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A Clinical Trial Evaluating the Efficacy of Prolistem® Supplement in Men with Non-Obstructive Azoospermia (Primary Testicular Failure)

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Abstract: ***Introduction: Non-obstructive azoospermia is a common cause of male infertility and remains one of the most challenging conditions to treat, despite advances in gynecology. Prolistem® is a novel treatment for this condition, which utilizes a unique mechanism called the "Spermatogenesis Restarting Process". Objectives: To evaluate the effectiveness of Prolistem® supplement in the therapy of infertile men with nonobstructive Azoospermia. Methods: Eighty-nine patients with non-obstructive azoospermia were enrolled in the study and received Prolistem® supplement for six months. Hormone parameters, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels, were measured before and after the procedure. Semen analysis was performed after the treatment, and in case of no sperm found, patients were recommended to undergo sperm retrieval techniques such as testicular sperm extraction (TESE) or micro-TESE. All the data required for the study were collected retrospectively from the patients or the hospital records. Results: After six months of Prolistem® supplementation, a significant improvement was observed in the hormone parameters of the patients. The mean FSH level decreased from 18.2 ± 5.6 to 8.4 ± 2.9 mIU/mL (p<0.001), while the mean LH level decreased from 10.1 ± 3.6 to 4.9 ± 2.1 mIU/mL (p<0.001). In contrast, the mean testosterone level increased from 3.6 ± 0.9 to 4.7 ± 1.2 ng/mL (p<0.001). Semen analysis performed after the treatment revealed that 32 patients (36%) had detectable sperm in their semen. Among the 57 patients with no detectable sperm in their semen, 47 patients (83%) underwent sperm retrieval techniques such as TESE or micro-TESE. The sperm retrieval was successful in 32 patients (68%), and viable sperm were obtained for assisted reproductive techniques. Conclusion: There is substantial evidence from both animal models and multiple studies that testosterone exerts an inhibitory effect on spermatogonial differentiation in cases of azoospermia. In the context of non-obstructive azoospermia due to primary testicular failure, Prolistem® supplement has shown promising results as a treatment option.***

Keywords: Azoospermia, Male Infertility, Prolistem, Testosterone, Primary Testicular Failure

1. Introduction

Male infertility accounts for approximately 50% of all infertility cases. While various techniques, including intracytoplasmic sperm injection (ICSI) and testicular sperm extraction (TESE) followed by ICSI, have been developed for male infertility, effective treatments for non-obstructive azoospermia, characterized by the absence of mature sperm in the testes, have yet to be found. Although a genetic predisposition has been suggested in many cases, the cause of non-obstructive azoospermia remains unclear in the vast majority of cases [1]. While recent studies using knockout mouse models have identified many genes associated with spermatogenesis, the applicability of these findings to most human cases remains uncertain. This is due to the fact that identifying affected genes in humans requires a retrograde genetic approach, and the knockout mouse phenotype is not always reliably reproduced in humans.

Human Male Infertility and Reasons

The decline in male fertility in advanced countries has been attributed by some researchers and clinicians to societal progress and worsening environmental conditions. Various occupational hazards, such as working in high temperatures, exposure to noise and radiation, and exposure to electromagnetic waves and chemical substances, have been reported as risk factors [2-6]. Comparisons of men with infertility (oligospermia or azoospermia) to healthy subjects have revealed potential risk factors, including air temperature, automobile driving time per day, air pollution, regional differences in residential population density, mumps, stress, and alcoholism [7-13]. However, some reports suggest no correlation between environmental factors and male infertility [14,15], leading to a lack of consensus on the role of environmental factors in male infertility.

In 1976, Tiepolo and Zuffardi identified microdeletions on the long arm of the Y chromosome in six patients with azoospermia, leading to the discovery of an important spermatogenesis gene, named the azoospermia factor (AZF) region [16]. Vogt et al. conducted subsequent studies and divided the AZF region into three subregions, AZFa, AZFb, and AZFc, based on the concentration of microdeletions in different testicular tissue types [17-19]. In 1995, Reijo et al. examined 89 patients with non-obstructive azoospermia and found that 12 (13%) had a deletion in the AZF region, which emphasized the close relationship between human azoospermia and this region [18].

Non-Obstructive Azoospermia

Non-obstructive azoospermia is a condition in which sperm production is severely disturbed to absent due to abnormal, atrophic, or absent testes. The interruption of the feedback loop results in elevated FSH levels (hypergonadotropic). This condition is observed in 49-93% of men with

azoospermia. Testicular failure can be caused by congenital issues such as certain genetic conditions (e.g., Klinefelter syndrome), cryptorchidism or Sertoli-cell-only syndrome, and acquired conditions resulting from infection (orchitis), surgery (trauma, cancer), radiation or other unknown causes. Mast cells releasing inflammatory mediators are thought to directly suppress sperm motility in a potentially reversible manner, and may be a common pathophysiological mechanism for many causes leading to inflammation. Men with unexplained hypergonadotropic azoospermia typically require a chromosomal evaluation. Until recently, men with non-obstructive azoospermia were thought to be untreatable, and options for these couples to have children were limited to the use of donor spermatozoa or adoption. However, several clinically relevant findings have changed the approach to this condition. Direct evaluation of testis biopsy specimens often demonstrates sperm in men with non-obstructive azoospermia, despite severe defects in spermatogenesis [20-22].

Current Azoospermia Treatment

In the management of non-obstructive azoospermia (NOA), the method of sperm retrieval is a crucial factor. Testicular sperm production, if present, is randomly and heterogeneously distributed throughout one or both testes. To obtain a wide sampling of the testicular parenchyma, surgical techniques have been developed. These techniques include percutaneous, incisional, and microsurgically assisted methods. Testicular sperm aspiration (TESA) is a percutaneous technique that involves using small- or large-bore needles to aspirate testicular tissue. The needle is attached to a syringe, and suction is created while the needle tip is moved around within each testis to obtain a wide sampling of the seminiferous tubular tissue. Conventional testicular sperm extraction (cTESE) and microdissection testicular sperm extraction (mTESE) are incisional methods. In cTESE, seminiferous tubular tissue is extracted through one or more testicular incisions. In contrast, microdissection TESE involves making a large testicular incision and selectively sampling the largest-diameter seminiferous tubules under optical magnification provided by an operating microscope.

The critical factor to consider when evaluating sperm retrieval methods is the sperm-retrieval rate. To date, there have been no randomized controlled trials that have directly compared the various techniques of sperm extraction. However, two recent systematic reviews have been conducted on surgical sperm-extraction techniques in men with non-obstructive azoospermia (NOA). Both reviews identified the same seven studies that compared microdissection testicular sperm extraction (mTESE) to conventional testicular sperm extraction (cTESE). The authors reported a successful sperm retrieval rate of 35% (range: 17%–45%) for cTESE and 52% (range: 45%–63%) for mTESE, estimating that mTESE was 1.5 times more likely to retrieve sperm (95% confidence interval) [23, 24]. Based on the combination of prospective and retrospective data, both reviews concluded that mTESE was superior to cTESE for surgical sperm extraction in men with NOA. The greatest benefit of mTESE appeared to be in men with limited sperm production, such as those with a Sertoli cell-only pattern.

Furthermore, the authors pooled data from seven studies to compare the sperm-retrieval rates between testicular sperm aspiration (TESA) and cTESE. The results indicated that cTESE had a higher retrieval rate (56%, range: 43%–64%) than TESA (28%, range: 7%–42%), and cTESE was found to be superior to TESA (relative risk [RR] 2.0, 95% CI 1.8–2.2). Although the sperm-retrieval rates varied for cTESE in each comparison group, the overall conclusion is that mTESE is superior to cTESE, which is superior to TESA.

Lastly, when a repeat procedure is necessary, data suggest that waiting at least 6 months can increase the retrieval rate (80% vs. 25%, P=.02 [calculated]) [25].

A diagnostic biopsy, whether open or percutaneous, is a recommended approach for identifying sperm in men with non-obstructive azoospermia (NOA). However, this approach requires a second procedure to obtain sperm for reproduction. Biopsy results can provide information about the likelihood of sperm retrieval at the time of sperm extraction. Men with hypospermatogenesis (79%-98%), maturation arrest (47%-94%), and Sertoli cell-only (5%-24%) pattern on biopsy have different sperm-retrieval rates [26, 27, 28].

Apart from sperm-retrieval rate, safety and complication rates are also important considerations. Complications from all sperm-retrieval techniques are generally uncommon and minor [29]. Percutaneous approaches are considered to have the lowest rate, with many studies reporting no complications [30-31]. However, a study of 267 procedures reported a 3% complication rate, including hematoma and syncope during the procedure [32]. Complications of testicular sperm extraction (TESE) include hematoma, hypogonadism, and wound infection. Few studies have compared complication rates between TESE groups. However, several studies suggest that postoperative intratesticular hematoma formation is higher with conventional TESE (cTESE) compared to microdissection TESE (mTESE) as assessed by scrotal ultrasonography [33-35]. The use of microsurgical techniques may reduce testicular parenchyma harvest and the risk of complications, including hypogonadism. Acute falls in serum testosterone levels have been observed after TESE, but these levels return to 95% of baseline after complete healing [36, 37].

Timing of sperm retrieval

The timing of sperm retrieval is an important consideration in the management of non-obstructive azoospermia (NOA). Surgical sperm retrieval can be performed during an in vitro fertilization (IVF) cycle to coincide with oocyte retrieval, allowing the use of fresh sperm for intracytoplasmic sperm injection (ICSI). Alternatively, sperm retrieval can be performed before ovarian stimulation with the plan for cryopreservation of sperm for future IVF cycles. Each strategy has theoretical advantages. The use of fresh sperm allows for avoidance of the stress of cryopreservation, while freezing sperm for later use separates timing of the IVF from sperm extraction, potentially avoiding unnecessary ovarian stimulation. Furthermore, it allows both partners to undergo gamete retrieval on separate days, avoiding the need for third-party involvement in transportation and assistance. However, scheduling a sperm extraction for a specific day or

time can be challenging due to the uncertainties in timing. Establishing the efficacy of frozen sperm can also allow for a single sperm extraction rather than a separate procedure for each cycle.

Several studies have compared outcomes for the use of fresh versus frozen sperm for assisted reproductive technologies (ART) in men with NOA. A meta-analysis of 11 studies involving 574 ICSI cycles (275 fresh and 299 frozen) reported no significant difference between fresh and frozen sperm in clinical pregnancy rate or fertilization rate. Additionally, three other studies involving 401 cycles also failed to identify any difference in outcomes using fresh versus frozen sperm in men with NOA. While not all studies reported the identification rate of sperm after cryopreservation, some reported rates ranging from 79% to 100%. Three studies reported post-thaw identification rates of 100% with an overall weighted average of 87% for all studies. Laboratory experience and comfort with cryopreservation of testicular tissue in men with spermatogenic failure are crucial to success. [38-45]

Andrology Research

Prolonged azoospermia can result from the destruction of spermatogonial stem cells inside the testis by infections or cytotoxic agents [46], or for reasons that are yet unknown. However, in some cases, the stem spermatogonia survive but fail to differentiate into sperm, as evidenced by the spontaneous re-initiation of spermatogenesis in some patients after many years of azoospermia [47]. The failure of differentiation of spermatogonia in azoospermic cases cannot be attributed to insufficient stimulation by gonadotropins or testosterone, as the hormonal status examination of azoospermic cases revealed that follicle-stimulating hormone (FSH) levels were 1.5-fold normal, while luteinizing hormone (LH) and testosterone levels remained unchanged [50,51].

Researchers hypothesized that testosterone may actually inhibit spermatogonial differentiation in azoospermia cases. In support of this hypothesis, studies have been conducted where testosterone was suppressed by treating azoospermic rats with gonadotropin-releasing hormone (GnRH) antagonists, which prevented the block in spermatogonial differentiation [52]. However, even though the spermatogonia differentiated, they could not progress past the round spermatid stage as long as testosterone was suppressed. It has been demonstrated that testosterone is critical for the late stages of spermatogenesis, but spermatogonia stem cells in the seminiferous tubules do not require testosterone to divide [53].

Moreover, researchers have found that testosterone is involved in the blockage of spermatogonia stem cells in abnormal conditions such as azoospermia [54]. Suppression of testosterone restores the spermatogenesis process, and in some cases, spermatogenesis was maintained after the cessation of hormonal treatment, leading to fertility restoration [54]. While hormones are responsible for the maintenance of sperm production in normal conditions, testosterone inhibits spermatogonial differentiation in abnormal conditions such as azoospermia [55-58].

Prolistem® Idea

Recent studies have revealed a surprising phenomenon in azoospermia cases, where the hormone testosterone, which is typically required to support normal spermatogenesis, appears to inhibit this process [59, 60]. Testosterone's role in normal spermatogenesis is to support spermatocyte and spermatid development, with minimal effects on spermatogonia [61, 62]. This newly discovered inhibitory effect is an additional action of testosterone and does not replace its usual role. It is important to note that germ cells generally lack androgen and follicle-stimulating hormone (FSH) receptors, meaning that these hormones act on somatic cells, such as Sertoli cells, which then affect spermatogonia through paracrine or juxtacrine interactions.

To address this issue, Prolistem® was developed as a natural supplement designed to support non-obstructive azoospermia (primary testicular failure) through a unique mechanism called the "Spermatogenesis Restarting Process." Prolistem® temporarily reduces testosterone levels, allowing for the crucial early stages of spermatogenesis to occur. Prolistem® Stage one and two focus on reducing testosterone levels to initiate sperm production, while Prolistem® Stage three provides the body with natural components and vitamins to support the production of healthy sperm. The exact mechanism of the testosterone-induced block on Spermatogonial Stem Cells is still unknown. [59, 60, 61, 62]

Prolistem® Rats Experiment

Animals and Non-Obstructive Azoospermia

In this study, male LBNF rats were subjected to irradiation with a 60Co gamma ray unit while under anesthesia and affixed to an acrylic board with surgical tape. The radiation field extended distally from a line approximately 6 cm above the base of the scrotum, with a dose of 6 Gy administered at a rate of approximately 1 Gy/min. The resultant effect was the induction of permanent non-obstructive azoospermia in the LBNF1 rats.

Prolistem® treatment

After a period of 10 weeks following radiation, the animals were treated with Prolistem®. Prolistem® (stage one) was dissolved in water and administered orally via gavage daily for one to two months. The control group was given only water.

Tissue processing

At the end of the treatment period, rats were euthanized with an overdose of a ketamine-acepromazine mixture at 1 and 2 months. Each testis was surgically removed and weighed with the tunica albuginea remaining intact. The right testis was fixed overnight in Bouin's fluid. It was then suspended by silk sutures and centrifuged for 30 minutes at 60 × g and 4°C to determine the weight of the collected fluid. The remaining weight of the testicular parenchymal tissue was measured after removal of the tunica albuginea. The tissue was homogenized in water for sperm head counts. The control group received only water and was subjected to the same procedures.

Evaluation of Spermatogenesis

Histological analysis was performed on the fixed right testis by embedding it in glycol methacrylate plastic, followed by cutting 4-µm sections, and staining them with periodicacid Schiff's (PAS) and hematoxylin. The recovery of spermatogenesis from irradiation was evaluated by scoring a minimum of 200 seminiferous tubules in one section from each animal for the most advanced germ-cell stage present in each tubule. The tubule differentiation index was calculated as the percentage of tubules containing 3 or more cells that had reached type B spermatogonial stage or later.

Rats Experiment Results

In this study, we investigated the impact of Prolistem® mixture on the recovery of spermatogenesis in LBNF1 rats. The treatment was initiated 10 weeks after irradiation with 6 Gy, and we observed a significant increase in the production of differentiated cells. After one month of treatment, there was a 9% increase in the number of differentiated cells, which further increased to 18% after two months (as illustrated in Figure 1). Conversely, the control group did not exhibit any significant recovery during the same period (as shown in Figure 2).

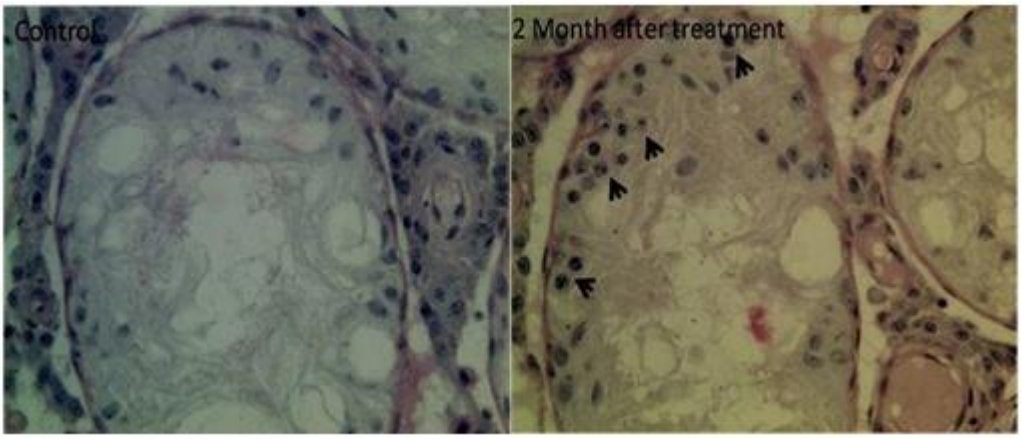

Figure 1: Recovery of spermatogenesis at 10 weeks after Prolistem treatment. Tubule differentiation index (TDI), defined as percentage of tubules differentiating to the B spermatogonial stage or beyond.

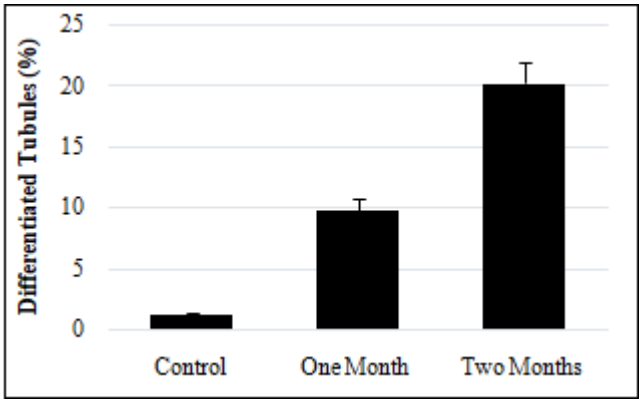

Figure 2: Histology of LBNF1 rat testes 2 months without (Control) or with (treated)Prolistem treatment. Control rats showed atrophic tubules and interstitial edema, most tubules contained only Sertoli cells (SC) but some contained a few type A spermatogonia. Prolistem treatment for two months induced recovery of spermatogenesis.

After one month of treatment, both the control and treated rats did not show any sperm count. However, the treated rats with Prolistem® showed a significant increase in sperm count, with values rising from zero to 100,000 sperm cells per testis (Figure 3).

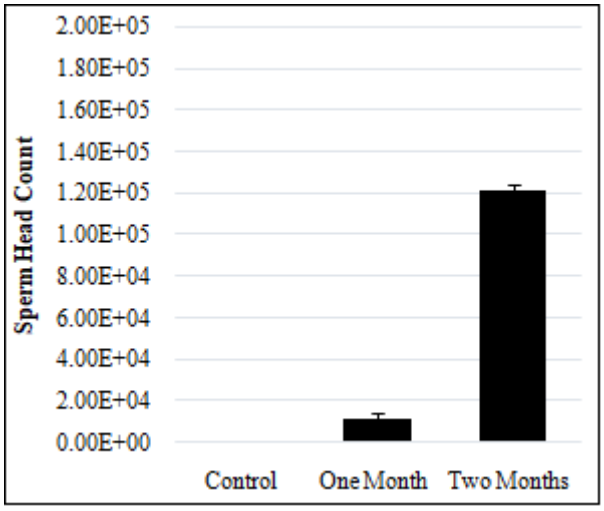

Figure 3: Testicular sperm production: numbers of sonicationresistant late spermatids per testis

Prolistem® Clinical Study

Purpose

The objective of this study was to evaluate the efficacy of a six-month treatment with Prolistem® in improving sperm production in semen and increasing sperm extraction in patients diagnosed with non-obstructive azoospermia (primary testicular failure).

Participants

Adults with non-obstructive azoospermia (primary testicular failure), patients with known genetic issues didn't included in the study.

Treatment with Prolistem®

The study involved administering six months of Prolistem® treatment to a total of 89 patients with non-obstructive azoospermia, who were located in various countries including the USA, Nigeria, Jordan, Israel, and India...

The patients either contacted the researchers directly or were referred by their respective clinics.

2. Data Analysis

The following parameters were collected from the patients basically through email or their doctor that collaborate with us:

- Levels of FSH, LH and Total Testosterone before starting the treatment and after the treatment (because the variability in reporting methods used by the various laboratories we used the terms "Norma" and "High" as an indication of hormones levels.
- Semen test (all reports were zero sperm in semen)
- Biopsy report if available

Results of Prolistem® Clinical Study

Patients who had undergone six months of Prolistem® treatment were subjected to semen analysis, and if no sperm were found, we recommended TESE or micro-TESE procedures. In 48% of the cases following the treatment, either through semen analysis or sperm retrieval, sperm was successfully found. Among the patients, 23% found sperm in their semen (Table 1), ranging from a few to a few million, while 25% underwent successful TESE or micro-TESE surgery to retrieve sperm (Table 2). However, for 52% of patients who received our treatment for six months, there was no observed effect, which may be attributed to unknown genetic factors.

Table 1: Shows the details of 21 patients who successfully found sperm in their semen after completing the six-month course of Prolistem® treatment. To protect their privacy, these patients were given special IDs. The table includes their age, country and previous treatments, Hormone levels were measured before and after the treatment, and the results are presented in the table below.

Patient information					Hormones levels before treatment			Hormones levels after treatment			Treatment result	
Patient ID	Country	Age	Treatments	Sperm Retrieval	Testosterone ng/dl	FSH mIU/ml	LH mIU/ml	Testosterone ng/dl	FSH mIU/ml	LH mIU/ml	Sperm Count	Sperm Retrieval
Az88932	Saudi Arabia	43	Other	NO	800	12.2	7.4	137	11.56	10.16	200	-
Az88965	India	40	NA	Yes, No Success	338	6.6	4.85	Normal	Normal	Normal	15 million	-
Az88777	Iraq	33	Other	NO	Normal	21	Normal	246	21.9	NA	20000	-
Az88522	USA	35	NA	Yes, No Success	581	23	5.8	620	37	6	10 sperm	-
Az87208	Pakistan	30	Other	NO	Normal	Normal	Normal	Normal	Normal	Normal	2.00E+07	-
Az86685	Bangladesh	27	Other	NO	147.9	3.58	NA	NA	NA	NA	15000	-
Az86162	Nigeria	29	NA	NO	NA	44.6	9.79	1093	79.8	12.7	20 sperm	-
Az85116	UAE	34	NA	Yes, No Success	830	74.23	28.72	NA	NA	NA	few sperm	-
Az84593	Nigeria	33	Other	NO	255	4.06	2.71	NA	NA	NA	81 million	-
Az84070	Iraq	33	Other	NO	Normal	37	Normal	Normal	23	Normal	10	-
Az83024	USA	29	Other	NO	Normal	Normal	Normal	Normal	Normal	Normal	10	-
Az82501	USA	35	Other	NO	Normal	Normal	Normal	Normal	Normal	Normal	1.00E+06	-
Az81978	Israel	39	NA	NO	677	21.3	9.7	Normal	Normal	Normal	3.20E+07	-
Az81455	India	28	NA	NO	Normal	High	Normal	Normal	Normal	Normal	1.80E+07	-
Az80932	UAE	33	Other	NO	Normal	Normal	Normal	Normal	Normal	Normal	10000	-
Az80409	Israel	32	Other	NO	Normal	Normal	Normal	Normal	Normal	Normal	few sperm	-
Az79886	Nigeria	33	NA	NO	Normal	High	Normal	Normal	Normal	Normal	few sperm	-
Az79363	Nepal	32	NA	NO	Low	Normal	Normal	Normal	Normal	Normal	1.00E+07	-
Az78840	Saudi Arabia	29	Other	NO	339	10.23	4.84	206	9.48	4.7	6	-
Az78317	UK	34	NA	NO	252	10.23	4.48	114	11.53	4.4	<10	-
Az77794	Saudi Arabia	31	Other	NO	Normal	High	Normal	Normal	High	Normal	1.30E+07	-

Table 2: shows the details of 22 patients who successfully found sperm by sperm retrieval after completing the six-month course of Prolistem® treatment. The table includes their age, country and previous treatments, Hormone levels were measured before and after the treatment, and the results are presented in the table below.

Patient information					Hormones levels before treatment			Hormones levels after treatment			Treatment result	
Patient ID	Country	Age	Treatments	Sperm Retrieval	Testosterone ng/dl	FSH mIU/ml	LH mIU/ml	Testosterone ng/dl	FSH mIU/ml	LH mIU/ml	Sperm Count	Sperm Retrieval
Az88976	USA	30	NA	Yes, No Success	202	6.88	2.65	NA	NA	NA	Negative	**Positive**
Az88721	Israel	36	NA	NO	142	24.7	8.4	Normal	Normal	Normal	Negative	**Positive**
Az88211	New Zealand	34	Other	Yes, No Success	Normal	Normal	Normal	Normal	Normal	Normal	Negative	**Positive**
Az88981	Algeria	33	NA	NO	760	21.83	10.05	684	14.86	NA	Negative	**Positive**
Az88254	Palestine	35	Other	NO	Normal	Normal	Normal	Normal	Normal	Normal	Negative	**Positive**
Az88233	Dominican Republic	40	NA	NO	NA	37.9	10.29	NA	NA	NA	Negative	**Positive**
Az76225	Saudi Arabia	38	Other	Yes, No Success	Normal	High	Normal	Normal	High	Normal	Negative	**Positive**
Az75179	Nigeria	33	NA	NO	Normal	High	Normal	Normal	High	Normal	Negative	**Positive**
Az74133	UK	36	NA	NO	Normal	Normal	Normal	Normal	Normal	Normal	Negative	**Positive**
Az73610	Jordan	34	Other	Yes, No Success	260	3.6	1.6	Normal	Normal	Normal	Negative	**Positive**
Az70995	USA	38	Other	NO	Normal	High	Normal	Normal	High	Normal	Negative	**Positive**
Az70472	Israel	32	NA	NO	399	17.23	4.75	770	21.86	7.35	Negative	**Positive**
Az69949	Jordan	31	Other	Yes, No Success	183.5	25.7	11.6	NA	NA	NA	Negative	**Positive**
Az67857	Jordan	29	Other	Yes, No Success	NC	2	4.3	Normal	Normal	Normal	Negative	**Positive**
Az67334	Nigeria	28	NA	NO	Normal	Normal	Normal	Normal	Normal	Normal	Negative	**Positive**
Az66811	India	26	NA	NO	NC	3.9	6.7	Normal	Normal	Normal	Negative	**Positive**
Az65765	Spain	40	NA	NO	121	10.1	NA	Normal	Normal	Normal	Negative	**Positive**
Az57397	Austrila	38	NA	NO	772	22.09	4.3	Normal	Normal	Normal	Negative	**Positive**
Az52167	Philippines	29	NA	NO	381	8.1	6.43	Normal	Normal	Normal	Negative	**Positive**
Az48506	Malaysia	28	NA	NO	202	6.88	2.65	Normal	Normal	Normal	Negative	**Positive**
Az45369	India	33	NA	NO	228	21.8	8.3	Normal	High	Normal	Negative	**Positive**
Az42232	France	33	NA	NO	Normal	High	High	Normal	Normal	Normal	Negative	**Positive**

3. Conclusion

The present study investigated the efficacy of Prolistem® treatment in an animal model of azoospermia and in a clinical trial involving 89 patients with non-obstructive azoospermia. The animal model demonstrated a 100% positive response to the treatment, which is consistent with our previous studies. However, the clinical trial showed a success rate of 48%, possibly due to unknown genetic factors.

Previous research has shown that testosterone has an inhibitory effect on spermatogonial differentiation in azoospermia cases. Additionally, suppression of testosterone with GnRH antagonist has been shown to stimulate spermatogonial differentiation, which is then reversed by exogenous testosterone. In contrast, testosterone plays a supportive role in normal spermatogenesis by promoting the survival and differentiation of spermatocytes and spermatids.

Chemical drugs that lower testosterone levels would be ideal for treating azoospermia, but their use may be associated with major side effects. Thus, physicians are cautious about testosterone supplementation in low testosterone cases, instead recommending natural ways to increase testosterone levels. In this regard, Prolistem®, which reduces testosterone levels naturally with no side effects, presents a promising treatment option for non-obstructive azoospermia.

Until recently, non-obstructive azoospermia was considered an untreatable condition. However, our study provides evidence that Prolistem® has the potential to restore fertility in affected individuals by reducing testosterone levels naturally.

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