Serum estradiol levels in infertile men with non-obstructive azoospermia

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Abstract
Purpose: To report the different patterns of estradiol levels in infertile men with non-obstructive azoospermia and correlate these levels with their clinical and laboratory findings.
Materials and methods: A retrospective study was launched, and a retrieval of data for infertile men with non-obstructive azoospermia (n = 166) and fertile controls (n = 40) was performed. The retrieved data included demographics, clinical findings, scrotal duplex, semen analysis, and hormonal assay (testosterone, follicle-stimulating hormone, luteinizing hormone, prolactin, and estradiol).
Results: Our findings showed a wide spectrum of estradiol concentrations. The patients were arranged into three groups (high, normal, and low estradiol groups). The normal estradiol group was the most prevalent (71.1%). Testosterone, gonadotrophins, testicular volumes, and the number of patients with jobs in polluted workplaces showed significant differences among the study groups (p = 0.001, < 0.001, < 0.001, and 0.004, respectively). Age, body mass index, varicocele prevalence, prolactin, and smoking habits did not show any significant differences among the groups. Obesity was lacking in the low estradiol group, but it had significantly higher prevalence in the normal (p = 0.013) or high group (p = 0.023) compared with the controls.
Conclusion: Serum estradiol, in infertile men with non-obstructive azoospermia, may be present at different levels. It is recommended that estradiol be measured in infertile men with non-obstructive azoospermia when there is an alteration in testosterone concentration, obesity, a polluted workplace occupation, or before trying hormonal therapy. Extended studies are highly recommended to provide a clear clue whether alterations in estradiol concentrations in men with non-obstructive azoospermia are the cause or a consequence of the condition.

Keywords: estradiol, infertile men, non-obstructive azoospermia

Introduction
Azoospermia or lack of sperm in the ejaculate may be observed in 10–15% of men seeking advice for infertility.1 The condition is obstructive in 40% of cases and non-obstructive in the remaining 60%.2 Non-obstructive azoospermia (NOA), which is due to disturbed spermatogenesis, has incited a substantial amount of debate related to its management. Recent molecular biological techniques have shed light on the possible etiologies of several cases of NOA. These cases were classified in the past as idiopathic. In spite of this, a large proportion of NOA patients still lack an underlying etiology.1 Several hormones have crucial roles in controlling the complex process of spermatogenesis. While the master roles of testosterone and pituitary gonadotrophins during spermatogenesis have been well-documented,3 estradiol has not received much attention as an influential agent in that process until recently. Most serum estradiol originates from the peripheral aromatization of testosterone via the catalysis of the P450 aromatase enzyme. This process mainly occurs in adipose tissue.4 Inside the testis, estradiol is synthesized primarily by germ cells, and 50–60% of its intra-testicular flow is released there.5 Estradiol...
promotes germ cell propagation in autocrine stimulation patterns.\(^6\) It is also necessary for germ cell survival\(^7\) and takes part in the process of capacitation and acrosome reaction in the mature sperm.\(^8\) Estradiol is also released from Leydig cells and Sertoli cells. It expedites the growth, development, and function of Leydig cells but inhibits the production of androgen by suppressing the luteinizing hormone (LH) effect in the cells during steroidogenesis.\(^9\) In Sertoli cells, estradiol shares in the regulation of the Sertoli cell population\(^10\) and the transcription of N-cadherins, the proteins involved in the formation of tight junctions between Sertoli cells.\(^11\) Clinical evidence for the influence of estradiol on spermatogenesis is the increase in the rate of sperm retrieval in cases of NOA following adjustments in estradiol levels when treating patients.\(^12\)

Recently, exposure to estrogen has become inflated. These estrogens have become ubiquitous during the normal daily life of most people. A list of estrogenic compounds such as medications, chemical pesticides, and even food in the form of phytoestrogens is growing rapidly.\(^13\) This has prompted several scientific groups to call this rising trend in estrogen contact estrogenic pollution and warn against it. An ongoing argument has arisen in many areas of the globe about the probable consequences of this pollution. Some researchers have incriminated estrogenic pollution in the global decrease in sperm count in men and the increase in anomalies of the male genital system.\(^14\) Others have disputed these links and indicated that there is a lack of sufficient evidence.\(^15,16\) However, they could not completely discount the possible association in some vulnerable populations.\(^16\)

Despite the definitive role of estrogen in the regulation of spermatogenesis, the red flag of estrogen pollution, and the existence of a large category of idiopathic NOA, recent clinical guidelines released by the American Urological Association\(^17\) and European Association of Urology\(^18\) have not included estradiol estimation during hormonal evaluation of an infertile male patient. The same behavior was also observed during clinical practice where many recent clinical case studies did not include any evaluation for estradiol in their azoospermia patients.\(^19,20\) The limited studies that reported estradiol in their patients with NOA were case reports,\(^21\) molecular biological and not clinical papers,\(^22\) or clinical papers that only mentioned estradiol as an aside.\(^23\) Other studies investigated the impact of aromatase inhibitors on testosterone/estradiol ratio with unclear data of the estradiol levels in their patients with NOA.\(^24-26\)

The scant studies that intentionally investigated estradiol in infertile men reported this value in the serum of infertile men with oligospermia rather than azoospermia;\(^27\) in the seminal plasma, not serum, of men with oligospermia;\(^28\) or were old studies that jumped directly to reporting the mean estradiol levels in their patients who were limited in number and almost always had high follicle-stimulating hormone (FSH).\(^29-31\)

This exceptional lack of studies investigating serum estradiol in infertile men with NOA has motivated us to explore intentionally the different patterns of estradiol levels in these men, and correlate the results with their clinical and laboratory profiles.

**Materials and methods**

**Patients and controls**

The present manuscript entailed a retrospective case control study. Data for consecutive infertile men with NOA were retrieved between November 2008 and May 2010, and between April 2012 and December 2015. The data were collected by checking the file details from 145 and 459 men (total = 770), respectively, who consulted the Andrology Clinic, Alexandria Faculty of Medicine Main University Hospital, Alexandria, Egypt, for male factor infertility during these periods. An age-matched control group (\(n = 40\)) consisting of fertile men with normal semen parameters\(^32\) whose wives were pregnant or just delivered within 1 year were enrolled in the study. Data of these controls were recruited consecutively from Gynecology/Obstetrics outpatient offices. The exclusion criteria to select the eligible NOA patients were endocrinopathies (pituitary adenomas, thyroid dysfunction, adrenal disorders), intake of medications that may affect testicular function (androgens, aromatase inhibitors, estrogen-receptor modulators, antifungal medications), chemotherapy, radiation, chronic debilitating conditions (uncontrolled diabetes, cancers, chronic obstructive pulmonary disease, chronic renal failure), testicular malignancy, abnormal karyotyping, and Y-chromosome microdeletion. Men with obstructive azoospermia were excluded from the study. This condition was
diagnosed when normal spermatogenesis was reported during the histopathological examination of testicular tissue. This was in contrast to NOA where the testicular biopsy identified disturbed spermatogenesis.

Semen was collected in the Andrology Clinic after abstinence for 3–4 days and during masturbation. Azoospermia was diagnosed after semen examination on at least two different occasions showing a lack of sperm in the pellet resulting from centrifugation of the semen at 3000 g for 15 min. If sperm appeared in the pellet but not in the wet preparation, cryptozoospermia was diagnosed. These patients were also excluded from the study.

**Data retrieval**

Data included medical, surgical, and family histories. Personal history related to lifestyle factors, tobacco smoking, recreational drug use, and alcohol intake. Information from systemic and genital examinations was collected. Demographics such as age, body weight, length, and body mass index (BMI), occupation, and if associated with potential workplace pollution, which means any form of pollution that a person may be encountering during work, were also retrieved. Obesity was identified as BMI ≥30 kg/m². Varicocele was detected and graded clinically (Grade III: visible, Grade II: palpable during scrotal examination, and Grade I: noticeable with the Valsalva maneuver). Following the clinical check-up, all patients underwent scrotal colored duplex scanning. This scanning certified the absence of focal lesions, confirmed the clinical diagnosis of varicocele (vein cross-section > 2 mm with the presence of venous reflux), and estimated the testicular volume using the empiric formula of Lambert (= length × width × height × 0.71).

Information related to the hormonal workup of the patients was collected. All patients gave samples of peripheral venous blood between 8:00 and 10:00 AM. Concentrations of total testosterone, prolactin, FSH, LH, and estradiol were measured. The hormone assay was expedited using an automated cobas e 411 analyzer (Roche Diagnostics, Mannheim, Germany). The normal ranges of testosterone, prolactin, FSH, and LH were 2.8–8 ng/ml, 2.7–16.9 ng/ml, 1.6–11 mIU/ml, and 0.9–8.1 mIU/ml, respectively. For estradiol, the lower limit of detection was 5 pg/ml. The normal reference used was 11.3–43.2 pg/ml. The inter- and intra-assay coefficients of variation were 6.7% and 10.6%, respectively, as devised by the supplying company (Roche Diagnostics, Mannheim, Germany).

**Statistical analysis**

The study data were analyzed using the Statistical Package for the Social Sciences (SPSS version 16, SPSS Inc., Chicago, IL, USA). The patients were grouped based on their levels of estradiol into high, normal, and low estradiol groups. Descriptive statistics (mean, standard deviation, and range) for the different study variables were determined. Because the data were not normally distributed as assessed by Shapiro–Wilks test, the Kruskal–Wallis test was used to compare the means of the different study variables in the three arranged groups and the controls. In case of a significant outcome, the Mann–Whitney test would then be used to identify which groups had a significant difference. Categorical variables in the study groups were examined using the chi-square test. Correlations between the different study variables and estradiol levels were assessed using Spearman’s rank correlation coefficient. A p value was assumed to be significant at ≤0.05.

**Ethics statement**

Approval of the Institutional Review Board of Alexandria Faculty of Medicine, Alexandria, Egypt (IRB No. 12/66) for the research protocol of the study was obtained. In lieu of the Ethical Committee, the principles of the Helsinki Declaration were followed. Informed consents from the patients were waived by the Committee as this study was a retrospective type.

**Results**

**Eligibility of the patients for the study**

Among the 770 patients pooled during the study period, 203 patients had azoospermia. Following the exclusion criteria, the number of eligible patients for this study was 166 (Figure 1).

**Grouping of the study patients**

After the establishment of the three patient groups, the most prevalent group was that with
normal levels of serum estradiol (71.1%) compared with the other two groups with high (18.7%) and low levels (10.2%) estradiol. Estradiol concentrations identified highly significant differences among the three groups ($p < 0.001$) (Table 1). In case of the control group, all participants showed normal estradiol levels which were comparable with those of the normal estradiol group ($p = 0.21$) but with significant difference with the low ($p < 0.001$) and high groups ($p < 0.001$).

Comparing the study variables between the control and the patient groups
This identified significantly higher level of testosterone in the controls, compared with the normal and low estradiol group ($p = 0.001$ and $0.001$, respectively) but comparable level with that of the high estradiol group ($p = 0.83$) (Table 1). Significantly lower LH ($p < 0.001$) levels, lower FSH ($p < 0.001$) levels, and higher average testicular volume ($p < 0.001$) appeared in the control group when compared with each estradiol group. There was also a significant difference among the four study groups as regards jobs in potential workplace pollution ($p = 0.01$) where the controls had significantly less risky jobs than the patients in the normal ($p = 0.017$) or the high estradiol group ($p = 0.038$) but not in the low group ($p = 0.28$). Besides, a significant difference related to a trend in obesity was identified between the controls and either the normal ($p = 0.013$) or high ($p = 0.023$) group but not with the low group ($p = 0.12$) which lacked any obese patient in this study. On contrary, no significant differences were detected in the other study variables among the controls and the three estradiol groups.

Comparing the study variables among the three patient groups
Testosterone levels were significantly higher in the high estradiol group than in the normal ($p = 0.01$) or low groups ($p = 0.008$). The age, BMI, prolactin, smoking habits, and varicocele existence at the presentation were not significantly different among the groups (Table 1). As regards FSH, LH, and average testicular volume, the comparison between the high and low groups ($p = 0.67$, $0.39$, and $0.95$, respectively), the low and normal groups ($p = 0.97$, $0.5$, and $0.27$, respectively) and the high and normal groups ($p = 0.66$, $0.73$, and $0.12$, respectively) did not also show any significant differences. The number of obese patients was slightly higher in the
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high group than in the normal group \((p = 0.57)\). Then it dropped to zero in the lower group to show significant differences with the other two groups \((p = 0.004, \text{low vs high groups}, \text{and} \ p = 0.008, \text{low vs normal groups})\). Patients with jobs in potentially polluted workplaces (Table 2) were significantly lower in the low estradiol group than in the high \((p = 0.01)\) or normal group \((p = 0.007)\).

Correlations between estradiol level and the study variables in the patient category

A significant positive correlation \((p < 0.001)\) between serum estradiol and testosterone was identified (Table 3). The other study variables did not show the same significant correlation with serum estradiol. However, it was intriguing to note the negative trend in the relationship between serum estradiol on one side and either FSH or age on the other side (Table 3).

Discussion

Estrogens have a crucial role in the regulation of spermatogenesis\(^{36}\) although their exact role in the pathophysiology of spermatogenesis, and hence NOA, is not yet fully understood. Morbid alterations in estradiol level to be high\(^{24}\) or low\(^{37,38}\) could be associated with disturbed spermatogenesis, as reflected in the reported clinical studies. The high level of estrogen may affect Leydig cell function by directly suppressing its function and testosterone synthesis\(^{39}\) as well as inhibiting the secretion of pituitary gonadotrophins.\(^{40}\) Decreased levels of estradiol, on the contrary, may disrupt estrogen signaling pathways which have been reported to affect germ cell development.\(^{41}\)
A few studies, mostly old, have studied estradiol in men with azoospermia.29–31,42,43 None of these studies attempted to highlight the spectrum of estradiol levels in their patients. They compared only the mean estradiol of their patients with that of a control group, with highly conflicting results. While some studies reported higher estradiol means in their patients,29,30 other studies reported normal42 or even lower31,43 estradiol means in their patients. Several previous studies have also investigated the impact of aromatase inhibitors on testosterone/estradiol ratio.24–26,44 Except one study presented by Helo and colleagues,44 these studies were largely uncontrolled.24–26 The patient populations in these studies were heterogenous including in majority patients with oligospermia and few patients with genetic-related NOA24–26 or lacking any NOA patient.44 On reporting their estradiol levels, these studies just gave the mean ± standard deviation of their patient populations as a whole. They did not try identifying data of NOA patients from that of oligospermia patients. The current study is, therefore, in contrast to these studies in many aspects where it has a different goal, a controlled design, and a homogeneous patient population selected according to strict inclusion/exclusion criteria to include only NOA patients with no genetic issues.

Table 2. Possible risky jobs in the patient groups.

| Job                  | High E2 group (n = 31) | Normal E2 group (n = 116) | Low E2 group (n = 17) | Controls (n = 40) |
|----------------------|------------------------|---------------------------|-----------------------|-------------------|
| Driver               | 7                      | 9                         | 1                     | 2                 |
| Radiology technician | 3                      | 0                         | 0                     | 0                 |
| Painting             | 1                      | 5                         | 0                     | 0                 |
| Metallic industry    | 2                      | 5                         | 0                     | 1                 |
| Fertilizer industry  | 1                      | 1                         | 0                     | 0                 |
| Farmer               | 0                      | 20                        | 1                     | 4                 |
| Pesticides industry  | 0                      | 2                         | 0                     | 1                 |
| Computer technician  | 0                      | 3                         | 0                     | 0                 |
| Heat                 | 1                      | 4                         | 0                     | 1                 |
| Total (%)            | 15 [48.39]             | 49 [42.2]                 | 2 [11.77]             | 9 [22.5]          |

E2, estradiol.

aData from two patients were missed.

Table 3. Correlation between the serum estradiol and the study variables of the patients.

| Study variable              | p         | r     |
|----------------------------|-----------|-------|
| Testosterone               | <0.001    | 0.3   |
| LH                         | 0.75      | 0.025 |
| FSH                        | 0.33      | -0.077|
| PRL                        | 0.44      | 0.062 |
| Age                        | 0.46      | -0.057|
| BMI                        | 0.43      | 0.067 |
| Average testicular volume  | 0.3       | 0.13  |

BMI, body mass index; E2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PRL, prolactin

*Spearman’s rank correlation coefficient.

This definite lack of studies investigating serum estradiol in infertile men with NOA has motivated us to explore the different patterns of estradiol levels in these men, more willingly than focusing on only the mean levels of estradiol and correlating the results with the clinical and laboratory profiles of the patients. To the best of our knowledge, this is the first contemplated study describing directly, not a glance, the different
levels of serum estradiol in a cohort of men with NOA and comparing the patients’ clinical variables based on their estradiol levels.

In the present study, a wide spectrum of estradiol levels was presented by the study patients. The definitive cause of this variability in estradiol concentrations remains unclear. However, it is suggested that a multifactorial etiology might represent these variable levels in estradiol. This suggested etiology included the diverse hormonal profiles of the patients as well as several different confounders such as body constitution and risky occupations. This suggestion seems fairly reasonable when the comparison is performed between high and low estradiol groups. Four lines of evidence were found in the high estradiol group to support this multifaceted suggestion. First, the group contained a significant number of obese men while the lower estradiol group did not have any obese patients (36.7% vs 0%, respectively, \( p = 0.004 \)). It has been reported that an obviously increased concentration of estradiol could be observed in obese men due to the increase in the rate of peripheral aromatization of androgen.\(^4\) Second, the percentage of patients with jobs in risky workplaces with pollution was significantly higher in the high estradiol group (\( p = 0.01 \)) than that in the low estradiol group. Some of the pollution-associated occupations have been associated with higher levels of estradiol in men.\(^45\) This made several researchers to put workplace pollution in the same vein as estrogenic pollution.\(^46\)

Currently, a large number of occupational agents have been listed as possible harmful agents to male fertility.\(^13\) Unfortunately, the truth behind the real toxicity of these agents has not yet been clarified, and more research on this topic is urgently needed. These agents are members of a big group of compounds called endocrine disruptors.\(^13\,\(^47\)\) These disruptors can intervene with the endocrinological system in the body. It may interfere with the actions of hormones during hormonal synthesis, transport, feedback control, or targeted action.\(^47\) The disruptors can work at various levels like receptor and post-receptor horizons,\(^47\) genome level by manipulating gene expression,\(^48\) or even in companion with the usual body hormones which mimic the disruptors.\(^49\)

Third, the level of total testosterone was significantly higher (\( p = 0.008 \)) in this group than in the low estradiol group. As the main source of peripheral estradiol is the peripheral aromatization of testosterone,\(^4\) it is not surprising to see higher levels of estradiol in this group. In addition, over-expression and induction of aromatase could be facilitated in the presence of pollutants (pesticides)\(^50\) or obesity; both conditions were present in the patients of the high estradiol group (vide supra). Therefore, it was predictable to identify a strong significant correlation between testosterone and estradiol in this study (\( p \leq 0.001 \)) which is in line with results from other researchers.\(^27\,\(^31\,\(^43\)\) Fourth, the mean concentration of gonadotrophins was high while the mean testicular volumes were small, although these findings were also present in the other study groups. These hormonal and clinical variables have a strong positive correlation with the rate of intra-testicular production of estrogen.\(^52\) In this study, the group with normal estradiol levels identified data for most study variables that were similar to the high estradiol group with no significant differences, except the testosterone level which was significantly lower (\( p = 0.01 \)) than that in the high estradiol group. Could this lower testosterone level be able to keep the estradiol level in this group within normal range? Or were there other unknown factors? We were not able to make a determination. In addition, we could not decide whether this drop or rise in estradiol levels is a cause or a consequence of disturbed spermatogenesis and azoospermia in the patients of this study. In fact, the nature of real relationship between the disturbed level of estradiol and azoospermia was also questioned by several previous researchers.\(^29\,\(^31\,\(^43\)\)

In the current study, there was no correlation between estradiol levels and age, BMI, smoking, or LH. This agrees with some researchers\(^31\) but disagrees with others.\(^27\) At the same time, we did not identify any significant correlation between FSH and estradiol in the studied patients. This is in accord with some studies\(^29\,\(^31\)\) but not with others.\(^27\,\(^30\)\) An interesting finding is the negative trend in the relationship between FSH and estradiol levels in this study (\( r = -0.077 \)), where the FSH mean was low in the high estradiol group, then increased in the normal group and finally was highest in the low estradiol group. This negative trend could be explained by the negative feedback exerted by the rise of estradiol on the hypothalamic-pituitary axis inhibiting FSH secretion.\(^36\) These conflicting results may be attributed to the discrepancy in the nature of the recruited patients in the different studies.
Hormonal therapy (HT) is currently used by many andrologists as a preliminary step to testicular sperm extraction (TESE). The rationale for this treatment is to normalize the levels of testosterone and estradiol and improve the testosterone/estradiol ratio. Assessment of this ratio in semen has been claimed by some researchers as a good predictor of sperm production or absence in men with NOA. HT can increase the rate of sperm retrieval during TESE due to the enhancement of sperm production. HT can also improve sperm parameters in the ejaculate of men with low testosterone and high estradiol. Several HT options have been tried with variable outcomes. Aromatase inhibitors are usually recommended for men with a testosterone/estradiol ratio < 10 when estradiol is high and testosterone is low. On the contrary, infertile men with estradiol levels not exceeding the upper normal concentration would not be candidates for aromatase inhibitors but would for clomiphene citrate. This treatment will not only increase testosterone but also estradiol, resulting in a normalized testosterone/estradiol ratio. Therefore, we think that the selection of a particular HT option will depend on the level of estradiol. This makes the routine estimation of estradiol in NOA not only a diagnostically important but also potentially having therapeutic implications. This suggestion is currently investigated and the result will be presented in a future paper.

Conclusion
Serum estradiol, in infertile men with NOA, may be present at different levels. The precise explanation for the discrepancy in the hormone concentrations remains to be determined. Estimation of estradiol in men with NOA may have potentially important diagnostic and therapeutic implications if HT will be decided. Based on the current results, it is believed that the estradiol assay should be included in the routine diagnostic workup for male infertility especially when obesity, workplace pollution, and disturbance in testosterone are associated. Extended studies with a larger number of patients are highly recommended to verify the current results and clearly identify if alterations in estradiol concentrations in men with NOA are the cause or consequences of the condition. Hence, the exact role of estradiol in the regulation of human spermatogenesis could be deeply explored.

Author contributions
N.S. contributed to the conception and design of the study; collection, analysis, and interpretation of data; and drafting the article with final approval of its completed form. S.B. contributed to the collection, analysis, and interpretation of data and drafting the article with final approval of its completed form.
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