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Low-Dose Lisinopril in Normotensive Men With Idiopathic Oligospermia and Infertility: A 5-Year Randomized, Controlled, Crossover Pilot Study

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The outcomes of drug treatment for male infertility remain conjectural, with controversial study results. Our pilot study employed a randomized, placebo-controlled, crossover methodology with intention-to-treat analysis. Thirty-three men with idiopathic oligospermia were randomized to start either daily oral lisinopril 2.5 mg ($n = 17$) or daily oral placebo ($n = 16$). Lisinopril was found to cause a normalization of seminal parameters in 53.6% of the participants. Although the mean ejaculate volume was unchanged ($P \geq 0.093$), the total sperm cell count and the percentage of motile sperm cells increased ($P \leq 0.03$ and $P < 0.001$, respectively), whereas the percentage of sperm cells with abnormal morphology decreased ($P \leq 0.04$). The pregnancy rate was 48.5%, and there was no serious adverse drug event. It is concluded, albeit cautiously, that prolonged treatment with 2.5 mg/day of oral lisinopril may be well tolerated in normotensive men with idiopathic oligospermia, may improve sperm quantity and quality, and may enhance fertility in approximately half of those treated.

Approximately 16.6% of patients at the primary health-care level are those seeking consultation for infertility.[1] Male factor infertility accounts for about 50% of all infertility problems.[2] Oligospermia of unknown cause is common, occurring in up to 60% of men with unexplained, seminopathic infertility.[3] Some subjects with oligospermia have fathered children,[4] but those with infertility have long posed a major therapeutic challenge.[5] The evidence base for using the various hormonal and nonhormonal drugs currently available is, at best, empirical, because most of the efficacy trials yielded conflicting results.[6,7] Although assisted fertilization techniques have now increased the number of therapeutic options available to couples with infertility problems, there is still a very serious limitation in access to the new technology, especially in low-income countries. Besides, there are additional concerns regarding the possible untoward effects of these procedures.[8] These lingering problems underscore the need for continuing to search for other effective treatment options that will not only be less expensive and more accessible but also noninvasive and less complicated.

This preliminary study was occasioned by our previous, independent observations (albeit fortuitous) that normalization of seminal fluid parameters occurred in two men with long-standing idiopathic azoospermia and that their spouses became pregnant. The common factor between the two men was treatment with low-dose (2.5 mg/day) lisinopril, an angiotensin-converting enzyme inhibitor (ACEI) that had been prescribed for concomitant hypertension.[9] A review of the available literature on the efficacy studies of various types of ACEIs on sperm count and quality, carried out in animals, revealed a near-consistent finding of improvement.[10–12] However, methodological flaws have rendered the results in the very scanty human studies extremely difficult to interpret.[13,14] Our preliminary study design was intentionally rigorous; we have made conscious efforts to control for most known confounding factors to the extent possible.

RESULTS

During the recruitment period, 131 men with idiopathic oligospermia volunteered to participate. They were screened for eligibility, and only 33 (25.2%) satisfied the inclusion criteria. These 33 men were enrolled in the study and randomized,

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with 16 in group A and 17 in group B. During the course of the study, five participants (two from group A and three from group B) were lost to follow-up at different stages, giving a total dropout rate of 15.2%. The percentages of dropouts were 17.6 and 12.5% in the lisinopril-onset participants and their placebo-onset counterparts, respectively. The average duration to dropout was 46 weeks in the lisinopril-onset group and 72 weeks in the placebo-onset group. The reasons for dropping out were transfer to distant locations ($n = 2$), death of intercurrent lymphocytic lymphoma ($n = 1$), marriage to another woman ($n = 1$), and loss to follow-up ($n = 1$). A total of 28 (84.8%) participants ($n = 14$ in each of the groups) completed the study. There was no misallocation of treatments.

Table 1 compares the baseline data for the treatment-onset and the placebo-onset groups. There were no significant differences in baseline parameters except for the mean percentage of motile sperm cells, which was significantly higher in the treatment-onset group as compared with their placebo-onset counterparts (95% confidence interval (CI) = 1.8–7.9%; $P < 0.01$).

Figure 1a–d represent plots of the seminal fluid parameters against the week of treatment, starting from week 0 through the crossover point at week 96 to the end of the study at week 282.

Figure 1a represents the changes in the ejaculate volume as mean values (95% CI) plotted against the duration of treatment in weeks for the treatment-onset and the placebo-onset groups. The mixed-model analysis of variance (ANOVA) showed no statistically significant differences in within-subject means, between-subject means, and their interaction ($P > 0.05$ for each).

Figure 1b represents the plot of total sperm counts as mean values (95% CI) plotted against the duration of treatment in weeks for the treatment-onset and the placebo-onset groups. The mixed-model ANOVA showed statistically significant differences in within-subject means, between-subject means, and their interaction ($P < 0.0001$ for each). The *post hoc* Bonferroni's multiple comparison test showed that, following a lag period from week 0 to week 12, the within-subject means began to increase with time in the lisinopril-onset group from week 24 to week 102 ($P \leq 0.03$) but did not change significantly in the placebo-onset group until week 138 ($P \geq 0.09$). By contrast, after an initial lag

Table 1 A comparison of the entrance data between the treatment and placebo groups of patients

Parameter	Patients in group A ($n = 16$)	Patients in group B ($n = 17$)	95% CI
Age (years)	26.93 ± 7.3	30.86 ± 8.8	−10.21, 2.35^{Ns}
Height (m)	1.48 ± 0.5	1.51 ± 0.4	−0.38, 0.32^{Ns}
Weight (kg)	64.26 ± 10.3	66.19 ± 11.2	−10.29, 6.48^{Ns}
Duration of infertility (years)	7.77 ± 3.1	8.20 ± 4.3	−3.31, 0.15^{Ns}
Ejaculate volume (ml)	3.01 ± 0.23^{GM}	3.09 ± 0.34^{GM}	−0.31, 0.15^{Ns}
Sperm cell count (millions/ml)	7.43 ± 3.97^{GM}	5.29 ± 2.6^{GM}	−0.47, 4.75^{Ns}
Sperm cells with good motility (%)	22.12 ± 4.4^{GM}	17.33 ± 3.2^{GM}	1.80, 7.78[a]
Sperm cells with abnormal morphology (%)	44.12 ± 2.6^{GM}	42.91 ± 5.1^{GM}	−1.94, 4.37^{Ns}

Comparisons are by Student's *t*-test. Results are expressed as mean ± SD and 95% CI.
CI, confidence interval; GM, geometric mean; Ns, not statistically significant.
[a]Statistically highly significant.

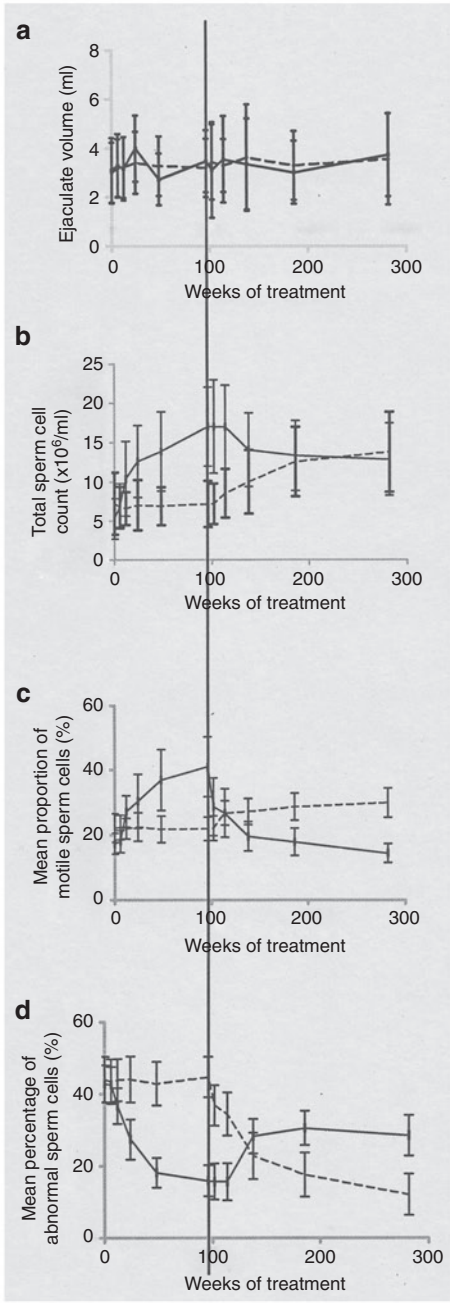

Figure 1 Plots of the changes in seminal fluid parameters with duration of treatment in weeks. Changes from baseline in relation to duration of treatment in weeks for (**a**) volume of the ejaculate (ml), (**b**) total sperm count (millions/ml), (**c**) the percentage of motile sperm cells, and (**d**) the percentage of sperm cells with abnormal morphology, all plotted against the number of weeks of treatment. Each plot shows the pattern of parameter changes with time in the lisinopril-onset group (dashed line) and in the placebo-onset group (dotted line). The vertical line parallel to the *y* axis indicates the crossover point. The points in the graph are mean values; the crossbars are the 95% confidence intervals of the means (95% CI).

from week 96 (i.e., the treatment crossover point) to week 138, the within-subject mean values of total sperm count began to decrease ($P \leq 0.02$) in the lisinopril-onset group (switched to placebo after the crossover) and began to increase ($P \leq 0.004$) in the placebo-onset group (switched to lisinopril after the crossover). The two curves intersected between week 186 and week 282. Interestingly, the mean sperm count in group B remained significantly higher at week 282 than the initial value at baseline (95% CI = 3.3–9.8 $(\times 10^6)$/ml; $P < 0.001$). This was despite the group having been placed on placebo from week 96 onward.

Figure 1c shows the percentages of motile sperm cells as mean values (95% CI) plotted against the duration of treatment in weeks for groups A and B. The mixed-model ANOVA showed statistically significant differences in within-subject means, between-subject means, and their interaction ($P \leq 0.001$ for each). The *post hoc* tests revealed that the first statistically significant increase in within-subject means for this parameter occurred in the lisinopril-onset group at week 12 (95% CI = −12.1 to −7.8 %) as compared with the value at week 0 ($P < 0.001$). Thereafter, the within-subject mean values increased progressively with time until week 48 ($P < 0.05$ for each interval of time). In the placebo-onset group, however, significant differences in within-subject means became noticeable only after the treatment crossover point (week 96), starting from week 114 ($P \leq 0.005$) and continuing up to week 282. Concurrently, a sequential decrease in the within-subject means ($P \leq 0.008$) was also observed in the treatment-onset group (group B).

Figure 1d shows the changes in the percentage of sperm cells with abnormal morphology (mean ± SD) plotted against the duration of treatment in weeks for both groups. The mixed-model ANOVA showed statistically significant differences in within-subject means and between-subject means ($P < 0.001$ for each). Bonferroni's *post hoc* tests showed mirror-image patterns when the within-subject mean differences in group A were compared with those in group B. In other words, statistically significant decreases ($P \leq 0.04$) were observed in the within-subject means of group B from week 12 to week 112, whereas in group A the increase was observed from week 102 to week 282 ($P \leq 0.03$ for each).

Further analysis was conducted using data for the 28 participants who completed the study in order to examine more closely the trend of each individual's seminal fluid characteristics over time in response to lisinopril exposure, as compared with the baseline values. Several subsets of participants were clearly identified. Whereas 15 (53.6%) exhibited an overall improvement—albeit to varying degrees—in the quantity and quality of sperm cells, it was found that 4 (14.3%) showed a decrease in both quantity and quality and 6 (21.4%) showed a decrease in quality with no significant change in the quantity of sperm cells. Other incongruous patterns of parameter variability were also encountered sporadically.

The incidence of unassisted pregnancies

During the 4-year period of the study, there were 21 confirmed cases of unassisted pregnancy in 16 families, giving a couple pregnancy rate of 48.5%. Between the onset of the study and the crossover point (week 96), three couples from the lisinopril-onset group and none from the placebo-onset group became pregnant. Between week 96 and the end of the study, 13 more pregnancies were confirmed: 5 in the placebo-onset group and 8 in the lisinopril-onset group. One of the couples reported three pregnancies during this period, and three other couples reported two pregnancies each. In addition, 11 couples reported one pregnancy each. There were 13 deliveries during the period of the investigation; one was by cesarean section, and the other 12 were normal vaginal deliveries. None of the babies exhibited any obvious anomaly. The pregnancies were reported after various durations of lisinopril treatment, ranging from 21 to 88 weeks (average = 63.2 weeks).

Adverse events reported

Table 2 represents the frequency distribution of symptoms and signs of adverse events reported with lisinopril and with placebo. An analysis comparing the two frequency distributions showed no statistically significant difference between the two groups ($U = 134.5$; $P = 0.74$). However, differences were found with respect to specific symptoms: chest pain, dizziness, and arthralgia were significantly more prevalent with lisinopril than with placebo. Cough, known to be one of the most troublesome side effects of ACEIs (including lisinopril), had a frequency of 7.8% with lisinopril. It was the fifth most frequently reported adverse event, and the frequency of cough, as reported, did not differ significantly between the two treatments (relative risk = 0.89; 95% CI = 0.50–1.38; $P = 0.62$). However, none of the events was adjudged as being severe enough to warrant hospitalization or withdrawal from treatment.

Changes in blood pressure

Figure 2 shows the mean arterial blood pressure (MAP) plotted against the duration of treatment in weeks in both groups. The mixed-model ANOVA showed no statistically significant differences either in within-subject mean values or in between-subject mean values of MAP, as measured with the participant in the supine posture and in the erect posture ($P > 0.31$ for each group).

Changes in serum potassium levels

No statistically significant difference was found in the within-subject means, between-subject means, or their interaction ($P > 0.16$ for each).

Compliance with treatment regimen

The compliance rates ranged from 79.1 to 92.3% (average = 83.5%). No significant difference was found in compliance rates between participants on lisinopril treatment and those on placebo, both before and after the crossover (81.7 ± 7.6% vs. 86.2 ± 9.4%; 95% CI = −10.59 to 1.59; $P = 0.14$, Student's t-test).

DISCUSSION

The pathophysiologic mechanisms involved in idiopathic seminal fluid defects (seminopathies) are complex and far from being well understood.[15] Oligospermia of unknown cause is

Table 2 A comparison of the symptoms reported by the patients during lisinopril treatment and during placebo treatment

Subject no.	Symptoms reported	With lisinopril treatment *n* (%)	With placebo treatment *n* (%)	Relative risk	95% Confidence interval of the relative risk
1	Anorexia	7 (5.4)	7 (4.4)	1.12	0.65–1.93
2	Constipation	3 (2.3)	1 (0.6)	1.72	0.96–3.08
3	Chest pain	7 (5.4)	0 (0.0)	2.30	2.02–2.63[a]
4	Cough	10 (7.8)	16 (10.0)	0.84	0.50–1.38
5	Catarrh	12 (9.3)	8 (5.0)	1.34	0.92–1.97
6	Diarrhea	6 (4.7)	8 (5.0)	0.96	0.52–1.78
7	Dizziness	6 (4.7)	0 (0.0)	2.30	2.02–2.63[a]
8	Epigastric pain	1 (0.8)	12 (7.5)	0.17	0.03–1.12
9	Fever	26 (20.3)	34 (21.4)	0.88	0.64–1.20
10	Headache	7 (5.4)	17 (10.7)	0.63	0.33–1.19
11	Joint pains	20 (15.6)	3 (1.9)	1.98	1.61–2.44[b]
12	Malaise	15 (11.7)	19 (11.9)	0.95	0.64–1.41
13	Myalgia	4 (3.1)	6 (3.8)	0.90	0.42–1.95

[a]Statistically highly significant ($P < 0.01$). [b]Statistically very highly significant ($P < 0.001$).

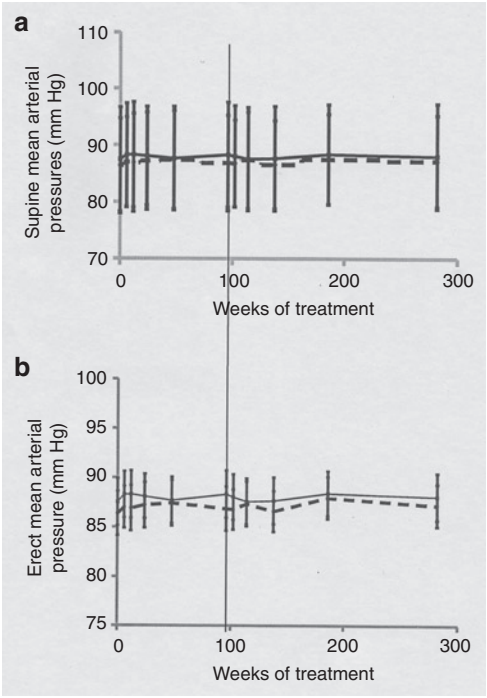

Figure 2 Plots of the changes in mean arterial pressures with duration of treatment in weeks. Changes from baseline in relation to duration of treatment in weeks for (**a**) the supine posture mean arterial pressures (supine-MAP) and (**b**) the erect posture mean arterial pressures (erect-MAP) for the lisinopril-onset group (dashed line) and for the placebo-onset group (dotted line). The vertical line parallel to the *y* axis indicates the crossover point. The individual points are mean values; the crossbars are 95% confidence intervals of the means (95% CI).

a commonly encountered form of idiopathic seminopathy,[3,4] and it poses a major therapeutic challenge.[5] Clomiphene, gonadotropins, bromocriptine, L-thyroxin, vitamin E, and B12 have all been tried, with not very encouraging results.[16] When drug treatments for infertility fail, intrauterine insemination and assisted reproductive therapies such as *in vitro* fertilization and intracytoplasmic sperm injection are recommended.[16] Access to these newer options is greatly limited—not by unwillingness but by inability to afford the cost, even when the treatment is available.[17–20] Besides, there are obvious concerns about their long-term safety.[21–26] These factors make it relevant to seek other solutions to this age-old problem that will be effective and also inexpensive and safe.

In 1999, we reported two separate observations in two male patients with hypertension who had long-standing infertility and azoospermia of unknown cause. In both men, there was normalization of seminal fluid parameters apparently resulting from therapy with 2.5-mg daily doses of lisinopril, and the spouses of both men became pregnant.[9] The current study was aimed at elucidating more clearly the nature of the relationship between treatment with lisinopril (an ACEI) and correction of the seminopathy. We used the same low dose of lisinopril 2.5 mg daily that had been taken by the two male patients discussed above.

This present study showed that treatment with a 2.5-mg daily dose of lisinopril, but not with the placebo, increased the total sperm cell count. Conversely, the withdrawal of lisinopril and its substitution with placebo led to a decrease in the total sperm cell count. Together, these findings indicate that lisinopril treatment is linked to the observed changes in sperm cell count. This observation is in agreement with some previous findings related to treatment with ACEIs and other kinin-enhancing drugs[27] but is contrary to the findings in other studies[28] (many of which had contentious methodological issues).[28] In an effort to avoid some of the identified methodological pitfalls in these earlier studies, we adopted a rigorous study design that enabled two types of controls, namely, within subjects and between subjects. The major limitations of the current study, however, are that the sample size is small and that the possibility of interaction

between lisinopril and some of the comedications cannot be conclusively ruled out.

The placebo-onset group and the treatment-onset group were found to have similar baseline characteristics, except for sperm cell motility, which was significantly better in the treatment-onset group than in the controls. Therefore, the two comparison groups were, for all intents and purposes, a good match in terms of the other variables. It is therefore unlikely that the observed changes in the sperm characteristics of the participants were caused by a random variation or by any comedication. A possible explanation for this observed action of lisinopril may be found in our current knowledge about the relationship between gonadal ACE and gonadal kininase II. All the products of the kinin–bradykinin–kallikrein system have been found in human male genital secretions. The kinins have been shown to increase spermatogenesis, sperm motility, and sperm metabolism even in minute concentrations.[27] It has recently been shown that these effects are mediated by a specific sperm membrane-integrated bradykinin receptor subtype B2. Kininase II, which is responsible for the degradation of the products of the kinin–bradykinin–kallikrein system, is identical to ACE.[27,28] It is possible that lisinopril, through the inhibition of kininase II, produces the observed effects on sperm quantity and quality by causing an accumulation of the products of the kinin–bradykinin–kallikrein system within the testicular milieu.

The current study also demonstrated that treatment with lisinopril increased sperm cell motility and improved morphology. Motility and morphology of the sperm cell have been proven to be major determinants of success in fertilization both *in vivo* and *in vitro*.[29–31] It is uncertain, however, whether—and, if so, how—these observations may have been influenced by the study sample size or whether the enhancement of kinin/bradykinin activity through ACE inhibition by lisinopril is the sole explanation. The modulation by ACEIs of some of the actions of, for example, sex hormones, cytokines, growth factors, and leptins has also been suggested as a mechanism.[32] It is possible that all these may act in an intricate web of combinations with the lisinopril to contribute to the observed changes in sperm characteristics. Although an adequate volume of the seminal fluid in the ejaculate is required to transport sperm into the female reproductive tract and allow for fertilization of the oocyte,[33] it does not appear likely that lisinopril has any significant effect on any of the processes regulating this parameter in humans. A possible alternative interpretation for this apparent lack of a lisinopril effect may be that most of the participants already had normal volumes of ejaculate, which may have constituted an inhibitory stimulus. Besides, the small size of the study sample may not have ensured sufficient sensitivity to detect small changes in parameters.

It is intriguing that in this study wide individual variations in parameters were observed in response to lisinopril exposure. Although a majority showed improvement in all the seminal fluid characteristics, there was an overall worsening of status in a few, with another small subset showing no significant change. There were also individuals who had one or two changes in parameters, sometimes in quite unexpected directions. Some previous studies have found lack of improvement, and even a worsening, in sperm quantity and quality after treatment with kinin-enhancing drugs.[13,14,28] This only further underscores the assertion that idiopathic oligospermia is not a homogeneous entity and that it possibly represents a conglomeration of entities with diverse pathophysiologic mechanisms.[3] Another possible explanation for the differences in the responses to lisinopril in this study may be related to a recently identified testicular isoform of ACE (*tACE*) expressed in the spermatozoa of humans and some other mammalian species.[34,35] This strongly suggests a potential role of *tACE* in at least some aspects of spermatogenesis. Inhibition of *tACE* activity would therefore tend to inhibit rather than enhance spermatogenesis. The validity of this hypothesis is supported by the various experiments involving *tACE* gene–knockout mice. The male animals lacked expression of the *tACE* isozyme and were found to have defective spermatogenesis and infertility.[36,37] This was rectified when the *tACE* gene was restored in these mice.[38] These apparently paradoxical findings are indicative of the pathophysiologic complexity of idiopathic oligospermia and reflect the immense difficulty involved in attempting to standardize drug treatment for the condition.

Spontaneous pregnancy is rare, although some series have reported its occurrence in up to 15.4% of couples with untreated oligospermia after the couple cohabited without achieving pregnancy for more than a year.[2,7] Hence, the pregnancy rate of 48.5% observed in this study is unlikely to be explained by chance. The factors responsible for infertility in couples with seminopathic problems are complex and continue to be the subject of intensive investigation.[3] It has been shown, for example, that an improvement in sperm quality does not necessarily improve the chances of achieving a pregnancy; nor does a deterioration in sperm quality necessarily reduce the chances.[39] It must be admitted, however, that in our study the paternity of the conceived babies was not established because this was not one of the objectives of the investigation. However, the fact that all the women who became pregnant had husbands with exposure to lisinopril is a finding that supports the possible effect of the drug in this regard.

Tolerability

Lisinopril is commonly prescribed for the treatment of hypertension and congestive heart failure.[40,41] The side effects of this ACEI are usually mild to moderate in therapeutic doses, the most serious being dry cough, angioedema, and hyperkalemia.[41,42] Of these potentially serious side effects, only cough was reported in this study, and it was the seventh most frequently encountered adverse event. However, all the events were mild. There are two possible explanations for the apparent attenuation of this side effect: (i) ACEI-associated cough is more often found in women than in men[43–48] and (ii) the intensity of cough, but not the frequency, appears to be dose-dependent.[49] The participants in our study were all men, and the dose of lisinopril used was low.

Conclusion

The pathophysiologic mechanisms involved in idiopathic oligospermia are complex and not well understood. However, lisinopril given orally at the dosage of 2.5 mg/day appears to be well tolerated among normotensive men with oligospermia and

may improve sperm quantity and quality and improve fertility in approximately half of those treated. However, the results should be interpreted with caution because of the small sample size of the study. These findings therefore require confirmation in larger, multicenter studies.

METHODS

The study was conducted at the University of Nigeria Teaching Hospital in Enugu. Prior approval of the detailed study protocol was obtained from the ethics committee of the hospital. Participants gave written informed consent before being enrolled. The study was a preliminary investigation designed as a longitudinal, randomized, double-blind, placebo-controlled, crossover clinical trial. The protocol has been published on ClinicalTrials.gov (identifier NCT01409837).

The subjects for this investigation were selected from a volunteer pool of male patients attending the hospital's fertility clinic. The criteria for selection were (i) age 24–34 years, (ii) being on treatment for oligospermia and regularly attending the fertility clinic for at least 2 years, (iii) total sperm count 5–10 million/ml, (iv) white blood cell count $<1 \times 10^6$/ml of the ejaculate, (v) evidence of comprehensive investigations to exclude secondary causes of low sperm count, (vi) evidence of comprehensive investigations to exclude female-factor infertility in the spouse, (vii) an assurance of a personal commitment to continue participating in the study until the end point, and (viii) normal blood pressure. The exclusion criteria were (i) nonconsent, (ii) total sperm cell count <5 million/ml or >10 million/ml, (iii) failure to fulfill any of the inclusion criteria during baseline assessments, and (iv) potential interaction between lisinopril and comedications. The main purpose of introducing these stringent criteria was to minimize the potentially confounding effects on sperm cell quantity or quality of factors such as wide differences in age[50,51] and in the initial seminal fluid characteristics.[52] At the time of enrollment, each subject was given explicit information about the study with respect to the intention, the expectations from the participant, the procedure, the planned duration of the investigation, and potential adverse reactions to the intended medication. Per the protocol, the recruitment of participants took place from March 1998 to September 2001, and the actual study lasted for 5 years, from January 2002 to December 2006. Before commencement of the study, seminal fluid analyses were carried out twice for each participant, with an interval of at least 2 weeks. Thereafter, the eligible participants were randomized into two groups, A and B. Treatments were also randomly allocated to the groups in a double-blind fashion. Group A was started on the coded drug "DY1," and group B was started on the coded drug "DZ2"; the drugs were identical in appearance. At week 96, the drugs were switched between the groups such that group A changed to drug DZ2 and group B changed to drug DY1. There was no intervening washout period. The drugs were procured, packaged, and coded by the Drug Compounding Unit of the hospital's pharmacy department. The codes were known only to the head of the department until after the data analysis.

In strict compliance with the design of the study, entry for all participants was complete within 7 days of starting the trial, and they were followed up concurrently. Throughout the period of the clinical trial, the participants mandatorily continued their various "background" fertility medications in the doses prescribed by their attending fertility physicians. The rationale for this was to avoid the unethical situation in which a group taking placebo would be denied medication. The seemingly superfluous strategy of combining a crossover design (which provides a within-subject control) with a separate between-subject control was deliberate. This was done in an effort to control, in one step, for two potentially confounding factors: concurrent background medications and any random, seasonal variation in the characteristics of the seminal fluid.[53] All-inclusive enrollment of eligible participants was a prior decision made while designing the trial.

The main outcome measures were the changes at various time points, relative to baseline, in ejaculate volume, total sperm cell count, percentage of motile sperm cells, and percentage of sperm cells with abnormal morphology. The incidence of pregnancy in a spouse in both groups was also one of the main outcome measures. The characteristics of the seminal fluid samples were assessed twice (with at least a 2-week interval between assessments) before the participants entered the study, and the average of the two results was entered as the baseline value (week 0 value). Evaluation of seminal fluid was also repeated in every participant during each of the scheduled visits at weeks 6, 12, 24, 48, and 96. The treatment was switched between the two groups at the end of week 96 (the treatment crossover point); subsequently, seminal fluid parameters continued to be assessed during each of the continuing scheduled visits at weeks 102, 114, 138, 186, and 282 (end point of the study). The choice of these intervals was arbitrary. The participants were advised to abstain from sexual intercourse for at least 4 days before each scheduled semen sample collection. Samples were collected via masturbation in a room near the laboratory. Throughout the entire study period, the investigators kept in close touch with the participants by telephone in order to continually motivate them, remind them of scheduled appointment dates, monitor compliance, and identify any possible adverse drug effects. A later modification was the decision to stop if there was no difference at week 96.

The randomization of the participants and the random allocation of treatment were carried out by a nonmedical student who had no further involvement in the study. The 33 participants were listed in alphabetical order of their surnames, and these numbers were marked on 33 table-tennis balls. The blindfolded student picked out balls at random and placed them alternately in two boxes marked A and B.

Assessment of compliance with the medication regimen

Compliance with the medication regimen was monitored through a combination of oral interviews and counting of the pills remaining in medication containers. These were done at every scheduled visit for every participant, and also between visits through sporadic phone calls and unscheduled home visits. The level of compliance of each participant over a period was expressed as a percentage, calculated as the actual dosage for the period divided by the expected dosage for the period, multiplied by 100.

Monitoring of adverse events

The participants were encouraged to report every adverse event promptly by telephone to one of the investigators (G.O.N.). The incidents were recorded according to the symptoms and signs. A physician examined each of these individuals and made recommendations with respect to further management and/or the need for withdrawing the participant or stopping the trial. Medical interventions, when needed, were made available free of cost to the participants.

Clinical measurements

Blood pressure was measured using mercury sphygmomanometers fitted with adult-size cuffs (Accoson, Essex, England), and the first and fifth Korotkoff sounds were used to determine systolic and diastolic blood pressures, respectively (because these had given more concordant results among the team members than the traditional first and fourth Korotkoff sounds). The MAP of each participant was calculated using the conventional formula: MAP = ((2 × diastolic) + systolic)/3.

Laboratory measurements

The semen specimens were incubated at 37 °C and allowed to stand for 1 h for thawing. Pipettes were used to determine the volume of the ejaculate, and microscopes were used to determine the total sperm cell count, the percentage of sperm cell motility, and the percentage of abnormal sperm cell morphology, in accordance with World Health Organization guidelines.[54] Serum potassium levels were estimated using the flame photometric method as described by Davidson and Henry.[55] The latter test was a safeguard against hyperkalemia, a well-documented, severe side effect of ACEI therapy.

Statistical analysis

Statistical analyses were carried out using SPSS 16 software (SPSS, Ashburn, VA).[56] All the data analyses were based on the intention to

treat (the latest observations after the baseline were carried forward to the end point). Before the analyses, all the parameter data were examined for distribution patterns, first visually, using quantal–quantal plots, and then quantitatively, using the Shapiro–Wilk normality test. The reason for this double check was that our sample size was small, which might have had a biasing effect on the reliability of the quantitative normality testing methods.[56] All the data with respect to seminal fluid parameters and serum potassium values were skewed and were normalized using logarithmic transformations. Two-group comparisons were performed using unpaired Student's *t*-test, and proportions were compared using Fisher's exact tests. The data from longitudinally measured outcome parameters were analyzed using two-way repeated-measures (mixed model) ANOVA. Bonferroni's *post hoc* multiple-comparison tests were done when a statistically significant difference was found with the ANOVA (at $P < 0.05$) in the within-subject means, the between-subject means, and their interaction.[56] The *post hoc* tests were done in order to explore further the patterns of within-subject changes in parameters with time points in both groups. The unwanted events reported during lisinopril therapy and those reported during placebo therapy were compared using Koch's adaptation of the Wilcoxon–Mann–Whitney rank sum test and Fisher's exact test. When the treatments were decoded, it was found that group B (referred to here as the lisinopril-onset group) started from week 1 to receive 2.5 mg of oral lisinopril daily (Zestril; AstraZeneca Pharmaceuticals, Washington, NC), whereas group A (referred to here as the placebo-onset group) started from week 1 to receive daily oral placebo. The results were expressed, for the continuous data, as means ± SD or as 95% CI as appropriate, and proportions were expressed as percentages (%).

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CONFLICT OF INTEREST

The authors declared no conflict of interest. We obtained the permission of Grassroots Healthcare Foundation of Nigeria (GHF), the grantor, to publish our findings in the peer-reviewed journal of our choice, with the only proviso that we acknowledge its financial contribution.

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