Decreased Sperm Motility is Associated with Increased Seminal Plasma IGF-I, IGF-II, IGFBP-2 and PSA Levels in Male Infertility

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Research Article

Abstract

Purpose

Previous studies have suggested the involvement of serum IGFs and IGFBPs in the regulation of the female reproductive system. Little is known of these peptides in the seminal plasma (SP) of men and their potential effects on fertility. We assessed SP levels of these peptides in infertile men with low sperm motility (asthenozoospermic; AZ) and low sperm counts (oligozoospermic; OZ), its effects on *in vivo* sperm motility, and whether aging affects these peptides.

Methods

Twenty eight infertile men (AZ; n = 18 and OZ; n = 10) and 20 fertile normozoospermic (NZ) men were studied. Seminal plasma IGF-I, IGF-II, IGFBP-2, IGFBP-3 and PSA levels were measured, and spermatozoa mRNA transcript patterns were examined.

Results

Asthenozoospermic men had higher SP IGF-I, IGF-II, IGFBP-2 and PSA levels than NZ and OZ men (all $P <$ 0.05), whereas SP IGFBP-3 levels were no different between the three groups. Sperm count positively correlated with SP IGF-I, IGF-II and IGFBP-2; sperm motility negatively correlated with SP IGF-II and IGFBP-2; and age correlated positively with SP IGF-II (all $P <$ 0.04). The expression of IGF-I and IGF-II mRNA and mRNA receptors was detectable, but no variations in transcript levels were noted.

Conclusion

Decreased sperm motility, but not sperm count, in infertile AZ men is associated with increased SP IGF-I, IGF-II, IGFBP-2 and PSA levels. Changes in SP IGFs and their interactions with IGFBPs and IGF receptors, and PSA levels suggest a role of these SP peptides in modulating sperm motility and possibly prostate disease development in aging men.

Introduction

Increasing evidence has implicated the role of the GH/IGF system in human reproductive physiology [1, 2]. Growth hormone modulates gonadotropin secretion and regulates longitudinal growth and function of secondary sexual organs, including activation of the formation of uterus in females and prostate and seminal vesicles in males [2]. Exogenous GH administration has been suggested as a potential treatment option for male infertility by increasing sperm count and total motile spermatozoa in infertile idiopathic oligozoospermic (OZ) men [3], improving sperm motility in asthenozoospermic (AZ) and OZ men [4, 5], and might even induce pregnancies in AZ men [6]. Although GH promotes the secretion of IGF-I and IGF-II mainly from the liver [7], localized testicular and prostatic production of these peptides are independent of GH induction [8] and can act in an autocrine/paracrine fashion [9]. Furthermore, circulating IGFs are bound by specific binding proteins (IGFBPs) that modulate their activity [10]. Production of IGFBPs is

ubiquitous and different IGFBPs are found in various body fluids. In serum, IGFBP-3 is predominant, whereas in seminal plasma (SP) and cerebrospinal fluid, IGFBP-2 is the most abundant binding protein [11, 12].

In the female reproductive system, studies have demonstrated the presence of IGFs and IGFBPs in granulosa cell cultures, follicular fluid, endometrium, and amniotic fluid, suggesting their involvement in the regulation of germ cell maturation, endometrial proliferation, and placental growth [13]. Conversely, the role of IGFs and IGFBPs in the male reproductive system has been less well studied. Although the presence of IGFs and IGFBPs have been demonstrated in SP [14-20], it remains unclear what these levels are in infertile men with low sperm motility *versus* those with low sperm counts, and if they exert a specific functional role in affecting sperm characteristics.

Previous studies have suggested that higher serum IGF-I levels is associated with a higher risk of benign prostatic hypertrophy (BPH) [21] and prostate cancer [22, 23]. Paradoxically, as men age, serum IGF-I levels decline [24] whereas the incidence of prostate diseases increases [25, 26]. We have previously shown that IGFBP-3 is present in SP and was completely proteolyzed into smaller molecular weight fragments by specific proteases [27], one of which was prostate-specific antigen (PSA), thus increasing bioavailable SP IGFs. It is, thus, possible that prostatic rather than serum IGFs may be more important in contributing to the pathogenesis of prostate diseases in aging men.

Therefore, to gain a better understanding of whether SP changes of IGFs, IGFBPs and PSA levels occurred in specific types of male infertility, we measured SP IGF, IGFBP and PSA profiles in fertile normozoospermic (NZ) men with normal sperm parameters, and infertile OZ men with low sperm counts and AZ men with low sperm motility. Additionally, we examined mRNA transcript patterns in mature motile spermatozoa to determine if there was a direct functional effect of SP IGFs and IGFBPs on *in vivo* sperm motility, and investigated if there are any age-related changes in SP IGF and IGFBP levels.

Subjects And Methods

Subjects

Twenty eight infertile men with abnormal semen analyses who attended the National University Hospital of Singapore Infertility Clinic were studied. All men were otherwise healthy on physical examination that included testicular examinations, non-smokers, not on any medications, and did not have any documented genitourinary tract infection at the time on enrolment. The infertile men were categorized into two groups based on their semen parameters, as classified by the World Health Organization (WHO) 2010 criteria [28]. The AZ men is classified as having normal sperm counts (> 20 x 10^6/mL) with low sperm motility (< 50% motile sperm), and the OZ men is classified as having low sperm counts (< 20 million^6/mL) with normal sperm motility (> 50% motile sperm). Twenty fertile NZ men, defined as having normal sperm counts (> 20 x 10^6/mL) and normal sperm motility (> 50% motile sperm) based on the WHO 2010 criteria [28], were also recruited that served as control subjects. These men were healthy male

volunteers whose wives/partners had produced an offspring within the previous 2 years, and were not on any medications. This study was approved by the National University Hospital of Singapore Institutional Review Board and informed consent was obtained from all subjects.

Seminal plasma collection and sperm extraction

Seminal plasma samples were collected from the semen of all subjects by masturbation after at least 48 hours of sexual abstinence into sterile specimen cups and placed in a 37°C heat block for 2 hours. After liquefaction, samples were centrifuged at room temperature for 7 minutes at 450 x g. Supernatants were re-centrifuged and the final supernatants filtered through 0.2 µmillipore filters and then frozen at -70°C. Viscous semen that could not be liquefied and semen with leucocytospermia were excluded, and these subjects were invited to return to the Infertility Clinic at another date for a repeat semen collection.

After the semen sample was liquefied at room temperature, the semen was examined for sperm motility characteristics using a mackler chamber and a phase contrast microscope. About 0.5- 1.0 mL of semen was stored from each sample for total RNA extraction. After collection, the semen samples were placed into small 2 mL tubes and mixed with 1 to 2 volumes of Phosphate Buffered Solution. These were spun in a microcentrifuge at 13000 rpm for 2 minutes, and this process of washing the spermatozoa was repeated on 2 occasions. Sperm counts (x 10^6/mL) and sperm motility (%) were assessed according to the WHO 2010 criteria [28] by a trained observer and sperm motility was assessed using the patient's own SP as media. The supernatant was then discarded and the pellet containing washed spermatozoa were subjected to RNA extraction.

Measurement of SP IGFs, IGFBPs and PSA levels

Seminal plasma IGF-I and IGF-II levels were measured by an immunoradiometric assay (IRMA) using commercially available kits (Diagnostic System Laboratories, Webster, Texas, USA), as previously described (Lee). For the measurement of SP IGFs, there was no interference from IGFBP-2 and IGFBP-3 as all these binding proteins were all washed out, as previously described [29]. Seminal plasma IGFBP-2 and IGFBP-3 was measured by a radioimmunoassay (RIA) kit (Diagnostic System Laboratories, Webster, Texas, USA). For the measurement of SP IGFBP-3, the RIA kit was previously validated [27] to measure low molecular weight fragments. Seminal plasma PSA levels were measured using an enzyme-linked immunosorbent assay, performed by using a commercially available PSA kit (Diagnostic System Laboratories, Webster, Texas, USA) according to manufacturer instructions.

Extraction of RNA

Total RNA was isolated from the spermatozoa using the RNAeasy mini kit (Qiagen, Germany) according to manufacturer's instructions and re-suspended in RNase-free water. Quantification and purity of the RNA was assessed by A260/A280 absorption and RNA quality was assessed by agarose gel electrophoresis. RNA samples with ratios > 1.6 were stored at -70°C for further analysis.

Reverse transcription (RT)

First-strand cDNA synthesis was carried out using Omniscript reverse transcriptase (Qiagen, Germany). Total RNA of 1-2 µg was incubated for 60 min at 37ºC in a 20 µL reaction volume. The reaction was stopped by incubating at 93ºC for 5 minutes and then quick-chilled on ice.

Polymerase chain reaction (PCR)

Polymerase chain reaction was performed in a final reaction volume of 50 µL containing the following reagents by use of the Taq PCR Core Kit (Qiagen, Germany): PCR buffer, Q-solution, dNTP mix, primer A, and taq DNA polymerase. The sequence of oligonucleotide primers are displayed in Table 1. Samples were heated to 94 ºC for 3 minutes and the denatured cDNA templates were amplified by the following cycles: 94°C for 1 minute (denature); 55°C (annealing temperature for IGF-I, IGF-II, IGF-I receptor, IGF-II receptor) for 1 minute; and then extended for 1 minute at 72°C. After 35 cycles, a final extension was performed for 10 minutes at 72°C, then cooled rapidly and stored at 4°C. Following amplification, 10 µL of the reaction products mixed with 2 µL of Blue/orange 6 X loading dye (Promega, USA) was applied to a 1.5% agarose (GIBCO BRL, NY, USA) gel containing a minimal amount of ethidium bromide. Molecular weight standards included 5 µg of '100 bp DNA Ladder[®]' (New England Biolabs Inc., USA). At the end of electrophoresis, the intensity of RT-PCR products was visualized on an ultraviolet box.

Statistical analysis

All statistical analyses were performed using SPSS for Windows (version 15.0, SPSS, Chicago, IL). To test for significant differences between the SP IGF-I, IGF-II, IGFBP-2, IGFBP-3 and PSA levels of the 3 subject groups, comparisons between groups were assessed using the one-way analysis of variance (ANOVA) with Dunnett's *post hoc* test. Correlation coefficients were calculated using Pearson's correlation for parametric data (sperm count, IGFs, IGFBP-3, PSA and age) and Spearman's correlation for non-parametric data (sperm motility). A two-tailed P-value of < 0.05 defined statistical significance.

Results

Baseline patient characteristics (Table 2)

The patients in the 3 groups (age range 22 to 57 years) were well-matched with no differences with regard to age and body mass index. The AZ men had a lower percentage of motile sperm (28.4 ± 2.5%) than the NZ (58.8 ± 1.3%) and OZ (57.6 ± 3.1%) men, whereas the OZ men had lower sperm counts (7.5 ± 1.8 x 10^6 /mL) than the NZ (45.0 ± 4.9 x 10^6 /mL) and AZ (57.9 ± 8.7 x 10^6 /mL) men (all $P < 0.0001$, ANOVA).

Seminal plasma IGF-I, IGF-II, IGFBPs and PSA levels (Figure 1)

Seminal plasma IGF-I levels were higher in the AZ (90.0 ± 13.5 ng/mL) compared to the NZ (62.6 ± 12.0 ng/mL) and OZ (45.6 ± 10.5 ng/mL) men ($P < 0.05$, ANOVA). Similarly, SP IGF-II levels were also higher in

the AZ (1441 ± 64 ng/mL) compared to the NZ (1130 ± 79 ng/mL) and OZ (1011 ± 129 ng/mL) men ($P <$ 0.05, ANOVA). Seminal plasma IGFBP-2 levels were higher in the AZ (7391 ± 665 ng/mL) compared to the NZ (5870 ± 549 ng/mL) and OZ (5010 ± 354 ng/mL) men ($P <$ 0.05, ANOVA). In contrast, SP IGFBP-3 levels did not differ between the 3 subject groups. Seminal plasma PSA levels were higher in the AZ (2.2 ± 0.2 mg/ml) compared to the NZ (1.4 ± 0.1 mg/ml) and OZ (1.2 ± 0.2 mg/ml) men ($P <$ 0.05, ANOVA).

Expression of IGF-I and IGF-II mRNA and receptor mRNA in mature motile spermatozoa (Figure 2)

The expression of IGF-I and IGF-II mRNA and IGF-I and IGF-II receptor mRNA in mature motile spermatozoa was detectable by RT-PCR. Using total RNA isolated from mature motile spermatozoa, low amounts of amplified cDNAs were detected using the IGF-I specific primers and abundant amplification of cDNA was observed with IGF-II specific primers. Specific primers for IGF-I and IGF-II receptors amplified cDNA from RNA were isolated from mature motile spermatozoa. No differences were observed in the transcript levels of IGF-I and IGF-II mRNA and mRNA receptors in the 4 spermatozoa samples tested.

Correlation analyses (Table 3)

Sperm count positively correlated with SP IGF-I, IGF-II and IGFBP-2 and sperm motility negatively correlated with SP IGF-II and IGFBP-2. Aging positively correlated with SP IGF-II, IGFBP-2 and PSA.

Discussion

In the present study, we found that AZ men with low sperm motility had significantly higher SP IGF-I, IGF-II, IGFBP-2 and PSA levels than NZ and OZ men implying that increased SP levels of these peptides was associated with decreased sperm motility, but not decreased sperm count. Conversely, SP IGFBP-3 levels (consisting mainly of low molecular weight IGFBP-3 fragments) were similar across the three groups suggesting that this peptide does not contribute to any meaningful effect in the pathogenesis of male infertility. The presence of IGF-I, IGF-II, and IGF-I and IGF-II receptors in mature motile spermatozoa suggests a direct role of these peptides in modulating *in vivo* sperm motility, and SP IGF-II, IGFBP-2 and PSA, but not SP IGF-I, levels increase with aging suggesting that SP IGF-II may be an implicating factor in the development of BPH and prostate cancer as men age.

Previous studies have investigated the role of IGF-I in sperm function, semen quality, and infertility in terms of a mitogenic, metabolic, and differentiating polypeptide with endocrine, paracrine, and autocrine effects [8, 30]. Animal studies have demonstrated that the proportion of normal spermatozoa was related to serum IGF-I levels in bulls [31]. Additionally, bovine SP IGF-I levels were related to semen quality [32] and motility in buffalos [33] and rams [34], and exogenous IGF-I administration improved sperm parameters in buffalo bulls [35]. Seminal plasma, the non-cellular component of semen, is a heterogeneous composite fluid built by secretions of the testis, the epididymis and the accessory sexual glands that is relevant for fertility [36]. Early human studies have shown the presence of IGF-I, IGF-II, IGFBP-2, IGFBP-3 fragments, IGFBP-4, IGFBP-5, IGFBP- protease activities and PSA in SP [14, 18, 19, 27, 37-39], while recent studies have reported that SP IGF-I levels do not differ between men with normal and

abnormal semen parameters [15, 20]. Evidence that vasectomized patients had lower SP IGF-I levels than intact patients suggests that SP IGF-I is mainly of testicular origin [39], whereas in patients with varicocele (a common cause of male infertility), higher SP IGF-I levels than healthy fertile men have been observed implying a role of localized IGF-I in the pathogenesis of male infertility [17].

We and others [30, 40] have shown that spermatozoa contains IGF-I receptors, so it is possible that the increased SP IGF-I levels can directly modulate sperm motility through these receptors. Although our data are in line with some studies where higher SP IGF-I levels are associated with abnormal sperm parameters [14, 17], others have reported either improved sperm parameters with increased SP IGF-I levels [9, 30, 33, 41] or unchanged SP IGF-I levels with abnormal sperm parameters [15, 20]. These discordant results suggest that there might be other local factors (e.g., IGF-II, IGFBPs and IGFBP proteolytic activity) that can influence the bioavailability and bioactivity of SP IGF-I.

To our knowledge, the present study is the first to investigate whether SP IGF-II might have any relationship with sperm function. The levels of IGF-II observed in the SP of fertile NZ men (1130 ± 79 ng/mL) are comparable with those reported by Ramasharma *et al.* [18] (1575 ± 66 ng/mL), and are notably 15-20 fold higher than the SP IGF-I levels. Our results concerning SP from AZ men with low sperm motility and increased IGF-II levels suggest that IGF-II, together with IGF-I, may act synergistically to inhibit sperm motility. Our findings are consistent with those reported by Henricks *et al.* [40] in which exogenous IGF-I and IGF-II increased the motility of washed bovine spermatozoa in an *in vitro* system devoid of IGFBPs. However, the *in vitro* system used in this study does not fully represent the *in vivo* environment because substantial amounts of IGFBPs are present in bovine SP that may modify the half-life and receptor interactions of IGFs.

Our study demonstrated that SP IGFBP-2 levels were significantly higher in AZ compared to NZ and OZ men. In normal men, there were no differences in SP IGFBP-2 levels before and after vasectomy, implying decreased IGFBP-2 secretion occurs in the testis rather than in the accessory sex glands [39]. This is consistent with the findings by Cohen *et al.* [42] who reported that IGFBP-2 was found in prostate epithelial cell-conditioned medium. Studies from both *in vivo* and *in vitro* models suggest that IGFBP-2 exerts inhibitory effects on IGF-mediated functions [43]. Hence, it is likely that SP IGFBP-2 may not induce any meaningful inhibitory effects on IGF-I and IGF-II actions in affecting sperm motility. Our data also indicated that SP IGFBP-3 levels were similar across the three groups. We have previously shown that SP IGFBP-3 is proteolyzed into low molecular weight fragments by PSA and other local proteases, resulting in a loss of intact IGFBP-3 [27]. These low molecular weight IGFBP-3 fragments do not to bind to IGF-I or IGF-II, and were comparable between normal and vasectomized males, and patients with idiopathic azoospermia, suggesting that the testicular contribution of SP IGFBP-3 is probably minimal [27]. Seminal plasma of proteolyzed IGFBP-3 would lead to increased IGF bioavailability [38] because intact IGFBP-3 would bind strongly to IGFs. Hence, the proteolyzed IGFBP-3 fragments in SP might not have a direct effect compared to the intact IGFBP-3 on sperm motility because other IGF independent actions of IGFBP-3 requires the intact molecule. The fact that no changes of IGFBP-3 levels in the SP of AZ, OZ and NZ men were observed in the present study suggests that proteolyzed IGFBP-3 did not exert any direct effects

on sperm motility *in vivo.* Conversely, elevated PSA levels were observed in the SP of AZ men, but whether there is a direct relationship between elevated SP PSA levels and reduced sperm motility remains to be clarified.

Miao *et al.* [16] first investigated the direct effects of IGFs and IGFBPs on *in vitro* sperm motility by using the 'swim up' technique utilizing washed sperm technique combined with computer video tracking methods. These authors found that IGF-I and IGFBP-3 caused significant and differing changes in sperm motility parameters, whereas IGF-II, IGFBP-2 and IGF-I/IGFBP-3 had no significant effects [16]. The mechanisms by which the IGF-I and IGFBP-3 exert these *in vivo* and *in vitro* effects on mature sperm is difficult to explain because of the uniqueness of the mature spermatozoa in not having a normal functioning nucleus, nor many of the normal processes of RNA transcription and translation. Additionally, the presence of IGF-I and IGF-II mRNA in the male prostate gland and testis have been reported [42, 44], but little is known about their function in mature spermatozoa. In light of these findings, we sought to evaluate the expression of mRNAs for the IGFs and IGF receptors in mature motile human spermatozoa. We found that IGF-I mRNA was detectable in low amounts in mature motile spermatozoa from fertile volunteers, whereas IGF-II mRNA was highly expressed. Furthermore, we found that spermatozoa can express type I and type II IGF receptors. Animal studies in bovine species have shown an increase in motile sperm when exposed to exogenous recombinant IGF-I and IGF-II [45]. The effects of IGF-I on sperm motility suggest increased flagella motion and thrust that is important in facilitating penetration of the zona pellucida [46] and in the transport through oviductal mucus [47]. Early studies have shown that mRNA for IGF-I and IGF-I receptor are expressed in oviducts, uteri and embryos [48-50]. Bongso *et al.* [51, 52] reported a strong correlation of sperm motility with fertilization, and showed that *in vitro* fertilization rates were higher when female tubal cells were added to sperm and oocytes in a co-culture system. The demonstration of the presence of IGFs and their receptors imply that complex interactions between the oviductal IGFs, sperm cell IGF receptors and sperm micro-anatomical structures in the tail/flagellum (e.g., axoneme, microtubules, and tubulin) may be at play in the regulation of sperm motility.

Previous studies have reported an association between male infertility and future prostate cancer [53, 54]. Dong *et al.* [55] also reported that IGF-II mRNA concentrations are 10-fold higher in prostate stromal cells from patients with BPH compared to normal men. Our findings that SP IGF-II increases with age suggest that the autocrine/paracrine actions of IGF-II may directly modulate prostate growth and possibly play a role in malignant prostatic changes. Our finding also helps to explain the paradox that although serum IGF-I has been shown to be a risk factor for prostate cancer, the incidence of BPH and prostate cancer increases whereas serum IGF-I levels decline with aging.

The limitations of our study are the small study numbers due to the rarity of AZ and OZ men in the general population and the fact that these men did not undergo specific examinations of the prostate and seminal vesicles by ultrasound or biochemical markers for improper function. The strengths, on the other hand, are the prospective design and the strict inclusion criteria of relatively healthy men over a wide age range with abnormalities of either sperm count or sperm motility, and no other ongoing medical

conditions. Our data, thus, apply to men with stable causes, and not to those with transient dynamic causes of male infertility.

In summary, our study has demonstrated that changes in SP IGFs, IGFBPs and PSA profiles are associated with decreased sperm motility but not sperm count, and that the presence of IGF-I, IGF-II, IGF-I receptor and IGF-II receptor in mature motile spermatozoa suggests a role via direct effects on sperm motility. The mechanisms of how differences in SP IGF-I, IGF-II, IGFBP-2, and PSA levels between AZ and OZ men come about remains to be elucidated. Furthermore, our results also suggest a possible autocrine/paracrine role of prostatic IGF-II and the development of prostate diseases with male aging. Further studies are needed to better understand the clinical significance and biological functions of these SP peptides on sperm characteristics in male infertility and prostate diseases as men age.

Declarations

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Tables

Table 1 Oligonucleotide primers used for the reverse transcription polymerase chain reaction assay.

Gene	Primer sequences	Product length (base pairs)
IGF-I	Sense 5'ACATCTCCCATCTCTCTGGATTTCCTTTTGC Antisense 5'CCCTCTACTTGCGTTCTTCAAATGTACTTCC	514
IGF-II	Sense 5'AGTCGATGCTGGTGCTTCTCACCTTCTTGGC Antisense 5'TGCGGCAGTTTTGCTCACTTCCGATTGCTGG	538
IGF-I receptor	Sense 5'AACCACGAGGCTGAGAAGCT Antisense 5'CAGCATAATCACCAACCCTC	447
IGF-II receptor	Sense 5'TCAACATCTGTGGAAGTGTG Antisense 5'GAATAGAGAAGTGTCCGGATCGGAGTC	428

Table 2 Baseline characteristics and semen parameters of fertile normozoospermic (NZ), and infertile asthenozoospermic (AZ) and oligozoospermic (OZ) men.

Patient groups	N	Age (years)	Body mass index (kg/m^2)	Semen parameters	
				Sperm count (x 10^6 /mL)	Sperm motility (%)
NZ	20	35.2 ± 5.3	24.5 ± 3.1	45.0 ± 4.9	58.8 ± 1.3
AZ	18	37.1 ± 8.1	23.2 ± 1.9	57.9 ± 8.7	**28.4 ± 2.5***
OZ	10	33.0 ± 5.9	24.3 ± 2.2	**7.5 ± 1.8***	57.6 ± 3.1

Data are presented as mean and standard deviation.

*statistically significant compared with the other 2 groups (*P* < 0.0001, ANOVA)

Table 3 Correlation analyses of seminal plasma IGFs, IGFBPs and PSA with sperm count, sperm motility and age.

Seminal plasma	Sperm count	Sperm motility	Age
IGF-I	**0.397***	-0.194	-0.013
IGF-II	**0.552****	**-0.475****	**0.305*****
IGFBP-2	**0.032*****	**-0.302*****	0.284
IGFBP-3	0.053	0.052	0.113
PSA	0.170	0.186	**0.368***

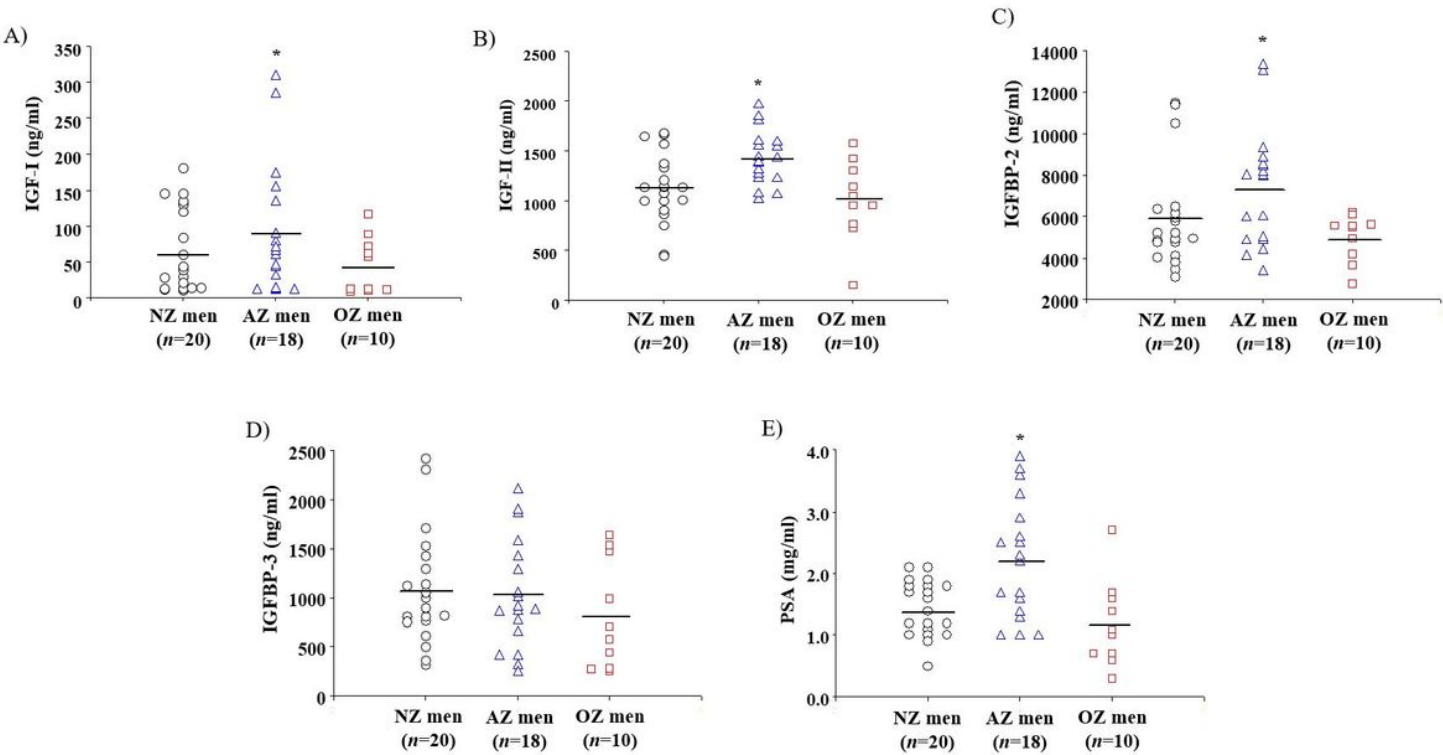*P* < 0.01; **P* < 0.001 and ***P* < 0.05.

Figures

Figure 1

Seminal plasma (A) IGF-I, (B) IGF-II, (C) IGFBP-2, (D) IGFBP-3 and (E) PSA levels of fertile normozoospermic (NZ), and infertile asthenozoospermic (AZ) and oligozoospermic (OZ) men. The horizontal line indicates the mean value and * indicates statistical significance (P < 0.05, ANOVA).
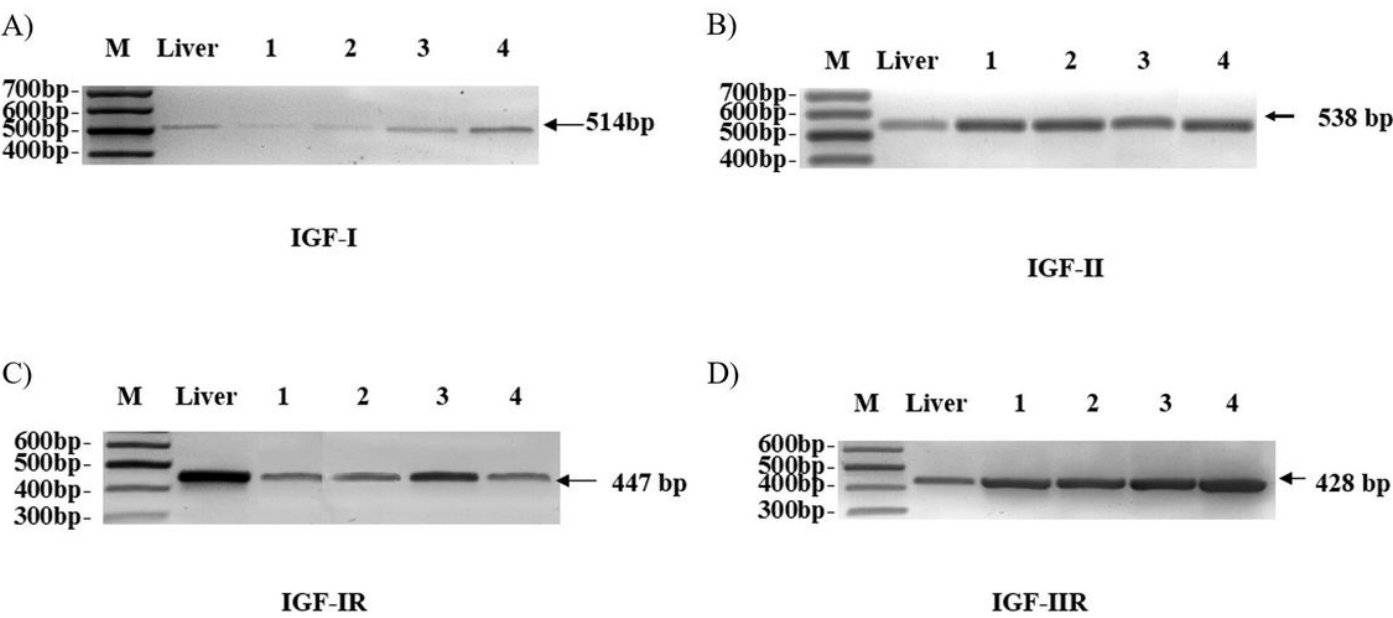

Figure 2

Agarose gel (1.5%) electrophoresis of RT-PCR products of (A) IGF-I, (B) IGF-II, (C) IGF-I receptor (IGF-IR) and (D) IGF-II receptor (IGF-IIR) from different sperm samples (Lane 1-4). A band corresponding to the RT-PCR products of (A) IGF-I (514 base pairs), (B) IGF-II (538 base pairs), (C) IGF-IR (447 base pairs) and (D) IGF-IIR (428 base pairs) are shown in each lane. Hepatic mRNA was used as a positive control. Lane M represents the molecular markers (100-base pair ladder).