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# Therapy with Growth Hormone: Major Prospects for the Treatment of Male Subfertility?

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**MALE** subfertility is a significant problem in reproductive medicine. The traditional clinical approach to remedy this problem has focused on the management of the female reproductive system and the use of *in vitro* technology. However, an effective direct therapeutic approach to improve male subfertility would have considerable clinical advantages. Treatment with hormones of the gonadotropic axis has given variable results with little improvement of the clinical condition [1]. However, there is increasing evidence that the somatotropic axis (the GH, GH receptor, insulin-like growth factor-1 (IGF-1) and IGF-1 receptor) may play an important role in the initiation and maintenance of normal male reproductive function.

GH plays an integral role in the regulation of a wide variety of biological functions including cellular differentiation, proliferation, metabolism, immune function, and normal reproductive function. GH also plays a major role in stimulating somatic growth at puberty. These effects appear to be interrelated with the pubertal increase in sex steroids and have been postulated to be partly mediated by stimulation of IGF-1 synthesis. A range of studies have demonstrated various associations between the somatotropic axis and normal reproductive development. Congenital GH deficiency results in short stature, phallic underdevelopment, diminished Leydig cell function, delayed puberty and delayed testicular development [2, 3]. Early GH treatment can often markedly increase statural growth rates, improve pubertal maturation and gonadal steroidogenesis. Indeed an interrelationship between GH and sex steroids was postulated more than 30 years ago [4]. Jansson *et al.* [5] showed that GH is required for some actions of testosterone and suggested that testosterone may stimulate body growth mainly by altering the secretory pattern of GH.

Work in rodents suggests that GH is necessary for normal spermatogenesis [6] and the normal development of male reproductive function [7]. More recent observations, both in humans and in laboratory animals, point to the contribution of IGF-1 to normal steroidogenesis and Sertoli cell function. However, the precise role and the mode of action of GH and IGF-1 in male reproductive performance are not known and there is increasing urgency to resolve a number of important questions before an appropriate therapeutic approach can be initiated. We firstly need to determine in well controlled direct experiments whether GH does indeed play a role in normal male reproductive function. Secondly, we need to define which distinct parameters of male fertility are regulated by GH and thirdly, if GH is shown to play a role in male fertility, we need to define its mode of action.

## Does GH Influence Male Fertility Parameters?

The rat has long served as a model species for the study of male fertility [8]. Of particular interest is a spontaneous mutant strain of dwarf rats with isolated GH deficiency (dw/dw rat) [9] which

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shows markedly reduced somatic growth and a reduction in endocrine parameters of the somatotropic axis [7]. However the dw/dw rat develops normally through puberty and, in the male, the secretion of LH, FSH and the action of steroids are normal [7]. This strain of rat therefore appears to be ideally suited to define changes in male fertility parameters resulting from GH-deficiency. We have compared the semen characteristics of approximately 100 days old male post pubertal dw/dw rats and Lewis rats (the appropriate GH-sufficient control strain out of which the dw/dw rat was derived). Spermatozoa were collected from the 6B region of the epididymis [10, 11] and analysed for concentration, normal morphology and motility (Fig. 1). The percentage of spermatozoa with normal morphology was slightly decreased in the dw/dw rat, while the concentration of spermatozoa was not significantly different between the dw/dw rat and the Lewis rat. However, the percentage of motile spermatozoa obtained from the 6B region of the epididymis was very low in the dw/dw rat in comparison to the normal Lewis rat of the same age and kept under identical conditions. These data suggest that in the GH-deficient state, the motility of spermatozoa is markedly decreased, while other semen characteristics are not particularly altered [12].

Our next approach was to treat GH-deficient male dw/dw rats at an age of approximately 80 days with recombinant bovine GH (rbGH) at a dose of 2 $\mu$g per g body weight per day for 21 days [12]. The rbGH was divided into two daily s.c. injections (0800 h and 1700 h). This treatment regime has been shown by this laboratory to be an appropriate replacement therapy in terms of somatic growth [13]. Two hours after the last rbGH injection, the animals were sacrificed under halothane anaesthesia and blood and semen were collected. The reduced motility of spermatozoa found in the dw/dw rat was markedly improved after 21 days of rbGH therapy (Fig. 2). Other semen characteristics, spermatozoa with normal morphology and the concentration of spermatozoa in the 6B region of the epididymis, were not significantly altered by rbGH treatment. These data suggest that GH therapy can reverse the low motility of spermatozoa observed in the GH-deficient state without negative effects on other semen characteristics. Thus, rbGH treatment markedly elevated the total number of motile spermatozoa with normal morphology. Since the weight of the testes and other semen characteristics, including morphology and concentration of spermatozoa, were not affected by rbGH therapy in this study [12], it is most likely that the action of rbGH observed on the motility of spermatozoa is

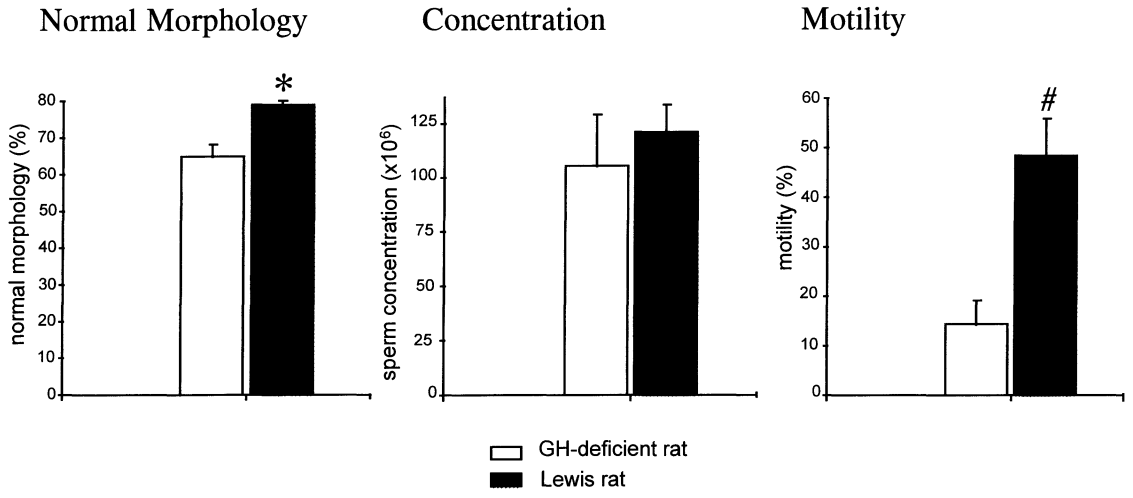


**Fig. 1.** Normal morphology, concentration and motility of spermatozoa in GH-deficient male dwarf rats and Lewis rats. Spermatozoa were collected from the 6B region of the epididymis and analysed for concentration, percentage with normal morphology and motility [12]. n=10 per group, data are mean ± SEM. $^{*}P<0.01$, $^{\#}P<0.001$.

not exclusively the result of altered spermatogenesis. We therefore propose that GH may improve spermatogenesis and the maturation process of spermatozoa, possibly through direct or indirect effects on the epididymides or other accessory glands.

While post-pubertal dw/dw rats show low motility of spermatozoa, this defect can be attributed to selective GH-deficiency since the endocrine parameters of the gonadotropic axis are functionally normal in the dw/dw rat [7]. Furthermore, detailed ligand binding studies from this laboratory revealed that rbGH binding to hepatic microsomal membranes from dw/dw rat livers is not affected by excess concentrations of prolactin, demonstrating that rbGH shows exclusively somatogenic but not lactogenic characteristics in dw/dw rats [13]. Thus, we believe that the beneficial effects of rbGH treatment observed in the GH-deficient rat are specific effects of GH and not mediated by GH binding to lactogenic receptors.

Recent clinical data are compatible with our research data in the rat. Oversen *et al.* [14, 15] treated subfertile men who showed a relative degree of GH deficiency with hGH for 12 weeks. They found that GH therapy did not change the concentration of spermatozoa but significantly increased semen volume and the motility of spermatozoa. These patients also showed elevated FSH levels before GH therapy which was interpreted by the investigators to reflect a primary impairment of spermatogenesis. They further suggest that GH therapy may not be effective in patients with an obstructive cause for oligozoospermia. This observation may explain why some studies of GH treatment of subfertile men were without beneficial effects [16] and implies that sound diagnostic procedures are imperative before the appropriate and effective therapeutic approach is selected. Further work is required to explore whether the marked GH-induced increase in motility of spermatozoa will lead to an increased rate of fertilization.

## What is the Mode of Action of GH-Induced Improvement of Male Fertility Parameters?

Considering the increasing evidence indicating that GH therapy can improve male fertility parameters, it is important to understand its mode of action. Indeed, there are strong indications that a range of components of the somatotropic axis are active throughout the male reproductive tract. The biological actions of GH are initiated when GH binds to specific GH receptors (GHR) on the plasma membrane of the target tissue. GHR have

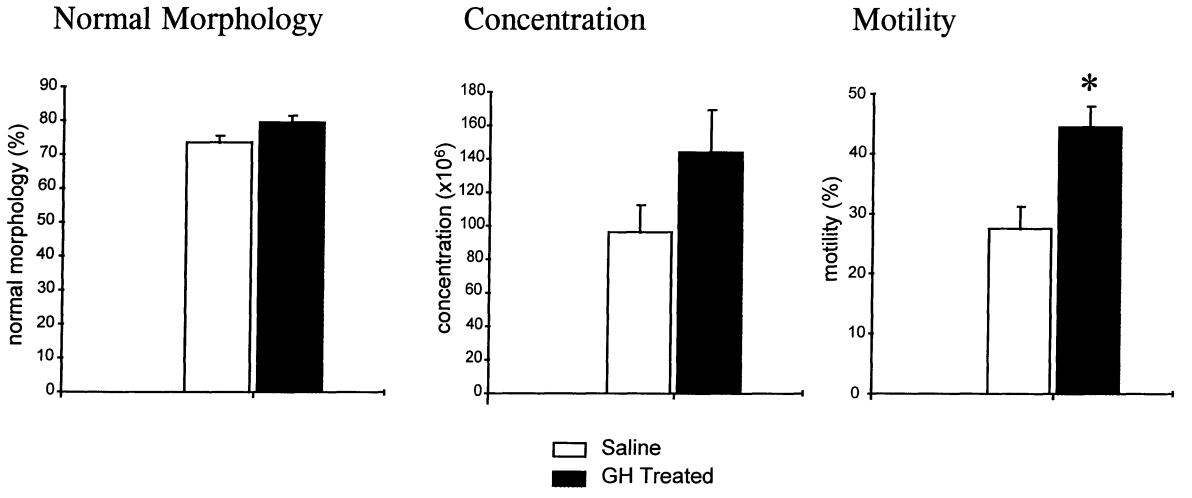


**Fig. 2.** Normal morphology, concentration and motility of spermatozoa in GH-deficient male dwarf rats treated with rbGH for 21 days. Spermatozoa were collected from the 6B region of the epididymis and analysed for concentration, percentage with normal morphology and motility [12]. n=10 per group, data are mean ± SEM. *$P<0.005$.

been found in multiple tissues including the liver, heart, kidney, muscle, ovary, testis and the epiphyseal growth plate. The GHR is regulated by numerous factors. GH itself increases the number of GHR in liver, muscle, and adipose tissue. Sex steroids enhance hepatic GHR number and this may be one mechanism operative in the pubertal growth spurt [17]. The interactions between GH and GHR initiate expression and secretion of IGF-1, which in turn promotes cellular differentiation and proliferation [18]. Thus, current notion suggests that GH may stimulate production of IGF-1 which may act in an endocrine or paracrine/autocrine fashion to enhance replication and/or differentiation and cellular metabolism.

The presence of GHR has been reported in a wide range of tissues in the male reproductive system of the GH sufficient rat. Lobie *et al.* [19] used immunohistochemistry to localize GHR and found intense immunoreactivity in Leydig and Sertoli cells, vas deferens, prostate, ductus epididymis and seminal vesicles. Using the same specific antiserum for the extracellular portion of the rat GHR, we found strong immunoreactivity in Leydig cells, Sertoli cells and in distinct phases during spermatogenesis in spermatogonia/spermatocytes of the testicular parenchyma in the GH-deficient dw/dw rat. Furthermore, additional immunohistochemical analysis using our highly specific antiserum 878/4 for IGF-1 [20, 21] shows strong immunoreactivity in the same histological sections and in the same areas where GHR was localised. Interestingly, treatment with rbGH of the male dw/dw rats resulted in a major increase of this IGF-1 immunoreactivity particularly in Sertoli cells, suggesting massive up-regulation of testicular IGF-1 synthesis with GH therapy (authors unpublished observations). Intense GHR and IGF-1 immunoreactivity in rat Sertoli cells and its responsiveness to GH therapy are of particular interest since the Sertoli cells regulate the development and maintenance of spermatogenesis. We speculate that Sertoli cells respond to GH treatment by increasing IGF-1 synthesis and transduce this stimulus to enhance differentiation and maturation of the germ cells through the intimate association that exists between these two cell types. Our hypothesis that the effects of GH therapy to increase the number of motile spermatozoa with normal morphology are mediated by IGF-1 is supported by our observations that the GH-deficient dw/dw rat shows markedly lower concentrations of IGF-1 not only in blood plasma but also in epididymal fluid and seminal vesicle fluid in comparison with the normal Lewis rat (Fig. 3). This relative deficiency in IGF-1 levels in the dw/dw rat is corrected with rbGH therapy (Fig. 4). Compatible data has been recently demonstrated in oligozoospermic men who showed an increase in the motility of spermatozoa after hGH treatment; this increase was accompanied by a rise in IGF-1 concentration in blood plasma and seminal fluid [14].

## Does IGF-1 Mediate the Effects of GH on Male Fertility Parameters through Endocrine or Paracrine Mechanisms?

There is increasing evidence for IGF-1 production, and the presence of IGF-1 receptors on different cell populations within the testes [25, 26]. It is therefore increasingly accepted that locally produced IGF-1 is a testicular growth factor involved in the differentiation, maturation and maintenance of both somatic and male gonadal cells including Leydig and Sertoli cells [23]. IGF-1 acts on both Sertoli and Leydig cells *in-vitro*, increasing FSH receptor number and responsiveness to FSH in Sertoli cells *in vitro* by increasing the cAMP response to FSH. *In vitro* treatment with IGF-1 also stimulates spermatogonial DNA synthesis during spermatogenesis. It has therefore been postulated that the FSH-dependent Sertoli cells produce apically secreted IGF-1 which contributes to the regulation of spermatogenesis [27]. IGF-1 receptors in testicular cells are also regulated by the gonadotropins *in vitro* [24]. This reciprocal upregulation of IGF-1 and the gonadotropin receptors, and of the gonadotropins on IGF-1 receptors, provides support for functional interactions between the somatotropic and the gonadotropic axes. These findings lead to the possibility that spontaneous disturbances in the testicular IGF-1 system may contribute to the underlying pathophysiology in some patients diagnosed with idiopathic subfertility.

However, there is considerable controversy over the mode of IGF-1 regulation in the testis. While some studies present evidence for paracrine regulation of IGF-1 secretion, there are other studies which propose an endocrine modulation, involving

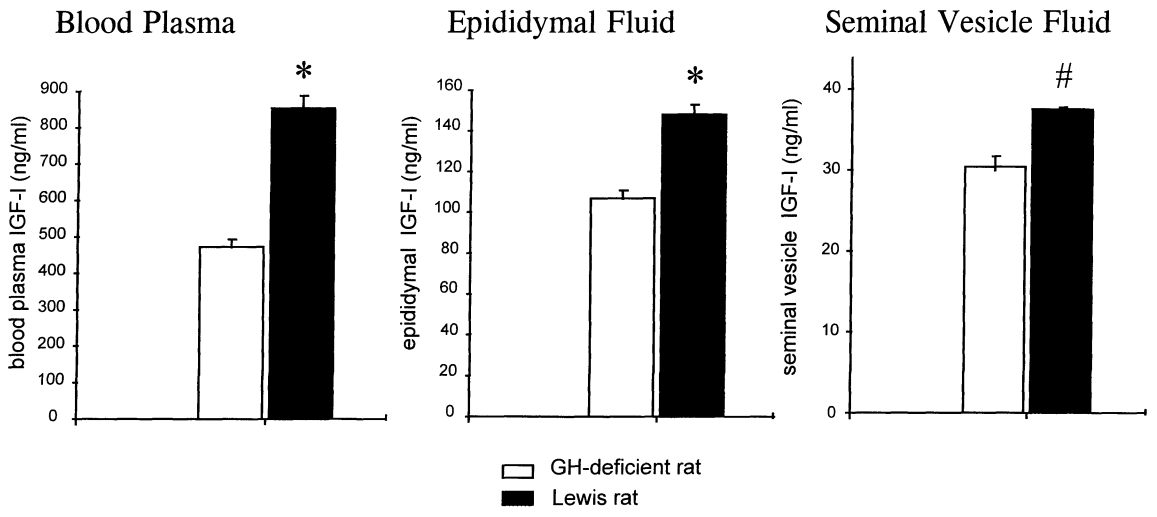


**Fig. 3.** IGF-1 concentrations in blood plasma, epididymal fluid and seminal vesicle fluid in GH-deficient male dwarf rats and Lewis rats. n=10 per group, data are mean ± SEM. *$P<0.001$, #$P<0.005$.

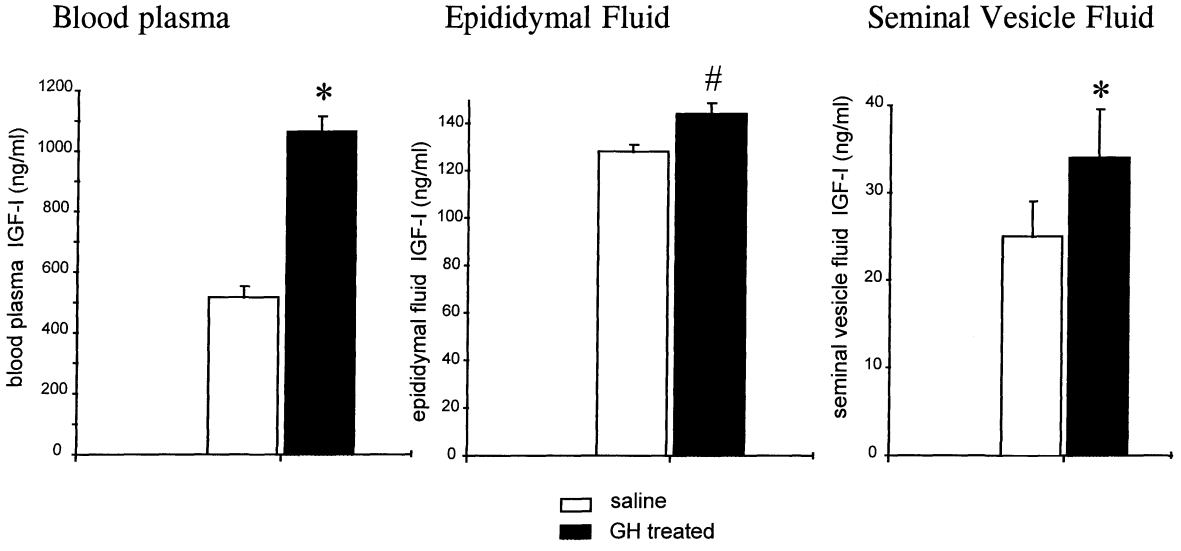


**Fig. 4.** IGF-1 concentrations in blood plasma, epididymal fluid and seminal vesicle fluid in GH-deficient male dwarf rats treated with rbGH for 21 days. n=10 per group, data are mean ± SEM. *$P<0.001$, #$P<0.05$.

trophic hormones including FSH, LH, GH and insulin. Following the early reports of testicular production of somatomedin-like activity [22], it was postulated that GH may be the prime regulator of testicular IGF-1 synthesis [23]. However, it has since become evident that other hormones or growth factors can also regulate IGF-1 production in different tissues. FSH and LH have been proposed to be important regulators of testicular IGF-1 production and GH was thought to play an indirect role by potentiating the actions of gonadotropins in regulating testicular IGF-1 content [24].

The presence of IGF-1 receptors in Leydig cells [28], Sertoli cells and in other accessory glands of the male reproductive system [6] certainly allows for endocrine, paracrine or autocrine mediation through IGF-1 of the effects seen with GH treatment. Whether the GH-induced increase in motile spermatozoa with normal morphology [12]

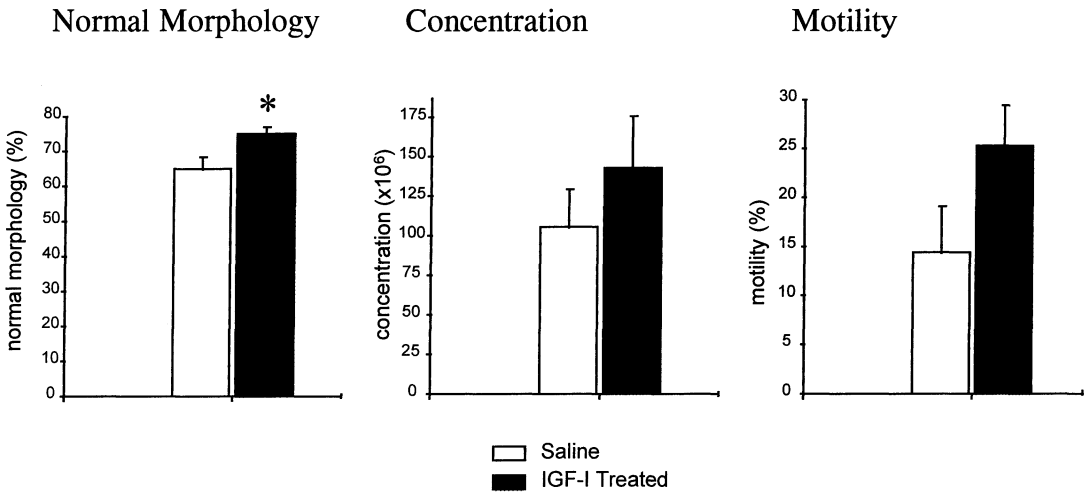


**Fig. 5.** Normal morphology, concentration and motility of spermatozoa in GH-deficient male dwarf rats treated with rhIGF-1 for 14 days. Spermatozoa were collected from the 6B region of the epididymis and analysed for concentration, percentage with normal morphology and motility [12]. n=10 per group, data are mean ± SEM. *$P<0.05$.

can be assigned to a direct effect of GH on the male reproductive system, or whether it is mediated by an increase in circulating IGF-1 was investigated in a further study. We treated approximately 80 days old male dw/dw rats with IGF-1. The rats were implanted with osmotic minipumps delivering 2 $\mu$g of IGF-1 per day for 14 days. The concentration of spermatozoa in the 6B region of the epididymis and the percentage of spermatozoa with normal morphology and the motility of spermatozoa were slightly increased (Fig. 5). However, the marked effects on the motility of spermatozoa observed after rbGH treatment were not achieved with IGF-1 therapy. While the dose of IGF-1 was sufficient to raise circulating concentrations of IGF-1 and those in seminal vesicle fluid to the same extent as observed after GH therapy (Fig. 6), the concentrations of IGF-1 in epididymal fluid were not increased. These results would suggest that IGF-1 treatment may not lead to an increase in local concentrations of IGF-1 in the testes and suggests further that the effects observed with GH therapy are not simply mimicked by raising blood plasma levels of IGF-1 by infusion of this growth factor. These data suggest that GH may act directly on the male reproductive system by inducing an increase in local production of IGF-1. This notion is supported by clinical data showing a marked decrease in seminal fluid IGF-1 after vasectomy, suggesting that the testes secrete considerable amounts of IGF-1 into seminal fluid [29]. Furthermore, the presence of IGF binding proteins (IGFBP) which may play a role in the delivery, distribution or modulation of local action of IGF-1 has been reported [30, 31]. In men the presence of IGFBP-1, -2, -3 and -4 have been documented in seminal fluid and IGFBP-3 was shown to decrease in seminal fluid after vasectomy [29], again supporting the notion of important paracrine or autocrine function of IGF-1 in the testis.

## Conclusions and Future Directions

There is increasing evidence for an interrelationship between gonadal function and the somatotropic axis. Independent studies performed by Ovesen *et al.* [14, 15] in men and our studies in the rat show important new evidence for a role of GH and IGF-1 in male reproductive function. These data further suggest that GH may act locally in a paracrine fashion in the testicular tissue and the accessory glands rather than via hepatic IGF-1 production and endocrine function of IGF-I. Furthermore, this research also suggests that the gonadal content of IGF-1 is likely to be GH dependent, rather than gonadotropin dependent. Some of the effects of GH at the testicular level may be similar to those described for the ovary,

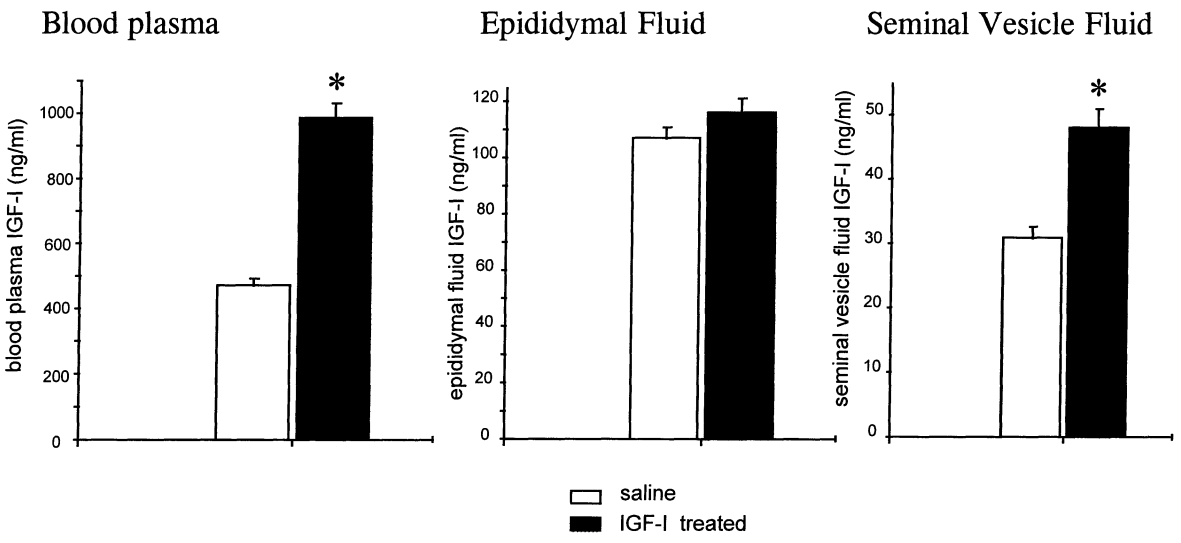


**Fig. 6.** IGF-1 concentrations in blood plasma, epididymal fluid and seminal vesicle fluid in GH-deficient male dwarf rats treated with rhIGF-1 for 14 days. n=10 per group, data are mean ± SEM. *$P$<0.001.

namely augmentation of the cellular response to gonadotropin stimulation [24]. However, following the demonstration of GH-binding sites in many compartments of the testes, including spermatogonia, Leydig cells and Sertoli cells [19 and authors unpublished data], and the expression and up-regulation of testicular and epididymal IGF-1 by GH therapy, we speculate that GH acts directly on testicular cells and accessory glands of the male reproductive system. These effects of GH may be mediated by local induction of IGF-1. The local stimulation of IGF-1 could be involved in spermatogenesis and or the maturation process of spermatozoa. These data suggest that further trials are warranted to explore the potential of GH and IGF-1 therapy for the treatment of male subfertility. Such studies should identify whether GH therapy can lead to increased fertilization rates. The effects of GH and IGF-1 on spermatogenesis and the role of the IGFBPs should also be considered in experimental paradigms of long-term treatment.

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