle stimulating hormone and luteinizing hormone) secretion (Anonymous, 1990; Jockenhovel et al., 1999; Matsumoto, 1990; Van dekerckhove et al., 2000). Thus, there is usually reversal of these androgen- induced symptoms when such drugs are discontinued in some cases.

In addition, most AAS and high dose testosterone intake exert an inhibiting effect on the hypothalomo-hypophysio-testicular axis with a resultant suppression in the normal testicular function which may further lead to a reduction in testosterone production, a decreased spermatogenesis, and a testicular atrophy. Although, this suppression is dependent on the duration of the steroid intake, the administered type of steroids and the dosage of the steroid used (Bra¨mswig et al., 1984; Mauro, 1998; Prader and Zachmann, 1978; Van dekerckhove et al., 2000)

Generally, anabolic and androgenic activities of AAS originate from their binding to and activation of the androgenic receptors (AR). However, the distinction between these two biological activities depends on the organs and target tissues involved. Thus, their anabolic impact concerns certain organs such as muscles, bones, the heart and kidneys with little 5 -reductase activity. More-over, the activity of AAS, especially testosterone will induce protein synthesis, muscle fiber development, erythropoiesis, and stimulation of bone growth (Keenan et al., 1993; Handelsman, 2000; Saudan et al., 2006; Urban et al., 1995).

On the other hand, anabolic steroids are in essence synthetic derivatives of testosterone, which are modified to enhance its anabolic activities (that is, promotion of protein synthesis and muscle growth) (Urban et al., 1995).

In addition, anabolic steroids are known to displace glucocorticoids from glucocorticoid receptors and inhibit muscle protein catabolism, leading overall to an anabolic or muscle building effect. Other mechanisms of direct and indirect anabolic effects of AAS include increases in the creatine phosphokinase activity in skeletal muscle, and increases in both circulating insulin-like growth factor (IGF)–1 (Arnold et al, 1996) as well as up-regulation of IGF-1 receptors (Urban et al., 1995).

Although, the abuse of AAS, especially dihydrotestosterone (DHT) analogues by athletes is on the increase (Anthony, 2004) . DHT has been found to contribute to androgen action on the testis with support for some resiual spermatogenesis in the rats` seminiferous tubules (Shittu, 2006; Shittu et al., 2006). However, proviron (mesterolone-DHT analogue, Schering, Germany) is also thought to enhance spermatogenesis in oligospermic patients (Lee and Kim, 1985).

In addition, DHT androgenic activity is found more potent than that of testosterone, by amplifying the activity of T to as much as 10-folds. This is because DHT has a higher relative binding affinity for and forms a more stable complex with the AR that is not easily dissociable thereby giving DHT a higher molar potency (Deslypere et al., 1992; Grino et al., 1990).

DHT is formed from the irreversible conversion of testosterone by the action of 5 -reductase enzymes, whose activity is important in testicles, skin, prostate, intestines, brain, bones, and adipose tissues etc. (Tenniswood et al., 1982; Warren and Ahmad, 1978). Therefore, androgenic effects of AAS predominate in these organs (Anthony, 2006; Proviron extract, 2006).

Moreover, DHT is shown to prevent the estrogen-dependent augmentation of the progesterone cytosol recaptors (PRc) in human breast cancer cells. While, both DHT and its metabolites also demonstrated a very high degree of inhibition of estrogen in human breast cancer cells (Hung and Gibbon, 1983). In addition, androgens are capable of inhibiting both the estrogenic induction and the ongoing stimulation of PRc receptor synthesis, but have no apparent effect upon basal concentrations of this receptor (MacIndoe and Etre, 1981).

Thus, DHT will certainly have beneficial effects on keeping our estrogen in check, hence reason for its abuse by sport machines. For example, with Proviron the athlete obtains more muscle hardness since the androgen level is increased and the estrogen concentration remains low. This effect is usually appreciated to be positively enhanced when taken with diet during the preparation for a competition. However, female athletes who naturally have a higher estrogen level often take Proviron as their steroid supplement resulting in increased muscle hardness (Anthony, 2006).

However, the conflicting outcome of AAS usages in clinical settings with their attendant reduction in spermatogenesis and induced hypopituitism usually observed in male infertility management coupled with AAS abuse by

sport machines during major sporting events have necessitated the need for this study. We therefore aim to carry out this study using the endocrine profile, histomorphometric and seminal analysis of adult matured male Sprague Dawley rats testis.

MATERIALS AND METHODS

Source of drugs

The proviron used for this study was bought from Agege pharmaceuticals in Agege. Proviron SCHERING (PTY) LTD is a product of Schering AG Germany/Allemagne, LOT # WEA6WX, manufactured date (MFD) : 06, 04, expiry date (EXP): 06, 2009. Each oral tablet contains 25 mg of mesterolone (17beta-hydroxy-1alpha-methyl-5alpha-androstan-3-one) with 20 tablets in a bottle and the preservatives methylparaben (0.02%) and propylparaben (0.01%).

Preparation of drug

A daily therapeutic dose of 20 mg of oral proviron tablet was used in this study. However, we calculated the human dose based on the physiological calculation for a 70 kg man, such that Proviron tablet was dissolved in 100 mls of distilled water to make up to 0.06 mg/kg bwt/day administered to the animal.

Animal

Twenty mature and healthy adult male Sprague Dawley rats weighing 120 to 200 g were procured from animal house of Lagos State University, College of Medicine, Ikeja and housed in a well ventilated wire-wooden cages in the departmental animal house. They were maintained under controlled light schedule (12 h Light: 12 h Dark) at room temperature (28°C) and with constant humidity (40 - 50%). The animals acclimatized for a period of 7 days before the start of treatment. During this period, they were fed with standard rat chows/pellets supplied by Pfizer Nigeria Ltd and water *ad-libitum*. Ear tag was used for individual identification of the entire group animals.

Experimental procedure

The rats were randomly divided into two groups of ten rats each. The control group received equal volume of 0.9% (w/v) normal saline daily while the treated groups received 0.06 mg/kg body weight /day of proviron solution via gastric gavage (oro-gastric intubation) daily for a period of 6 weeks.

All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (World Medical Association and American Physiological Society, 2002) and approved by the Departmental Committee on the ethics and research.

Animal sacrifice

The rats were anaesthetized after post over night fasting using the procedure described in our previous study (Shittu et al. , 2006). Weekly weighing of the animals carried out all through the experi-experimental period and prior to the sacrifice.

Organ harvest

The testes was initially dissected out whole via midline abdo-minal incision, cleared of fats and blotted dry. Their weights were measured on a sensitive digital balance with volume measured by water displacement using a 10 ml measuring cylinder. Later, the sizes (length and width) were recorded by use of a sliding gauge (d = 0.1) before eventually fixed in freshly prepared 10% formol saline solution The two testes of each rat were measured and the average value obtained for each of the two parameters was regarded as one observation as described earlier in Shittu et al. (2006).

Tissue processing for light microscopy

Serial paraffin sections of 5 μm were obtained from fixed processed testicular tissues blocks and stained with H & E stains as prepared and previously described in our earlier studies (Shittu, 2006; Shittu et al, 2007).

Cauda sperm forward motility

After anesthetizing the rat, epididymis was exposed by scrotal incision, and spermatozoa were expressed out by cutting the distal end of the cauda epididymidal tubule. Spermatozoa with epididymal fluid diluted with physiological saline was placed on a thin glass slide and forward motility (rate and percentage) of 100 spermatozoa/mouse was observed under microscope at X 400 magnification as previously described in Shittu et al. (2007, 2008).

However, the motility index was carried based on the method described according to WHO (1999).

Sperm count

Spermatozoa were counted as per the method described in our earlier studies (Shittu et al., 2007; 2008). Briefly, sperm suspension drops were placed on both sides of Neubauer's hemocytometer, allowed to settle by in a humid chamber (wet) for 1 h. The numbers of spermatozoa in the appropriate squares of the new improved neuber haemocytometer were counted under the microscope at X100 magnification as previously described by Shittu et al. (2007, 2008)

Hormonal assay

The estimation of serum Testosterone was carried out using the procedure enclosed with the specific commercial kit purchased from Amersham International Plc. (Bucking- hamshire, United Kingdom) by ELISA method. While, FSH estimated by RIA techniques as previously described in our studies (Shittu, 2006; Shittu et al., 2007, 2008).

Statistical analysis

The weight data were expressed in Mean ± S.D while other data were expressed as Mean ± S.E.M. Comparison between groups were done using the student t- test and non-parametric Mann- whitney U test as the case may be. All the data input into SPSS 12 software Microsoft computer (SPSS, Chicago, Illinois). Statistical significance was considered at $P < 0.05$ (Shittu et al., 2006).

RESULTS

No obvious toxicity signs such as weakness, lethargy, tremors, refusal of feeds, weight loss, hair-loss, coma and death were seen in any of the animals. However, most of the animals exhibited calmness; improve appetite

Table 1. Summary of body and organ weights of animals.

GROUP	Pre-experimental body weight (g) (Mean + S.D)	Final body weight(g) (Mean + S.D)	Raw Testicular weight. (g) (Mean + S.E.M)	Testicular-body/body weight (wt/100g bwt) (Mean + S.E.M)
Control	27.3 ± 6.40	184.4 ±12.6	1.24 ± 0.01	0.67 ± 0.01
proviron	159.4 ± 5.16	185.2 ± 12.8*	0.89 ± 0.09*	0.55 ± 0.01*

N = 10 rats per group.
*P < 0.05 was considered significant statistically.

Table 2. Summary of mean sperm parameters.

Parameters group D	Number of rats	Proviron group	Control group
Sperm Count (X 10^6 m/l)	10	58 ± 24.5*	80 ± 17.6
Sperm motility (%)	10	53 ± 10.2*	174 ± 46.9
Motility index	10	23 ± 4.9*	52 ± 9.7
Morphology (% NORMAL)	10	83 ± 5.9*	88 ± 1.7

*P <0.05 is considered significant.
100 - % normal = % abnormal morphology.

Table 3. Summary of hormonal profile of the animals.

Hormone Types	Number of animals	Proviron group	Control group
Testosterone (ng/ml)	10	0.3 ± 0.1*	0.8 ± 0.03
FSH (I.U)	10	3.0 ± 0.4*	11.0 ± 2.3

*P <0.05 is considered significant.

Table 4. Summary of % spermatogonial profile in animals

Group	Number of animals	Mean ± SEM
Proviron	10	39.6 ± 6. 76
Control	10	62.6 ± 5.76

*P <0.05 is considered significant.

for food and water and general sense of well-being all through the duration of the study.

Evidence of weight gained was observed in all the animals in both groups. Although, the mean average raw body weight gained of animals in the proviron treated animals were lower in the control. However, the proviron treated group weight was not significantly different from that of the control, using ANOVA as shown in Table 1.

Moreover, both the raw testicular weight and relative testicular weight decreased significantly (P < 0.05) in the proviron treated group as compared to the control as shown in Table 1.

Proviron group has the sperm count of about 1.4 times significantly (P< 0.05) lower than the control. The sperm motility of proviron is about 2.8 times significantly lower than the control.

In addition, the percentage normal sperm morphology in the proviron group was significantly lower than the control as shown in Table 2.

Proviron has lower significant (P < 0.05) % spermatogonial count than the control as shown in Table 4.

However, testosterone level in the proviron group was about 2.7 folds significantly (P<0.01) lower than control. While, the FSH level in proviron was 3.7 folds significantly (P<0.05) lower than the control as shown in Table 3.

Histology of control rats' testis (Figure 1a): Numerous round tubules with a fewer elongated ones. Most of the tubules are regularly shaped with evidence of active spermatogenic and spermiogenic activity seen.

Histology of the proviron treated animals testis (Figure 1b): Regressive changes were seen in the tubular epithelia that affected both the germinal and sertoli cells. Some evidence of spermatogenesis and spermiogenesis were equally seen in some of the seminiferous tubules with expanded intracellular spaces. Moreover, there was a lot of liberation of germinal cells during development with abnormal luminal dilatation and gradual reduction in epithelial heights of some of the tubules with

Figure 1a. Photomicrographs of testicular histology of control animals.

There are numerous regularly round shaped seminiferous tubules with a fewer elongated ones present within the testis. Moreover, most of the tubular lumens of the seminiferous tubules were filled with spermatozoa and evidence of active spermatogenic and spermiogenic activity were seen in both the X400 (right) and X100 (left) control animal slides.

Figure 1b. Photomocrographs testicular histology of the proviron treated animals.

Regressive changes were seen in the tubular epithelia that affected also both the germinal and sertoli cells of the testis. Some evidence of spermatogenesis and spermiogenesis were equally seen in some of the seminiferous tubules and most of them with fewer spermatozoa within their tubular lumens. Moreover, there was a lot of liberation of germinal cells during development with abnormal luminal dilatation and gradual reduction in epithelial heights of some of the tubules with thickened basement membrane were observed in both the X400 (right) and X100 (left) proviron treated animal slides.

thickened basement membrane observed. However, imamture germ cells with variable nuclear sizes and irregular profiles associated with atrophic changes were seen in some seminiferous tubules. In addition, localized accumulation of leydig cells were seen around the atrophic tubular area. Active hyperchromatic leydig cells with increased activity were seen.

DISCUSSION

We now know that combination of well-characterized animal models with stereological techniques always allow for proper quantitative study of any hormonal impact on male reproductive system (McLachlan et al., 1995; Shittu et al., 2006).

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In addition, rat appears to be a more suitable animal model in studying the roles of the androgenic hormones within the male reproductive system. Hence was used for this study because, it operates on a two-way androgen model (DHT and T) for its sexual differentiation. However, unlike mouse model that is dependent on testosterone action alone for the differentiation of its male urogenital tract (George et al., 1989; Shittu et al., 2006; Tenniswood et al., 1982).

The proviron treated group showed an insignificant (P> 0.05) gain in body weight, this is similar to the findings in other studies where proviron is thought to enhance weight gain from water retention in the body and decreased high-density lipoprotein among others (Anthony, 2006; Lee and Kim, 1985; Proviron extract, 2006).

The proviron group showed evidence of significant reduction in both raw and relative testicular weights as compared to control. However, these differential changes in testicular weights observed were well correlated with the seminiferous tubular profile/densities of the testis for each group of animals as observed in our previous studies (Shittu, 2006; Shittu et al., 2006).

These findings also implied that a major intratesticular change is taking place during proviron therapy, which accounted for the decrease in seminiferous tubule size/diameters and testicular sizes (Barham and Berlin, 1974; Heller et al., 1950).

Both sperm motility and percentage normal sperm morphology were significantly lowered in the proviron -treated groups compared to the control. These were similar to the findings in other studies where testosterone undecanoate and high dose of T-esters were used in rats and human models (Bra¨mswig et al., 1984; Mauro, 1998; Prader and Zachmann, 1978; Van-dekerckhove et al., 2000; Yang et al., 2004). However, in another similar study, a significant increased in sperm motility was observed without any significant increased in sperm morphology in proviron treated adult oligo-asthenozoospermic male human subjects (Lee and Kim, 1985).

The low level of testosterone found in the treated group in this present study must have been responsible for the low sperm density and motility obtained as reflected in Tables 2 and 3. However, a positive correlation exist between T and sperm count including motility in other studies (Doshi et al., 1994). In addition, the significant reduction in sperm count in the proviron group was found to be well correlated to the significant reduction in spermatogonia count and decreased in spermatocyte count seen in testicular tissues histology as shown in Figure 1b, Table 4 and in other similar study (Shittu, unpublished data).

Moreover, the above finding may be as a result of the reduction in proliferation of stem cells or spermiogenesis as large masses of seminiferous tubules epithelium appeared to be sloughing into the tubular lumen of the proviron groups with associated evidence of testicular atrophy as shown in Figure 1b.

However, on the contrary, Lee and Kim (1985) noticed a rather significant, increased in the sperm count of their proviron treated oligospermic-patients. Moreover, the action of proviron on the AR in the testis is responsible for the features stated above with a rebound effect and may account for the reduced sperm count and quality seen in the proviron treated group as observed in other similar study (Shittu et al, 2006).

In addition, studies have shown that the administration of T-esters at high doses is associated with both morphological/structural and cytological changes in adult testes of rat and humans (Barham and Berlin, 1974; Heller et al., 1950; Jezek et al., 1993). Such that, the degenerative features like increased luminal dilatation (up to 35%) and reduced epithelial height seen in other previous studies (Shittu, 2006; Shittu et al., 2006), were amongst the other factors responsible for the testicular atrophy (Oliveira et al., 2001) observed in some of the seminiferous tubules of proviron treated groups in this study.

Russell and co-workers (1993) had also speculated that both FSH and T might co-operate and thus have a common post receptor pathway of action. Moreover, other studies have shown that both FSH and androgen act in a co-existing additive and synergistic manners in regulating spermatogenesis and Sertoli cell activity (Abel et al., 2008). However, the Sertoli cell still retain a significant capacity for activity that is independent of direct hormonal regulation (Abel et al, 2008).

Although, spermatogenesis in the adult male is a complex hormonal interplay that is FSH and androgen dependant (Shittu et al., 2006). However, ablation of either hormone has deleterious effects on sertoli cell func-tion and the progression of germ cells through sperma-togenesis. Thus, a reduction of intratesticular androgen is an essential factor needed for the inhibition of spermato-genesis as reflected with the low sperm quality associa-ted with a significantly low T as shown in Table 1 and 3.

However, DHT has the tendency to amplify the effects of T with its binding on AR (Grino et al., 1996; Shittu et al., 2006). This may also account for the reason why some degree of residual spermatogenesis and spermio-genesis took place within some of the seminiferous tubules of the proviron treated groups in Figure 1b (Abel et al., 2008; Grino et al.,1996; Shittu, 2006; Shittu et al., 2006).

It is obvious that there is a complex hormonal interplay existing at the level of the hypothalamic testicular axis with negative feedback and rebound effects, which may be the cause of the significant low FSH observed in the present study as shown in Table 3. Moreover, we know that FSH has a role in facilitating the transport and loca-lization of testosterone within Sertoli cells involved in spermatogenesis.

Other studies also showed that previously androgen-treated tall men had significantly higher FSH levels compared with controls normal subjects and further observed

that there is a significant negative correlations between plasma FSH levels and sperm concentration as well as the age at start of therapy in the androgen treated men (Stenvertl et al., 1998). On the contrary, in the present study, we obtained a low FSH level in proviron treated normal adult male rats compared with control and a concomitant reduction in sperm quality. Thus, may imply that there is a positive correlation between FSH and sperm quality in the present study.

Moreover, other previous study has shown that, the double inhibition of spermatogenesis, a phenomenon that is usually active and effective in both monkey and humans after gonadotrophins withdrawal (a low FSH or low LH as the case may) was found to be rather ineffective in rats (Yang et al., 2004). This is because of its ineffective inhibition effects on both the Leydig cell population and intra-testicular testosterone level, thus may enhance spermatogenesis in rats` testis (Yang et al., 2004).

In addition, we know that FSH is involved in increasing spermatogonial number and maturation of spermatocytes including meiosis process. However, spermatid maturation is essentially T-dependent, a step, which cannot be, completed in-spite of the presence of high doses of FSH in the male reproductive system (Bartlett et al., 1989; Cameron and Muffly, 1991). Thus, FSH and testosterone act in synergism, which implied that a lower dose of either of the two hormones is equally effective when the other is available. Nevertheless, all these factors may have accounted for the observed residual spermato-ge-netic activity seen in some of the seminiferous tubules of the proviron treated animals as shown in Figure 1b. In addition, with associated evidence of mild significant increaseed in tubular diameters compared to control rats (Abel et al., 2008; Bartlett et al., 1989; Sinhan -Hikim and Swerdloff, 1999; Shittu, 2006; Shittu et al., 2006; Sun et al., 1989).

Moreover, Brown and Chakraborty (1988) have suggested that clomiphene intake (antiestrogenic agent) decreased the synthesis and/or release of gonadotrophins with decreased serum LH and testosterone concentration in male rats. This was similar to the case with the proviron (antiestrogenic- androgen) treatment in the present study where chemical castration level was reached with T< 0.5 ng/ml (T = 0.3 ng/ml) and with associated low FSH level compared to control as shown in Table 3.

However, the significant low testosterone level observed in the proviron as shown in Table 3 was thought to be due to selective conversion of testosterone to dihydrotestosterone through increase in its 5a reductase enzymes activity or reduction in DHT metabolism by enzymes.

Perhaps, this could also be because of the increased competitive binding affinity of the exogenous DHT (proviron) to the serum sex-steroid binding hormone (SHB) and AR sites. Thereby displaying the testosterone with a resultant reduction in T activity in the male reproductive system or probably be due to reduction in the leydig cells population or impact from the low FSH effect as the case may be in this study. Therefore, the effect observed with

proviron (DHT) above may be purely receptor-based action in nature.

In addition, several other studies have equally shown that the negative impacts caused by the use of AAS are likely to be reversible. This is because testicular volume which was initially reduced following androgen usage in adult men (Mauss et al, 1975) normalized after discontinuation of therapy (Zachmann et al., 1976; Bra¨mswig et al., 1984; Prader and Zachman, 1978; Mauss et al., 1975). However, few studies proved otherwise that the conditions are usually permanent with significantly smaller testicular sizes found in previously treated men (Willig et al., 1991; Willig et al., 1992).

Therefore to maximize the positive effect of proviron at the level of the hypothalamic-pituitary- testicular axis with resultant improvement in semen quality, it would be advised that intermittent rather than continuous treatment for about 6 weeks maximum be initiated with a pre-and post-hormonal check up as the case may be in any clinical setting.

Thus, real battle and way forward is educating our youth and athletes on the danger of taking AAS or other performance enhancers and to re-focus their energy on taking of adequate good diets and trainings.

Conclusion

The role of proviron in management of male infertility will need to be reviewed based on results of present study. In fact, proviron could probably serves as hormonal contraceptive in the light of these present findings.

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