Review Article

Can serum 17-hydroxyprogesterone and insulin-like factor 3 be used as a marker for evaluation of intratesticular testosterone?

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Abstract: Serum testosterone values vary considerably with little correlation to intratesticular testosterone (ITT). ITT is approximately ~100 times that of serum testosterone and is critical for spermatogenesis. Unfortunately, the only method to accurately measure ITT is invasive testicular aspiration and therefore is not performed routinely. The identification of a serum biomarker for ITT would allow serial monitoring during hormonal manipulation and the ability to assess the effectiveness of a male contraceptive agent. Prior studies have evaluated several serum biomarkers for their ability to accurately reflect ITT with data supporting 17-hydroxyprogesterone (17-OHP) and insulin-like factor 3 (INSL3) as a potential marker. Because evaluation of serum 17-OHP is readily available through commercial laboratories, in this review, we present the evidence for 17-OHP and how it can play a pivotal role in the management of male infertility.

Keywords: 17-hydroxyprogesterone (17-OHP); insulin-like factor 3 (INSL3); intratesticular testosterone (ITT); biomarker; infertility

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Introduction

Currently there exists no reliable and readily available serum biomarker for measurement of intratesticular testosterone (ITT) other than invasive testicular aspiration. The identification of a serum biomarker may allow further elucidation into the causes of male infertility, monitoring patients on therapy for male infertility (e.g., clomiphene citrate), as well as the development and monitoring of an effective male contraceptive. The adult testis serves two primary functions: the production of spermatozoa and the secretion of testosterone (T) (1). T is known to play a vital role in the initiation and maintenance of spermatogenesis (2,3). Healthy testicular function is dependent upon hormonal control of spermatogenesis through the intratesticular activity of the pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (2). LH stimulates Leydig cells of the testicular interstitium to synthesize and secrete T (2). FSH activity is also critical for normal spermatogenesis through its action on Sertoli cells, though its specific role is unclear (1,4,5). Withdrawal of gonadotropin stimulation, namely LH, results in a decrease in ITT, which then decreases sperm production (5). It is believed that ITT directly stimulates spermatogenesis in men, however, the amount of ITT required to initiate spermatogenesis has yet to be elucidated (5). Additionally, it is important to note that ITT concentrations in normal men are approximately 100 times greater than T in serum, which suggests that serum T may not be an accurate marker for ITT (3). A 2004 study examined the relationship between intratesticular fluid and serum T and found that ITT levels comparable with serum

T were not sufficient to support normal spermatogenesis (4). A reliable biomarker for ITT can help elucidate the currently obscure intratesticular hormonal milieu.

While ITT is thought to serve as a central role to spermatogenesis, the relationship between LH, ITT and spermatogenesis is far from understood (6). This gap in literature is most likely attributed to the difficulty in assessing the intratesticular microenvironment, especially with repeated measurements of individuals during hormonal manipulation (6). Existing options for evaluating ITT include repeat surgical testicular biopsies, which risk testicular injury. A safe and accurate method is bedside testicular aspiration with local anesthesia. Although minimally invasive, there are risks associated with the procedure including pain, bleeding, infection and injury to the testicle (7). However, due to the risks and overall difficulty involved, ITT concentrations are rarely evaluated in men (3,4,6). In clinical practice, men are treated with human chorionic gonadotropin, recombinant FSH and clomiphene citrate to increase ITT to either initiate spermatogenesis (i.e., hypogonadotropic hypogonadism, anabolic steroid abuse), maintain spermatogenesis (i.e., preserve spermatogenesis in men on testosterone replacement) or to improve spermatogenesis (i.e., idiopathic oligospermia). Identification of a serum biomarker has the potential to identify men deficient of ITT who may respond to therapy and to serve as a means of monitoring these patients for a therapeutic response (7-9).

Control of the intratesticular hormonal environment

The hypothalamus and the pituitary gland are the primary regulators of the intratesticular microenvironment through negative feedback of T (2,10). Exogenous T administration at both physiologic and supraphysiologic doses can dramatically suppress gonadotropin release (11,12). This can lead to a decrease in sperm in 65% of men to levels sufficient for contraception (13,14). Treatment with the LH receptor agonist, human chorionic gonadotropin (hCG), stimulates Leydig cells in a similar fashion as LH (6). The resultant increase in ITT stimulates spermatogenesis in men with hypogonadotropic hypogonadism from hypothalamic and/or pituitary failure and in men with experimentally induced gonadotropin deficiency (2). Therefore, treatment with hCG, usually in conjunction with recombinant FSH can be useful to treat infertility as it leads to initiation of spermatogenesis (15). Thus, the identification of serum biomarker to potentially predict response and monitoring of therapy would provide a benefit to infertility patients. Despite the fact that ITT concentration is key to induce spermatogenesis, the dose of hCG is often titrated to serum T levels. Given the poor association between serum T and ITT, the situation may arise where administration of hCG may normalize serum T levels without normalizing ITT (16). This occurrence is also consistent in normal men with experimentally-induced hypogonadotropic hypogonadism (3).

17-hydroxyprogesterone (17-OHP) as a marker of ITT

Intratesticular steroids are comprised by approximately 70% T, 20% 17-OHP, and smaller percentages of other hormones (6). Approximately 70% of T and 17-OHP are thought to be of testicular origin (17,18). The remainder of 17-OHP production is thought to be adrenal origin (16). *Figure 1* illustrates the synthesis of 17-OHP from its steroid precursors. Amory *et al.* suggested that serum 17-OHP strongly reflects ITT concentrations in normal men receiving gonadotropin suppression and hCG (6). This study assessed ITT concentration by testicular aspiration before and after treatment through the random allocation of healthy men receiving exogenous T into hCG dosage groups of either 0, 125, 250, or 500 IU every other day for 3 weeks. It was found that serum 17-OHP decreased by about 60% in men receiving placebo and increased by 70% in men at the highest dose of hCG. Their results found 17-OHP did not correlate to ITT at baseline, but was very strongly correlated during treatment, specifically when taking into consideration change from baseline and post-treatment measurements. While the overall correlation between ITT and serum 17-OHP was strong, the ITT increased much more than the serum 17-OHP in the lowest dose hCG group. This difference implies that serum 17-OHP may not be as sensitive to changes in ITT mediated by lower doses of hCG stimulation as it is to the higher doses of hCG (6). The authors highlighted the limitations of their study findings which included being a small, single institution study with a large variance in the measurement of ITT. They further discuss how suppression of the hypothalamic-pituitary-gonadal axis with the use of exogenous testosterone may not reflect accurate measurement of ITT. Nonetheless, their results are compelling and suggest further evaluation into this biomarker.

A recent paper by Roth *et al.* sought to evaluate the

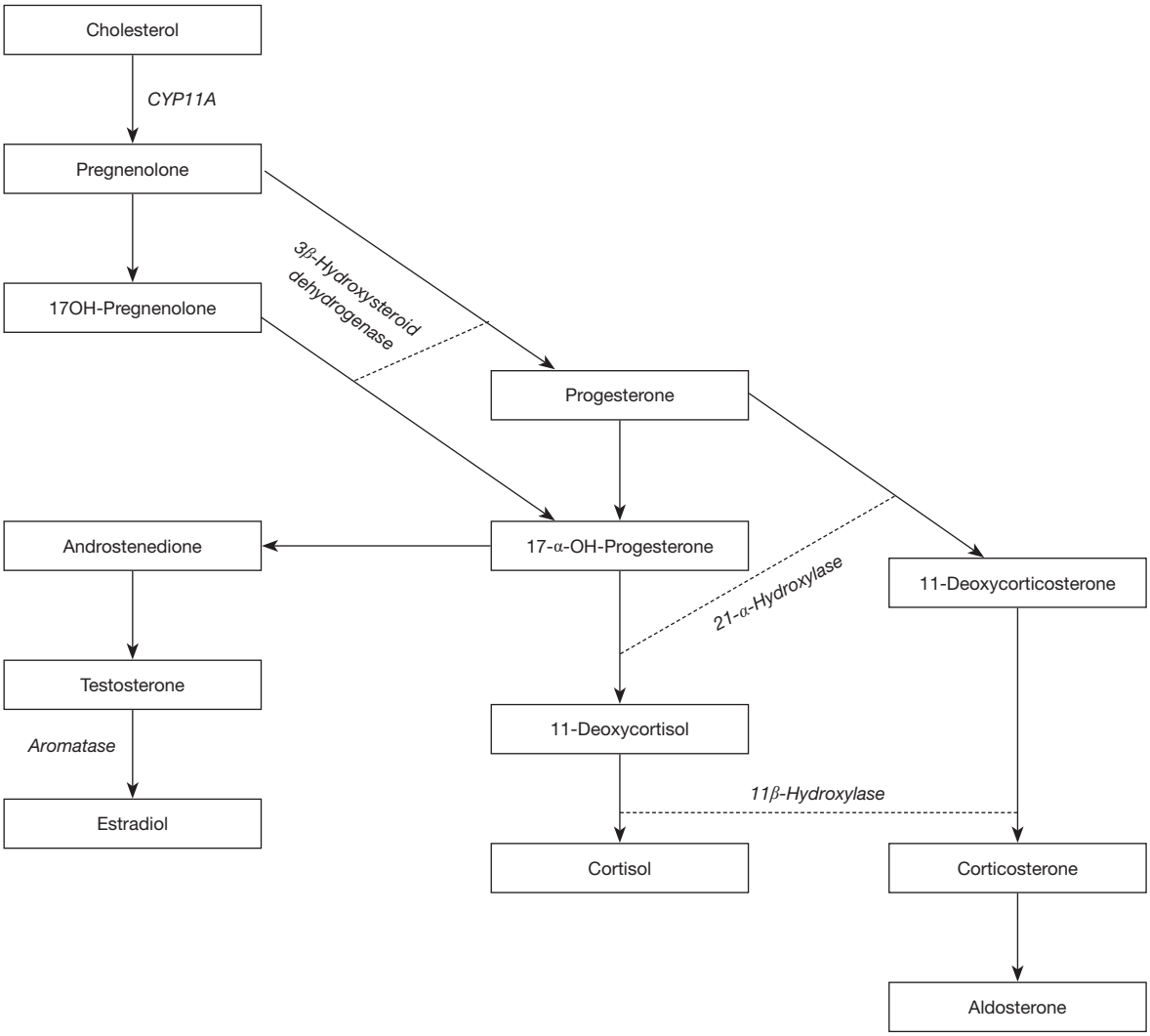

Figure 1 Synthesis of 17-hydroxyprogesterone (19).

minimal amount of hCG required to stimulate ITT in men with experimental gonadotropin deficiency. They treated 37 men with a GnRH antagonist to induce gonadotropin insufficiency and subsequently randomized them to receive 1 of 4 low dose hCG treatments (0, 15, 60 or 125 IU SQ every other day) or to 7.5 g of daily testosterone gel for 10 days. Their results found minimal amount of hCG was necessary for the production of ITT likely between 15–60 IU. Their results found very little correlation between ITT and 17-OHP. However, as the authors state the dose of hCG was quite minimal to their previous work and the incongruity in results is likely due to the lower doses of hCG used by Roth *et al.*, which in turn would have induced dramatically lower ITT concentrations. As previously stated this may indicate that serum 17-OHP correlates with ITT in the presence of normal or near-normal hCG stimulation (16).

Insulin-like factor 3 (INSL3) as a marker for ITT

INSL3 is a peptide hormone produced by mature Leydig cells in mammals and is another promising serum biomarker of ITT. Prior data by Bay *et al.* investigated INSL3 through the delivery of gonadotropin suppression with exogenous testosterone and progestin and then assessment of spontaneous or assisted recovery with exogenous gonadotropins (15). They found no increase in INSL3 after administration of supraphysiologic hCG in normal men which suggests that INSL3 may be maximally produced in

men with normal testicular physiology (15). Their results corroborate their prior finding of differential expression of T and INSL3 (20). INSL3 also declined drastically after gonadotropin deprivation and was responsive to hCG in the short-term (4 days). This finding provides some evidence to support INSL3 as a marker for ITT, however, serum T recovered significantly better (80% baseline) compared with serum INSL3 (38.9% baseline) in the presence of fully recovered serum LH, signifying that INSL3 is more sensitive than T to impairment in Leydig cell function (15). Interestingly, INSL3 has been suggested to be a marker of Leydig cell differentiation since it is constitutively secreted and thus not subject to variability like testosterone (21). INSL3 has the capability to reflect the number of Leydig cells present which is evidently unique from testosterone, which is homeostatically regulated by the hypothalamic-pituitary-gonadal axis (15). Other studies have reported stimulation of INSL3 production in men with hypogonadotropic hypogonadism treated with high doses of hCG monotherapy who would have had normally suppressed levels (15). Roth *et al.* built upon the findings of Bay *et al.* and delivered a co-treatment of acyline, a GnRH antagonist, with very low-dose hCG and found that serum INSL3 concentrations in normal men effectively decrease with gonadotropin suppression and increase in a dose-response relationship with low-dose hCG stimulation, strongly correlating with ITT and serum T concentrations (16). This finding provides evidence that serum INSL3 might be useful as a biomarker for the effect of hCG therapy and LH-like stimulation of Leydig cells. The utility of INSL3 as an alternative and superior serum marker to serum T might allow for more accurate monitoring of hCG therapy in infertile men, however further research is required to validate its reflection of ITT and Leydig cell function.

Clinical implications

Identification of the minimum concentration of ITT necessary for spermatogenesis in men has important clinical implications. The ability for a serum biomarker to accurately reflect ITT concentrations has the potential to identify men deficient of ITT who may respond to therapy to initiate spermatogenesis (i.e., hypogonadotropic hypogonadism), maintain spermatogenesis (i.e., men on testosterone replacement) and improve spermatogenesis (i.e., idiopathic oligospermia). Furthermore, it may also identify men with impaired sperm production who may not benefit from hormonal manipulation (i.e., men with normal ITT pre-treatment with oligospermia). The development of an effective male hormonal contraceptive could be possible with identification of the minimum concentration of ITT necessary for spermatogenesis. Some men do not respond to gonadotropic suppression, despite maximal suppression (2). The cause behind this is unknown, which presents a consequential barrier in the production of a male contraceptive (22,23). Through the use of a biomarker for ITT, more information about the intratesticular hormonal microenvironment can be discerned which can fill gaps in knowledge that are currently preventing the development of male contraceptive agents. Not only this, but a serum biomarker would lead to safer and more reliable methods for repeated sampling of the intratesticular environment. However, even in the absence of LH or hCG, ITT concentrations are still 100 times higher than normal serum T (16). These concentrations are sufficient to support spermatogenesis which must be accommodated for when developing a male hormonal contraceptive. Perhaps, T biosynthesis inhibitors, such as ketoconazole, may be useful to efficaciously decrease ITT concentrations (3). A serum biomarker for ITT would be of great value to investigate this phenomenon as a method to assess the associations between low ITT concentrations and spermatogenesis. A clearer understanding of the interactions between intratesticular hormones and spermatogenesis can be advantageous to overcome hurdles in the development of a male contraceptive.

Conclusions

Both the minimal and optimal concentrations of ITT necessary for spermatogenesis remain unknown. A serum biomarker to evaluate ITT could be useful to investigate the intratesticular hormonal milieu. A serum ITT marker would also be clinically useful in conjunction with serum T measurements to identify the ideal dosage of hCG required to treat infertility in men with hypogonadism. Finally, the identification of an appropriate serum marker for ITT has the potential to overcome the obstacles of and aid future research in developing effective hormonal male contraceptives.

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Footnote

Conflicts of Interest: R Ramasamy: Coloplast—Consultant; Boston Scientific—Investigator; Endo—Investigator, Advisory Board; Aytu Biosciences—Investigator, Advisory Board; Direx—Investigator. P Patel—Advisory board; Aytu Biosciences. The other authors have no conflicts of interest to declare.

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