Clinical Study of Prolistem® Supplement in Men with Non-Obstructive azoospermia (Primary testicular Failure).

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Abstract

Introduction: Non-obstructive azoospermia is a cause of male infertility and despite the advancement in gynecology it is still one of the most challenging conditions to treat. Prolistem is a novel treatment for this condition with unique mechanism called "Spermatogenesis Restarting Process"

Objectives: To evaluate the effectiveness of Prolistem supplement in the therapy of infertile men with nonobstructive Azoospermia.

Methods: Eighty-nine patients received Prolistem supplement for six months. Hormones parameters such as FSH, LH and Testosterone levels of the patients were measured before and after the procedure. Semen test was performed after the treatment. In case of no sperm found semen, the patients recommended to perform sperm retrieval such as TESE or micro-TESE. All the required data for the study was collected retrospectively from the patient or the hospital records.

Results: 23% of NOA cases success to find sperm in semen after the six months supplement. 25% of NOA cases success to extract sperm from the testis with the help of sperm retrieval techniques.

Conclusion: Numerous studies and our previous studies using animal models have proven that that testosterone had an inhibitory effect on spermatogonial differentiation in azoospermia cases. Prolistem supplement has been successful for the treatment of cases of non-obstructive azoospermia type primary testicular failure

Introduction

Infertility in the male partner contributes to approximately half of all cases. To date, various techniques, such as in vitro fertilization (particularly, intracytoplasmic sperm injection or ICSI) and so-called TESE-ICSI involving the harvesting of sperm from the testes, have been developed for male infertility. Although these methods are steadily producing results, no technique has proven effective for patients with non-obstructive azoospermia, in which there is an absence of mature sperm in the testes. Evidence suggests that many patients with azoospermia have a genetic predisposition to the condition, although the cause has not been elucidated in the vast majority of cases [1]. Conversely, studies using knockout mouse models have recently linked many genes to spermatogenesis, the mechanisms of which are currently being clarified. These animal findings have yet to be shown applicable to most human cases. This is because identifying the affected genes in humans requires a retrograde genetic approach and because the knockout mouse phenotype is not always faithfully reproduced in humans.

Human Male Infertility and Reasons

Many researchers and clinicians have asserted that societal progress in advanced countries and worsening of the natural environment have likely resulted in decreased male fertility. Long-reported risk factors include working in high temperatures [2], noise associated with manufacturing [3], exposure to radiation [4], electromagnetic waves [5], and a variety of chemical substances [6]. Numerous studies have compared patients with male infertility (oligospermia or azoospermia) to healthy subjects (normal sperm count). To date, proposed risk factors include air temperature [7], automobile driving time per day [8], air pollution [9], regional differences in residential population density [10], mumps [11], stress [12], and alcoholism [13]. On the contrary, many reports indicate the absence of a correlation between environmental factors and male infertility [14, 15]. Thus, there is presently no consistent view on the role of environmental factors and male infertility.

In 1976, Tiepolo and Zuffardi first proposed an explanation for the role of the human Y chromosome in spermatogenesis [16]. They microscopically identied the presence of micro deletions on the long arm of the Y chromosome in six patients with azoospermia and proposed an important spermatogenesis gene in this region. They named this the azoospermia factor (AZF) region. Various subsequent studies have been conducted, particularly by Vogt et al. [17], and in 1995, Reijo et al. examined 89 patients with non-obstructive azoospermia and found that 12 (13%) had a deletion in the AZF region.
These results brought recognition to the close relationship between human azoospermia and this region [18]. Vogt et al. further showed the micro deletions to be concentrated in three regions according to the testicular tissue type and divided the AZF region into subregions, AZFa, AZFb, and AZFb [19].

Non-Obstructive Azoospermia

In non-obstructive azoospermia the testes are abnormal, atrophic, or absent, and sperm production severely disturbed to absent. FSH levels tend to be elevated (hypergonadotropic) as the feedback loop is interrupted. The condition is seen in 49-93% of men with azoospermia [20]. Testicular failure includes absence of failure production as well as low production and maturation arrest during the process of spermatogenesis.

Causes for testicular failure include congenital issues such as in certain genetic conditions (e.g. Klinefelter syndrome), some cases of cryptorchidism or Sertoli-cell-only-syndrome as well as acquired conditions by infection (orchitis), surgery (trauma, cancer), radiation [21] or other causes that we don’t know yet. Mast cells releasing inflammatory mediators appear to directly suppress sperm motility in a potentially reversible manner, and may be a common pathophysiological mechanism for many causes leading to inflammation [22] generally, men with unexplained hypergonadotropic azoospermia need to undergo a chromosomal evaluation.

Until recently, it was assumed that men with non-obstructive azoospermia were untreatable. The only options offered to these couples to have children were the use of donor spermatozoa or adoption. Several clinically relevant findings have changed our approach to this condition. Direct evaluation of testis biopsy specimens often demonstrates sperm in men with non-obstructive azoospermia, despite severe defects in spermatogenesis.

Current Azoospermia Treatment

The method of sperm retrieval may be critical in the management of NOA. Because testicular sperm production, when present, is randomly and heterogeneously distributed throughout one or both testes, surgical methods for sperm retrieval have been developed to achieve wide sampling of the testicular parenchyma. Percutaneous, incisional, and microsurgically assisted techniques have been described. Percutaneous methods such as testicular sperm aspiration (TESA) involve aspiration of testicular tissue using small- or large-bore needles. The needle is typically attached to a syringe that is used to create suction while the needle tip is moved around within each testis to achieve wide sampling of the seminiferous tubular tissue. Incisional methods are generally referred to as conventional testicular sperm extraction (cTESE) or microdissection testicular sperm extraction (mTESE). In cTESE, seminiferous tubular tissue is extracted through one or more testicular incisions. Microdissection TESE is performed by making a large testicular incision and then selectively sampling the largest-diameter seminiferous tubules using optical magnification provided by an operating microscope.

The most important outcome when assessing sperm extraction is sperm-retrieval rate. No randomized controlled trials have been performed to compare techniques of sperm extraction. Two recent systematic reviews have been performed examining surgical sperm-extraction techniques in men with NOA; both identified the same seven studies comparing mTESE to cTESE. The authors report successful sperm retrieval in 35% of cTESE cases (range: 17%–45%) and 52% of mTESE cases (range: 45%–63%), estimating that the performance of a mTESE was 1.5 times more likely to retrieve sperm (95% confidence interval) [23, 24]. Using a combination of prospective and retrospective data, the authors of both reviews concluded that mTESE was superior to cTESE for surgical sperm extraction in men with NOA. It was noted that the greatest advantage seemed to be in men with limited sperm production such as Sertoli cell−only pattern. In addition, seven studies were also pooled to provide a comparison in sperm-retrieval rates between TESA (28%, range: 7%–42%) and cTESE (56%, range: 43%–64%), concluding the superiority of cTESE vs. TESA (relative risk [RR] 2.0, 95% CI 1.8–2.2). Although sperm-retrieval rates were different for cTESE in each of the comparison groups, the conclusions suggest the superiority of mTESE over cTESE and of cTESE over TESA. When a repeat procedure is necessary, data suggest that allowing at least 6 months to pass increases the retrieval rate (80% vs. 25%, P=.02 [calculated]) [25].

A diagnostic biopsy (either open or percutaneous) has also been advocated. Although it may allow men to avoid a more extensive procedure to identify sperm, a diagnostic biopsy obligates men to undergo a second procedure to obtain sperm for reproduction. Data suggest that a diagnostic biopsy may provide information about the likelihood of sperm retrieval at the time of sperm extraction. Men in whom biopsy results demonstrate hypospermatogenesis (79%–98%), maturation arrest (47%–94%), and Sertoli cell−only (5%–24%) have different sperm-retrieval rates [26, 27, 28].

In addition to the sperm-retrieval rate, safety and complication rates are also important considerations. Overall, complications from all sperm-retrieval techniques are uncommon and minor [29]. Percutaneous approaches are thought 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 TESE have been reported as hematoma, hypogonadism, and wound infection. Few studies have been reported that compare complications rates between TESE groups. However, higher

postoperative intratesticular hematoma formation with cTESE compared to mTESE as assessed by scrotal ultrasonography has been suggested by several studies [33-35]. The use of the microsurgical technique may allow decreased testicular parenchyma harvest and reduced sequelae including hypogonadism. Serum testosterone levels do fall acutely after TESE but return to 95% of baseline after healing is complete [36, 37].

Timing of sperm retrieval

Another important consideration in the management of NOA is the timing of sperm retrieval. Surgical sperm retrieval can be performed during an IVF cycle to coincide with oocyte retrieval with the intent of using fresh sperm, if identified, for ICSI. Alternatively, sperm retrieval can be performed before ovarian stimulation with the plan for cryopreservation if sperm are identified for use in future IVF cycles. There are theoretical advantages of each strategy. The use of freshly extracted sperm allows sperm to avoid the stress of cryopreservation. Freezing the extracted sperm for later use separates timing of the IVF from sperm extraction so that if sperm is not found, the female partner can potentially avoid an unnecessary ovarian stimulation. In addition, both members of the couple will be undergoing gamete retrieval on separate days, allowing each to help the other rather than involving a third party for transportation/assistance. Moreover, due to the inherent work flows of coordinating an operating room, scheduling a sperm extraction for a precise day or time can be challenging when the exact timing is known only a few days prior. Establishing the efficacy of frozen sperm can also allow men to undergo a single sperm extraction rather than a separate procedure for each cycle.

Outcomes for the use of fresh vs. frozen sperm for ART in men with NOA have been compared. A meta-analysis compiled data from 11 studies reporting on 574 ICSI cycles (275 fresh and 299 frozen) that involved injection of 4,177 oocytes [38]. No difference between fresh and frozen sperm was identified in clinical pregnancy rate (RR 1.00, 95% CI 0.75–1.33) or fertilization rate (RR 0.97, 95% CI 0.92–1.02). Three additional studies involving 401 cycles also failed to identify a difference in outcomes using fresh vs. frozen sperm in men with NOA [39-41]. Identification of sperm after cryopreservation was not reported by all studies, but five groups report identification ranging from 79% to 100% [42-45]. Three studies reported post-thaw identification rates of 100% with an overall weighted average of 87% for all studies. Laboratory comfort and experience with cryopreservation of testicular tissue in men with spermatogenic failure are crucial to success.

Andrology Research

Many cases of prolonged azoospermia appear to be a result of killing all the spermatogonial stem cells inside the testis by infection or cytotoxic agents [46] or others reasons that we don’t know. In other instances, however, 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]. There is evidence of arrest at the spermatogonial [48] or the spermatocyte [49] stages during the azoospermic period caused by cytotoxic agents or other reasons. The trigger for the spontaneous recovery is not known, but its existence supports the proposal that more rapid or additional recovery from surviving but arrested germ cells can be induced.

Examination of the hormonal status of Azoospermic cases revealed that the failure of differentiation of spermatogonia could not be a result of insufficient stimulation by

gonadotropins or testosterone. Follicle-stimulating hormone (FSH) levels were 1.5-fold normal, luteinizing hormone (LH) and testosterone levels remained unchanged fold normal [50,51]

Researches hypothesized that, in azoospermia cases, testosterone might actually be inhibiting spermatogonial differentiation. Based on this hypothesis, many studies have been done and researches suppressed testosterone by treating Azoospermic rats with gonadotropin releasing hormone (GnRH) antagonists that [52] prevented the block in spermatogonial differentiation. Although the spermatogonia differentiated, they could not progress past the round spermatid stage as long as testosterone was suppressed.

Studies have shown that testosterone is critical for the late stages of spermatogenesis, Spermatogonia Stem Cells in the seminiferous tubules do not need testosterone to divide [53]. Researchers have also 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 and fertility restoration [54].
Hormones are responsible for the maintenance of sperm production in normal conditions; however, in abnormal conditions the testosterone inhibits the spermatogonial differentiation [55-58].

Prolistem® Idea

It was surprising that the testosterone, which is required to support normal spermatogenesis, appeared to inhibit this process in azoospermia conditions. Therefore, more studies were performed to confirm that testosterone was indeed inhibitory in azoospermia cases [59, 60]. It should be pointed out that the action of testosterone in normal spermatogenesis is to support spermatocyte and spermatid development; it has only small quantitative effects on spermatogonia [61,62]. So, the newly discovered inhibitory phenomenon is an additional action of testosterone and does not replace his usual action. Furthermore, it should be noted that the germ cells are generally believed to lack androgen and FSH receptors, so that the hormones act on the somatic cells, likely the Sertoli cell, which then affects the spermatogonia by paracrine or juxtacrine interactions.

Prolistem® is a natural supplement that designed to support non-obstructive azoospermia (primary testicular failure) by a unique mechanism called “Spermatogenesis Restarting Process”. Prolistem® works by temporarily reducing testosterone levels to allow for the crucial early stages of spermatogenesis to take place.
Prolistem® Stage one and two works on the reduction of testosterone that will push the body to restart sperm production while Prolistem® Stage three supplies the body with natural components and vitamins to increase the production of healthy sperm. The testosterone-induced block to Spermatogonial Stem Cells mechanisms is still unknown

Prolistem® Rats Experiment

Animals and Non-Obstructive Azoospermia
LBNF rats were anesthetized and affixed to an acrylic board with surgical tape; then the lower part of the body was irradiated by a 60Co gamma ray unit. The field extended distally from a line about 6 cm above the base of the scrotum. Dose 6 Gy was given at a dose rate of approximately 1 Gy/min, the radiation caused permanent azoospermia to the LBNF1 rats (Non-Obstructive Azoospermia).

Prolistem® treatment
Prolistem® treatment was performed after 10 weeks of radiation; Prolistem® (stage one) was dissolved in water and administered as daily by gavag for one and two months (by the mouth). Control group received only water.

Tissue processing
Rats were killed by an overdose of a ketamine-acepromazine mixture after 1 month and 2 months. Each testis was surgically excised and weighed with the tunica albuginea intact. The right testis was fixed overnight in Bouin's fluid. The testis was suspended by silk sutures and centrifuged for 30 min at 60 × g at 4°C, and the weight of the fluid collected was determined. The remaining weight of the testis parenchymal tissue was measured after removing the tunica albuginea. The tissue was then homogenized in water for sperm head counts.

Evaluation of Spermatogenesis
For histological analysis, the fixed right testis was embedded in glycol methacrylate plastic and 4-µm sections were cut and stained with periodicacid Schiff's (PAS) and hematoxylin. To evaluate the recovery of spermatogenesis from irradiation, we scored a minimum of 200 seminiferous tubules in one section from each animal for the most advanced germ-cell stage present in each tubule. we computed the tubule differentiation index, which is the percentage of tubules containing 3 or more cells that had reached type B spermatogonial stage or later.

Rats Experiment Results

We examined the effects of Prolistem® mix on spermatogenic recovery in LBNF1 rats; Prolistem® treatment starting after 10 weeks after irradiation with 6 Gy restored the production of differentiated cells in 9% after one month and 18% after two months (fig.1). Control rats didn’t show any recovery (fig. 2).

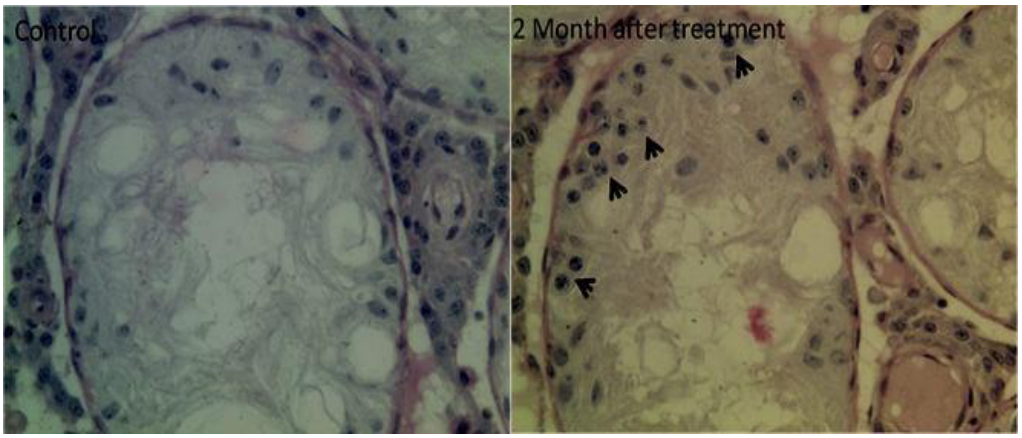

FIG. 1. Recovery of spermatogenesis at 10 weeks after Prolistem treatment. Tubule differentiation index (TDI), defined as percentage of tubules differentiating to the B spermatogonial stage or beyond.

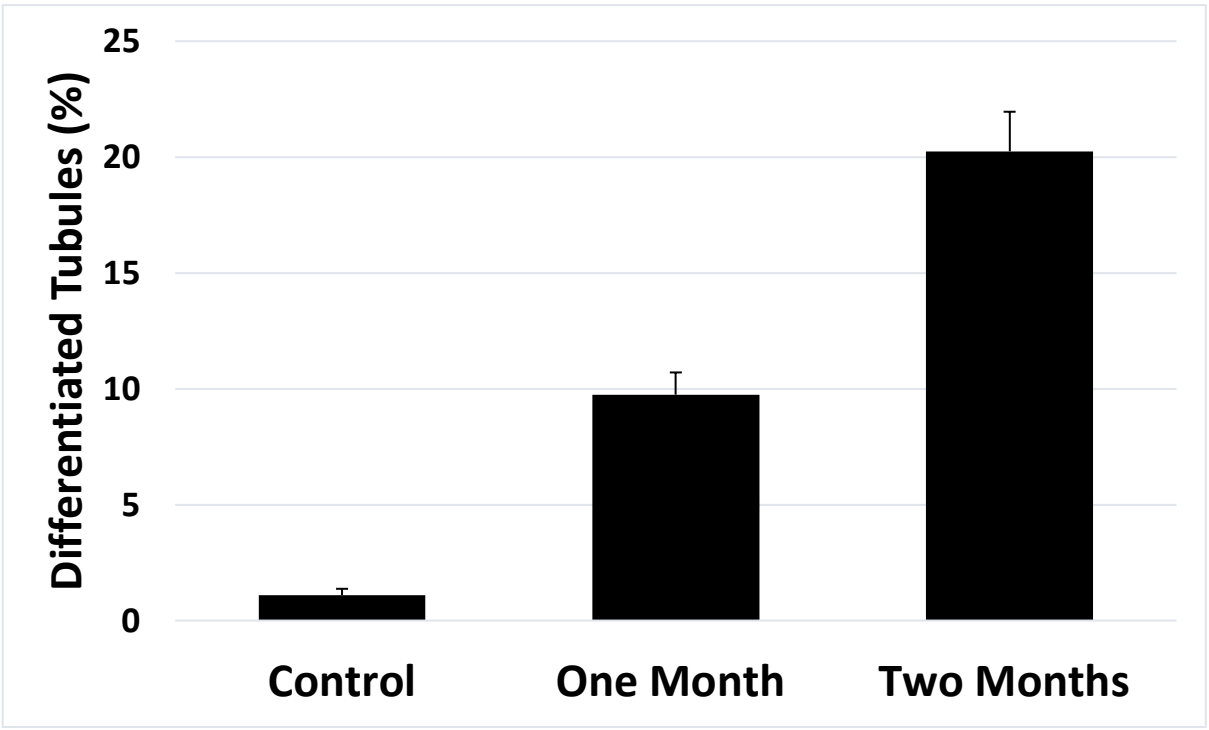

FIG. 2: Histology of LBNF1 rat testes 2 months without (Control) or with (treated) Prolistem treatment. Control rats showed atrophic tubules and interstitial edema, most tubules contained only Sertoli cells (SC) but some contained a few type A spermatogonia. Prolistem treatment for two months induced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

Control and treated rats did not show any sperm count after one month of treatment. However, treated rats with Prolistem® showed increase in sperm count from zero to 100,000 sperm cells / testis (Figure 3).

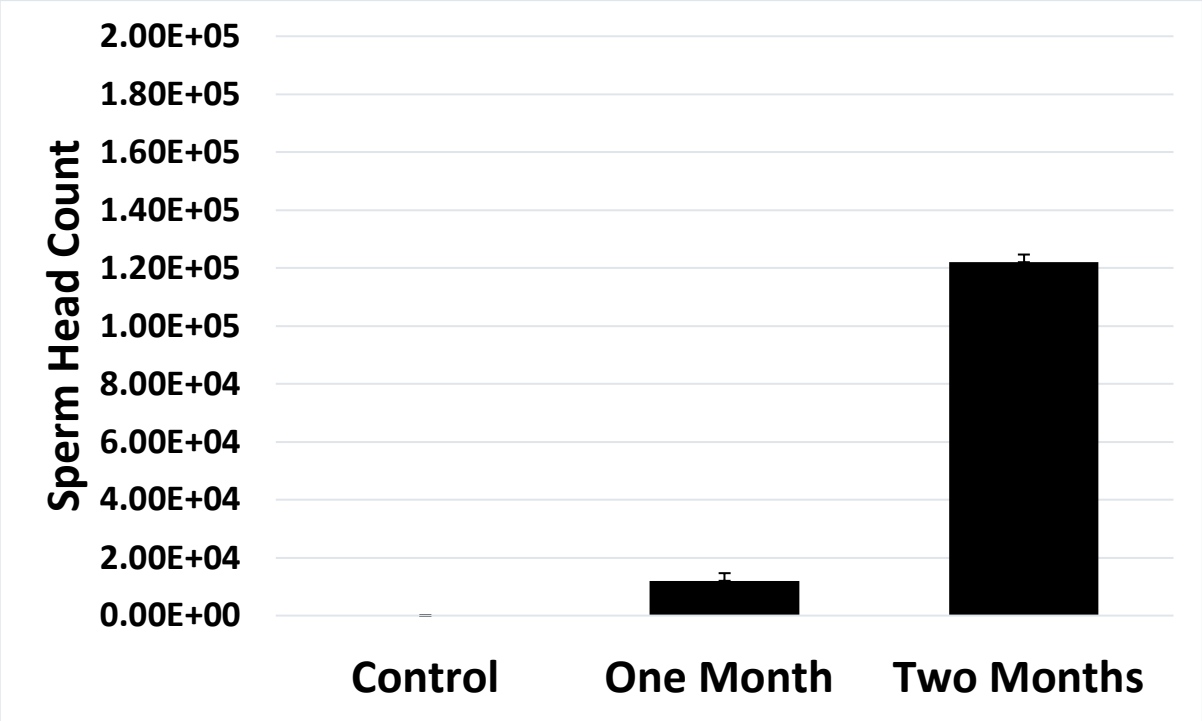

FIG. 3: Testicular sperm production: numbers of sonicationresistant late spermatids per testis.

Prolistem® Clinical Study

Purpose:
The purpose of this trail was to determine whether 6 months treatment with Prolistem® improve sperm production in semen or increase the sperm extraction from patients 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 treatment of six months of Prolistem® shipped to 89 patients around the world (such as: USA, Nigeria, Jordan, Israel, India…), the patients of azoospermia contact us directly or through their clinic.

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

After six months the patients performed semen analysis, if no sperm found then they performed TESE or micro-TESE directly after the treatment based on our recommendations.
23% of the patients found sperm in their semen (from few sperm to few millions) and 25% of the patients performed success TESE or micro-TESE (sperm were extracted by surgery) There is no effect in 52% of the patients that used our treatment for six months, this could be related to unknown genetic issues. Example of success reports described in table 1 to 10.

Table 1:

Case # Az88932	**Age:** 43		**Country:** Saudi Arabia
Patient History	*FSH*	12.2	Range (1.27 – 19.26) MIU/ML
	LH	7.4	Range (1.24 – 8.62) MLIU/ML
	Testosterone	0.8	Range (1.75– 7.81) ug/L
	Ultrasound	Normal	
Genetic History	*karyotype test*	Normal	
	AZF test	Normal	
	Family	No family members with infertility	
Treatments History	Clomid tab, Proxeed, Evit 400mg, Merional 75 and choriomon 5000 IU		
Biopsy History	Not done		
After taking Prolistem® for six months			
Hormones After Prolistem®	*FSH*	11.56	Range (1.27 – 19.26) MIU/ML
	LH	10.16	Range (1.24 – 8.62) MLIU/ML
	Testosterone	1.37	Range (1.75– 7.81) ug/L
Sperm in Semen	**200 sperm**		
Sperm Retrieval	Not done		

Table 2:

Case # Az88965	**Age:** 40		**Country:** India
Patient History	*FSH*	6.60	Range (1.4 – 18) mIU/mL
	LH	4.85	Range (1.5 – 9.3) mIU/mL
	Testosterone	338	Range (241– 827) ng/dl
	Ultrasound	Normal	
Genetic History	*karyotype test*	Normal	
	AZF test	Normal	
	Family	No family members with infertility	
Treatments History	Sperm Retrieval No Success		
Biopsy History	Maturation Arrest		
After taking Prolistem® for six months			
Hormones After Prolistem®	*FSH*	Normal	Range (1.27 – 19.26) MIU/ML
	LH	Normal	Range (1.24 – 8.62) MLIU/ML
	Testosterone	Normal	Range (1.75– 7.81) ug/L
Sperm in Semen	**15 million in semen**		
Sperm Retrieval	Not done		

Table 3:

Case # Az88976	**Age:** 30		**Country:** USA
Patient History	*FSH*	6.88	Range (1.55 – 9.47) muI/mL
	LH	2.65	Range (0.8 – 7.6) muI/mL
	Testosterone	202	Range (262– 1593) ng/dl
	Ultrasound	Normal	
Genetic History	*karyotype test*	Normal	
	AZF test	**AZFc deletion**	
	Family	-	
Treatments History	Sperm Retrieval No Success		
Biopsy History	Sertoli cells only		
After taking Prolistem® for six months			
Hormones After Prolistem®	*FSH*	-	-
	LH	-	-
	Testosterone	-	-
Sperm in Semen	**-**		
Sperm Retrieval	Sperm found by Micro-TESE		

Table 4:

Case # Az88522	**Age:** 35		**Country:** USA
Patient History	*FSH*	23	Range (1.55 – 9.47) muI/mL
	LH	5.8	Range (0.8 – 7.6) muI/mL
	Testosterone	581	Range (262– 1593) ng/dl
	Ultrasound	Normal	
Genetic History	*karyotype test*	Normal	
	AZF test	Normal	
	Family	-	
Treatments History	Sperm Retrieval No Success		
Biopsy History	Biopsy Tubular findings; Azoospermia in 100 % of tubules and Sertoli cells only. Conclusion: Germ cell aplasia /Azoospermia		
After taking Prolistem® for six months			
Hormones After Prolistem®	*FSH*	37	Range (1.55 – 9.47) muI/mL
	LH	6	Range (0.8 – 7.6) muI/mL
	Testosterone	620	Range (262– 1593) ng/dl
Sperm in Semen	**10 sperm in semen**		
Sperm Retrieval	-		

Table 5:

Case # Az88721	**Age:** 36		**Country:** Israel
Patient History	*FSH*	24.7	Range (1.55 – 9.47) muI/mL
	LH	8.4	Range (0.8 – 7.6) muI/mL
	Testosterone	142	Range (262– 1593) ng/dl
	Ultrasound	Normal	
Genetic History	*karyotype test*	Normal	
	AZF test	Normal	
	Family	No Family History	
Treatments History	-		
Biopsy History	-		
After taking Prolistem® for six months			
Hormones After Prolistem®	*FSH*	-	Range (1.55 – 9.47) muI/mL
	LH	-	Range (0.8 – 7.6) muI/mL
	Testosterone	-	Range (262– 1593) ng/dl
Sperm in Semen	-		
Sperm Retrieval	**11 Sperm Found by Micro-TESE**		

Table 6:

Case # Az88211	**Age:** 34		**Country:** New Zealand
Patient History	*FSH*	Normal	Range (1.55 – 9.47) muI/mL
	LH	Normal	Range (0.8 – 7.6) muI/mL
	Testosterone	Normal	Range (262– 1593) ng/dl
	Ultrasound	Normal	
Genetic History	*karyotype test*	Normal	
	AZF test	Normal	
	Family	No Family History	
Treatments History	TESA in march 2012/ Micro-TESE in July 2012		
Biopsy History	Maturation Arrest		
After taking Prolistem® for six months			
Hormones After Prolistem®	*FSH*	Normal	Range (1.55 – 9.47) muI/mL
	LH	Normal	Range (0.8 – 7.6) muI/mL
	Testosterone	Normal	Range (262– 1593) ng/dl
Sperm in Semen	-		
Sperm Retrieval	**10 Sperm Found by Micro-TESE on April 2013**		

Table 7:

Case # Az88981	**Age:** 33		**Country:** Algeria
Patient History	*FSH*	21.83	Range (1.55 – 9.47) muI/mL
	LH	10.05	Range (0.8 – 7.6) muI/mL
	Testosterone	760	Range (262– 1593) ng/dl
	Ultrasound	Normal	
Genetic History	*karyotype test*	Normal	
	AZF test	Normal	
	Family	No Family History	
Treatments History	-		
Biopsy History	-		
After taking Prolistem® for six months			
Hormones After Prolistem®	*FSH*	14.86	Range (1.55 – 9.47) muI/mL
	LH	Not tested	Range (0.8 – 7.6) muI/mL
	Testosterone	684	Range (262– 1593) ng/dl
Sperm in Semen	-		
Sperm Retrieval	**Few Sperm Found by Micro-TESE**		

Table 8:

Case # Az88233	**Age:** 40		**Country:** Dominican Republic
Patient History	*FSH*	37.9	Range (1.55 – 9.47) muI/mL
	LH	10.29	Range (0.8 – 7.6) muI/mL
	Testosterone	Not tested	Range (262– 1593) ng/dl
	Ultrasound	Normal	
Genetic History	*karyotype test*	Normal	
	AZF test	Normal	
	Family	No Family History	
Treatments History	-		
Biopsy History	-		
After taking Prolistem® for six months			
Hormones After Prolistem®	*FSH*	-	Range (1.55 – 9.47) muI/mL
	LH	-	Range (0.8 – 7.6) muI/mL
	Testosterone	-	Range (262– 1593) ng/dl
Sperm in Semen	-		
Sperm Retrieval	**Successful TESE and IVF (baby girl)**		

Table 9:

Case # Az88777	**Age:33**		**Country:** Iraq
Patient History	*FSH*	21	Range (1.55 – 9.47) muI/mL
	LH	Normal	Range (0.8 – 7.6) muI/mL
	Testosterone	Normal	Range (262– 1593) ng/dl
	Ultrasound	Normal	
Genetic History	*karyotype test*	Normal	
	AZF test	Normal	
	Family	No Family History	
Treatments History	Different supplements were prescribed (Proxeed Plus , fertiman and others)		
Biopsy History	-		
After taking Prolistem® for six months			
Hormones After Prolistem®	*FSH*	21.9	Range (1.55 – 9.47) muI/mL
	LH	Not tested	Range (0.8 – 7.6) muI/mL
	Testosterone	246	Range (262– 1593) ng/dl
Sperm in Semen	**20,000 Sperm in semen**		
Sperm Retrieval	**-**		

Table 10:

Case # Az88254	**Age: 35**		**Country: Palestine**
Patient History	*FSH*	Normal	Range (1.55 – 9.47) muI/mL
	LH	Normal	Range (0.8 – 7.6) muI/mL
	Testosterone	Normal	Range (262– 1593) ng/dl
	Ultrasound	Normal	
Genetic History	*karyotype test*	Normal	
	AZF test	Normal	
	Family	No Family History	
Treatments History	Micro-TESE no sperm found		
Biopsy History	Sertoli cell only		
After taking Prolistem® for six months			
Hormones After Prolistem®	*FSH*	Normal	Range (1.55 – 9.47) muI/mL
	LH	Normal	Range (0.8 – 7.6) muI/mL
	Testosterone	Normal	Range (262– 1593) ng/dl
Sperm in Semen	**-**		
Sperm Retrieval	**Micro-TESE: Few sperm for IVF**		

Conclusion

Our animals model using azoospermic rats showed 100% response to the Prolistem treatment. The same effect also reported in our previous studies [63,64] while our clinic trail showed about 48% success and this may be due to unknown genetic issues.

Various studies have indicated that testosterone had an inhibitory effect on spermatogonial differentiation in azoospermia cases. The stimulation of spermatogonial differentiation by suppression of testosterone with GnRH antagonist was reversed by exogenous testosterone.

In azoospermia conditions, it appears that testosterone act additively to inhibit the differentiation of spermatogonia, whereas in normal spermatogenesis testosterone act to support survival and differentiation of spermatocytes and spermatids.

Chemicals drugs that lower testosterone would be ideal for use to treat azoospermia in humans but we don't know what are the major side-effects. For example, in low testosterone cases, physicians trying to avoid testosterone supplementation or at least delayed and given in as low a dose as possible, because testosterone supplementation has many side-effects that we trying to avoid, researches and physicians recommend to increase the testosterone levels naturally if it possible. For the same reason we found formula from natural sources that reduce the testosterone levels in human body to acceptable levels with no side-effects to treat non-obstructive azoospermia.

Until recently, it was assumed that non-obstructive azoospermia was untreatable, here we showed that Prolistem® has the ability to restore fertility by reducing the testosterone levels naturally.

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