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# Semen Quality, Hormonal Levels, and Androgen Receptor Gene Polymorphisms in a Population of Young Male Volunteers from Two Different Regions of Poland

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**Background:** The population of healthy Polish men has not been frequently and systematically investigated for fertility status. The aim of this study was to assess the quality of semen in a randomly recruited population of young males. The most important task was to find a relationship between semen parameters, sex hormones, and AR gene polymorphism.





**Material/Methods:** Semen and blood samples from young men from the Poznań (n=113) and Lublin regions (n=89) were collected for semen analysis, assessment of hormonal concentrations, and calculation of the CAG and GGN repeats of the AR gene.

**Results:** Statistical comparisons of the hormones and circulating proteins and the seminological parameters revealed significant differences between the regional groups of males studied. Among the correlations found, we emphasize the positive relationship between inhibin B levels and both the number of spermatozoa per ml ( $R=0.37$ ;  $p=0.0001$ ) and the total sperm concentration ( $R=0.40$ ;  $p=0.00003$ ). Positive correlations between IGF1 and sperm morphology was also found ( $R=0.40$ ;  $p=0.000004$ ). The mean number of CAG repeats in our tested groups was  $21.93 \pm 2.79$ , in a range from 16 to 31. The mean number of GGN repeats was  $23.2 \pm 1.66$  and ranged from 16 to 29. Numerous significant correlations were found between CAG or GGN repeats and blood hormones or circulating proteins and semen parameters; however, Spearman's rank correlations revealed rather weak coefficients.

**Conclusions:** This report attempted to determine the quality of semen samples and sex hormones in a population of Polish young men. The results were found to be similar to data obtained in Scandinavia. The calculated means and range of CAG or GGN repeats of the AR gene in Polish males were similar to West European epidemiological data.

**MeSH Keywords:** **Hormones • Receptors, Androgen • Semen Analysis**

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## Background

Epidemiological studies of human semen and male infertility in both Europe and North America have a long tradition, and has verified the decline in semen quality over the past sixty years [1,2]. The population of healthy Polish men has not been frequently investigated to date, but infertile Polish males have been examined more often. The possible effects of environmental factors on the male reproductive functions were the main subjects of study. Thus, we have no data for systematically monitoring trends in human semen quality in Poland. However, the data collection here could potentially be helpful for demographic analysis.

The quality of semen and male reproductive functions depends mainly on hormones, which operate in a network of complicated interrelationship with other factors. The most important role has been assigned to the hypothalamic-pituitary-testicular axis. Pulse secretion of LH, FSH, and GH stimulates testis function and develops male phenotype and spermatogenesis. Inhibin B appears as a main serological marker of male gonad function, which is produced by Sertoli cells. It can negatively regulate FSH, exhibiting crucial feedback between the gonad and pituitary levels. Androgens are crucial for the development and differentiation of the male genital organs, testicular descent, initiation of spermatogenesis, and growth of the accessory glands. Testosterone is the main circulating androgen. Testosterone can be converted to 5 $\alpha$ -dihydrotestosterone (DHT), and 44% of circulated testosterone is bound to sex-hormone-binding globulin (SHGB).

Testosterone and DHT act through the androgen receptor. Functional androgen receptors may be responsible for the enhancement or impairment of the transcriptional activity of at least a few genes. Androgen receptor function is modified by two polymorphic sequences, a polyglutamine encoded by trinucleotide repeat (CAG) and a polyglycine coded by another trinucleotide repeat (GGN). The CAG repeat length has been inversely related to the risk of prostate cancer, and shorter CAG repeat length is associated with its higher incidence [3,4]. Greater than average CAG repeats show an association with infertility [5] but there are studies in which it is difficult to confirm this interpretation [6–8]. Longer CAG repeats have rather been associated with idiopathic hypospadias or bilateral undescended testes [9]. The GGN repeat has been less frequently studied, and inconsistent data have been reported. Rajender revealed no association between GGN repeat length and infertility [10]. The Castro-Nallar group showed that GGN 23 was the predominant allele and that GGN 24 was the second most common allele in men with idiopathic infertility. GGN 24 was thus identified to be the prevalent allele in cryptorchidism and infertility [11].

The present study was designed to assess the quality of semen in a population of young men in Poland. The most important goal was to find a relationship between sex hormones, AR receptor gene polymorphism, and seminological parameters.

Our aim was therefore to evaluate the main seminological parameters and level of sex hormones and circulating serum proteins (paracrine factors), together with the genetic polymorphism of androgen receptor (AR) in a randomly recruited population of young males (18–35 years old) from Poznan and Lublin regions.

## Material and Methods

### Cohort recruitment

Young men (18–35 years of age) were invited to participate in a study to assess their semen quality, sex hormonal levels, and CAG and GGN (length) repeats. The cohort of young males was divided into two subgroups, depending on the site (region) of volunteer recruitment: the first group was recruited in Poznan (n=113) and the second group in Lublin (n=89). The Poznan-based population was recruited by the Andrology Unit of the University of Medical Sciences in Poznan and through media notices. The subpopulation from the Lublin region was recruited through advertisements supervised by private clinics dealing with infertility.

**Semen samples** (n=202) were obtained by masturbation after 3–4 days of sexual abstinence.

**Blood samples** (n=202) were obtained on the day that the semen samples were collected. Blood samples were drawn without anticoagulant and were partly placed onto EDTA for DNA studies.

**Seminological evaluation** was performed using standard light microscopy according to WHO [12] recommendations. Semen samples were left for 30 minutes after ejaculation to liquefy at room temperature. Sperm concentration and motility types A, B, C, and D were determined using a Makler counting chamber. Sperm viability was assessed by eosin-fixed smears. The presence of leukocytes was evaluated by the Endtz test [13]. Sperm morphology was assessed according to Kruger's strict criteria [14] following Papanicolaou staining of the semen smears. Antisperm antibodies were determined by the mixed antiglobulin reaction (MAR) test [12].

**Medical interview and andrological examination.** During the medical interview, the volunteers were asked to fill out the previously prepared questionnaires. These included questions on previous and current diseases, lifestyle, and possible environmental disrupters. Physical andrological examination of all

participants was performed and the following features were estimated: testicular volume, testis position, pubic hair, varicocele, hydrocele, etc.

**Serum concentrations of hormones and circulating proteins** were determined by means of enzyme-linked immunosorbent assay (ELISA) using an EL-808 scanner, (BioTek Instruments Inc., USA). Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), sex-hormone-binding globulin (SHGB), total testosterone, and estradiol were thus determined. Serum samples were also analyzed for IGFBP-3, IGF-1, DHEA, DHEAS, and inhibin B. Commercially available kits from DRG Diagnostics (Marburg, Germany) and Diagnostic Systems Laboratories, Inc., (Webster, USA) were used. Hormones and proteins were measured with the following kits: DRG DHEA ELISA EIA-3415 and Active DHEA EIA DSL-10-9000, DRG DHEA-S ELISA EIA-1562, DRG Estradiol ELISA EIA-2693, Active IGFBP-3 ELISA DSL-10-6600, DRG FSH ELISA, EIA-1288, DRG Testosterone ELISA EIA-1559, Active SHGB ELISA DSL 10-7400, Active Inhibin B ELISA DSL-10-84100i, Active Prolactin ELISA DSL-10-4500, Active LH ELISA DSL-10-4600, Active IGF-1 ELISA DSL-10-5600, Active Non Extraction IGF-1 ELISA, and DSL-10-2800. The results were statistically evaluated based on Gen 5 Microplate Data Collection & Analysis Software (BioTek Instruments, Inc., USA).

**Determination of the CAG and GGN repeats within exon 1 of the AR gene** was performed by polymerase chain reaction (PCR). The study population consisted of 180 young men from the Poznan (n=103) and Lublin regions (n=77).

DNA was extracted from the blood samples using the salt precipitation method. The CAG and GGN repeat regions of the AR gene were amplified by PCR (Peltier Thermal Cycler, PTC-200, MJ Research) utilizing primers flanking the polymorphic CAG and GGN regions labeled with fluorescent dyes by HEX (GGN) and FAM (CAG). (Only forward FW primers were labeled.) For GGN repeat length analysis, we used forward primer (FW) 5'-TCCTGGCACACTCTCTCAC-3' and reverse primer (RV) 5'-GCCAGGGTACCACACATCAGG-3'. For CAG repeat length analysis, forward primer (FW) 5'-TCCAGAATCTGTCCAGAGCG-3' and reverse primer (RV) 5'-CTGTGAAGGTTGCTGTCTCTCA-3' were used. The amplification of the AR gene for CAG was performed in 20 µl reaction volumes, containing (apart from the primers) 10 mM dNTP, 5 U/µl polymerase Taq D-6677 (Sigma), buffer with 15 mM MgCl<sub>2</sub>, and 200 ng genomic DNA. The PCR conditions included an initial denaturation step at 95°C for 5 min; the 30-cycle PCR consisted of: denaturation at 95°C for 45 s, annealing at 60°C for 30 s, and elongation at 72°C for 30 s. The final extension step was performed at 72°C for 1 h.

GGN amplification was performed in 25 µl reaction volumes consisting of (apart from the primers) 10 mM dNTP, a buffer containing MgCl<sub>2</sub>, GC-RICH buffer, 5 U/µl polymerase FastStart Taq

(no. 04738314001, Roche Diagnostics, Mannheim, Germany), and 200 ng genomic DNA. PCR conditions included initial denaturation at 95°C for 3 min, while the 30-cycle PCR consisted of a denaturation step at 95°C for 1 min, annealing at 55°C for 2 min, and elongation at 72°C for 2 min. The final extension step was performed at 72°C for 1 h.

The PCR products were separated using ABI 310 capillary electrophoresis and the fragment lengths were analyzed using the Peak Scanner v1.0 Applied Biosystems program.

### Data analysis

Statistical calculations were performed using Statistica data analysis software system (version 10; www.statsoft.com., StatSoft, Inc. (2011). Significant differences were assessed using the Mann-Whitney test. All results were expressed as means ± SEMs. P-values below 0.05 were taken as the accepted level of significance. Correlations between the semen parameters and serum hormone levels were evaluated by Spearman's rank – order correlation.

## Results

**The semen parameters of the studied male cohort** are shown in Table 1.

In total, 2 groups of men contributed their data to this analysis. Statistical evaluation of some seminological parameters in these groups revealed statistically significant differences. Lower sperm concentration per milliliter of ejaculate was detected in a group of males from Lublin group as compared to Poznan (p=0.04). Among others, the total sperm concentration per ejaculate revealed high statistically significant differences between the groups (p=0.003). The Poznan group exhibited superior key semen parameters (sperm concentration per ml, total sperm concentration per ejaculate, motility type A) over the Lublin group but, surprisingly, the vitality and morphology parameters were statistically lower than in the Lublin group. All the observed seminological parameters (means) in the tested group were correct.

**Hormonal evaluation.** For 161 randomly selected men: PRL, FSH, LH, IGF-1, estradiol, testosterone, DHEA and DHEAS and SHGB concentrations were evaluated (Table 2). The levels of IGFBP-3 and inhibin B were examined only for the Poznan group.

There was a range of statistically significant differences in the hormonal levels measured in the groups. The differences between the Poznan and Lublin groups in seminological parameters, shown in Table 1, were reflected by FSH levels. Statistically, the highest level of FSH was found for the Poznan

**Table 1.** Seminological parameters in tested groups of healthy young male volunteers.

Semen parameters	Poznan means $\pm$ SD	Lublin means $\pm$ SD
Sperm concentration spermatozoa/ml	49.6 $\pm$ 34.52	40.9 $\pm$ 32.44
Sperm concentration (total per ejaculate)	209.0 $\pm$ 270.67	121.4 $\pm$ 100.84
Motility type A (rapid progressive motility)	24.5 $\pm$ 15.10	22.7 $\pm$ 12.06
Motility type B (slow or sluggish progressive motility)	17.8 $\pm$ 8.47	24.8 $\pm$ 7.55
Motility type C (nonprogressive motility)	12.1 $\pm$ 9.17	10.9 $\pm$ 6.46
Motility type D (immotility)	43.6 $\pm$ 16.91	40.5 $\pm$ 15.70
Vitality	82.5 $\pm$ 12.78	88.5 $\pm$ 18.97
Good morphology	32.1 $\pm$ 28.11	34.6 $\pm$ 18.17
WBC: white blood test	0.44 $\pm$ 1.18	0.27 $\pm$ 0.58
ORC: other round cells	1.5 $\pm$ 1.71	0.47 $\pm$ 0.66
pH	7.8 $\pm$ 0.30	7.4 $\pm$ 0.18
Volume	3.5 $\pm$ 1.50	3.1 $\pm$ 1.45
MAR IgA	0.9 $\pm$ 1.82	–
MAR IgG	0.5 $\pm$ 1.93	–

Statistically significant differences were as following: Sperm concentration, spermatozoa/ml: Poznan vs. Lublin  $p=0.04$ ; Sperm concentration, total per ejaculate: Poznan vs. Lublin  $p=0.003$ ; Motility type A: Poznan vs. Lublin ns; Motility type B: Poznan vs. Lublin  $p=0.0000001$ ; Motility type C: Poznan vs. Lublin ns; Motility type D: Poznan vs. Lublin ns; Vitality: Poznan vs. Lublin  $p=0.00006$ ; Morphology: Poznan vs. Lublin  $p=0.0004$ ; WBC: Poznan vs. Lublin  $p=0.002$ ; ORC: Poznan vs. Lublin  $p=0.0000001$ ; pH: Poznan vs. Lublin  $p=0.0000001$ ; Volume: Poznan vs. Lublin  $p=0.003$ .

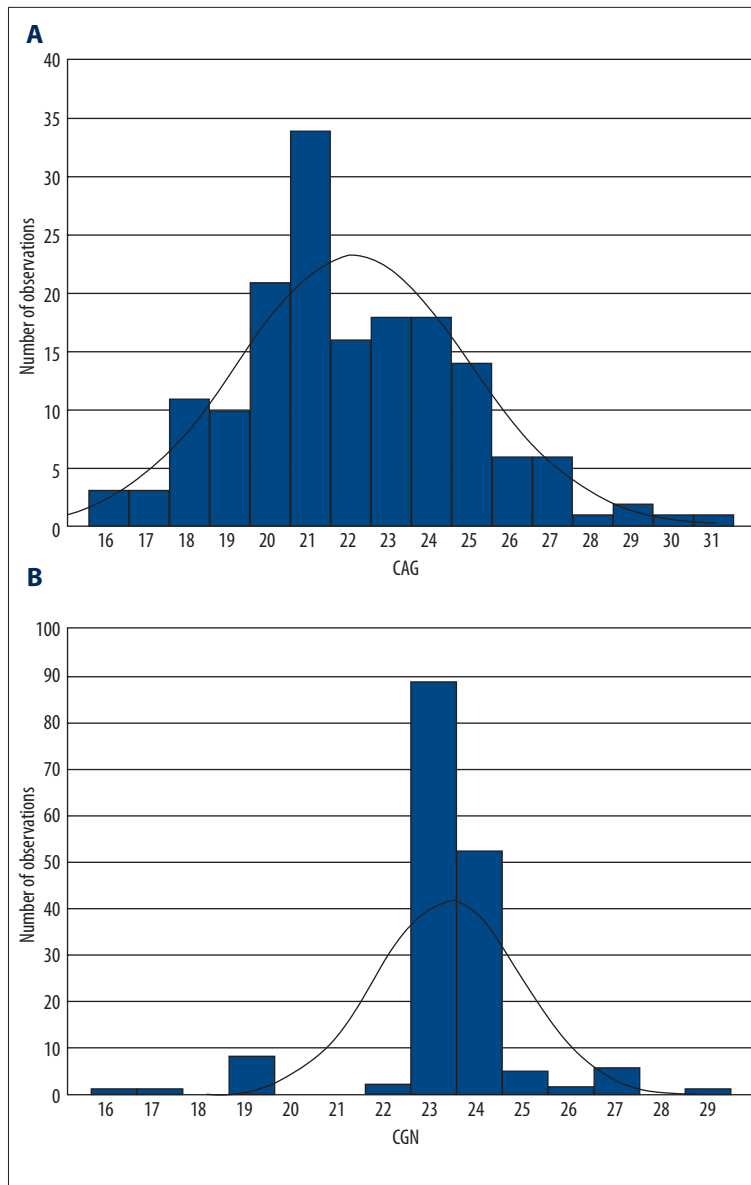
**Table 2.** Hormonal and circulating proteins levels studied in selected groups of males.

Evaluated factor	Poznan mean $\pm$ SD	Lublin mean $\pm$ SD
PRL (ng/ml)	12.30 $\pm$ 5.08	11.34 $\pm$ 5.19
FSH (mIU/ml)	6.23 $\pm$ 7.47	4.42 $\pm$ 3.71
LH (mIU/ml)	6.88 $\pm$ 8.55	3.74 $\pm$ 2.93
IGF1 (ng/ml)	189.02 $\pm$ 63.65	313.90 $\pm$ 29.31
Estradiol (pg/ml)	22.46 $\pm$ 16.98	34.93 $\pm$ 9.14
T (ng/ml)	7.13 $\pm$ 12.8	3.91 $\pm$ 1.49
DHEA (ng/ml)	17.59 $\pm$ 7.78	18.81 $\pm$ 20.40
DHEAS ( $\mu$ g/ml)	2.66 $\pm$ 1.04	3.22 $\pm$ 1.27
SHGB (nmol/l)	45.91 $\pm$ 22.58	24.68 $\pm$ 14.83
Inhibin B (pg/ml)	116.73 $\pm$ 52.25	nd
IGFBP3 (ng/ml)	52.98 $\pm$ 17.39	nd

Statistically significant differences were as following: PRL: Poznan vs. Lublin, ns; FSH: Poznan vs. Lublin  $p=0.01$ ; LH: Poznan vs. Lublin  $p=0.000001$ ; IGF-1: Poznan vs. Lublin  $p=0.000001$ ; Estradiol: Poznan vs. Lublin  $p=0.000001$ ; Testosterone: Poznan vs. Lublin  $p=0.000001$ ; DHEA: Poznan vs. Lublin ns; DHEAS: Poznan vs. Lublin  $p=0.002$ ; SHGB: Poznan vs. Lublin  $p=0.000001$ ; nd – no data; ns – not significant.

**Table 3.** Correlations found between seminological parameters, hormones, and proteins.

	R Spearman	p
<b>Variables (whole tested population)</b>		
Spermatozoa/ml & motility A	0.580091	0.0000001
Spermatozoa/ml & inhibin B	0.367542	0.0001
Spermatozoa/ml & motility D	-0.444178	0.0000001
Spermatozoa/ejaculate & motility A	0.552507	0.0000001
Spermatozoa/ejaculate & motility D	-0.424421	0.0000001
Spermatozoa/ejaculate & volume	0.551282	0.0000001
Spermatozoa/ejaculate & inhibin B	0.402745	0.000027
Motility A & FSH	-0.319015	0.000042
Motility D & morphology	-0.420014	0.0000001
IGF1 & morphology	0.404741	0.000004
PRL & morphology	-0.258234	0.001
FSH & inhibin B	-0.379181	0.000078
<b>Variables in Poznan group</b>		
Spermatozoa/ml & motility A	0.590780	0.0000001
Spermatozoa/ml & inhibin B	0.350368	0.0001
Spermatozoa/ml & motility D	-0.411146	0.000006
Spermatozoa/ejaculate & motility A	0.566404	0.0000001
Spermatozoa/ejaculate & motility D	-0.421141	0.000004
Spermatozoa/ejaculate & volume	0.646913	0.0000001
Spermatozoa/ejaculate & inhibin B	0.368785	0.00006
Motility A & FSH	-0.337942	0.0003
PRL & inhibin B	0.240358	0.01
PRL & morphology	-0.237498	0.01
FSH & inhibin B	-0.336896	0.0003
<b>Variables in Lublin group</b>		
Spermatozoa/ml & motility A	0.566934	0.0000001
Spermatozoa/ml & motility D	-0.505906	0.0000001
Spermatozoa/ejaculate & motility A	0.542665	0.0000001
Spermatozoa/ejaculate & motility D	-0.471267	0.000003
Spermatozoa/ejaculate & volume	0.406468	0.00009
Motility A & viability	0.49027	0.000003
Motility A & morphology	0.672386	0.0000001
Viability & PRL	-0.376848	0.0004



**Figure 1.** (A, B) Allelic frequency distribution of CAG and GGN in a population of healthy Polish men.

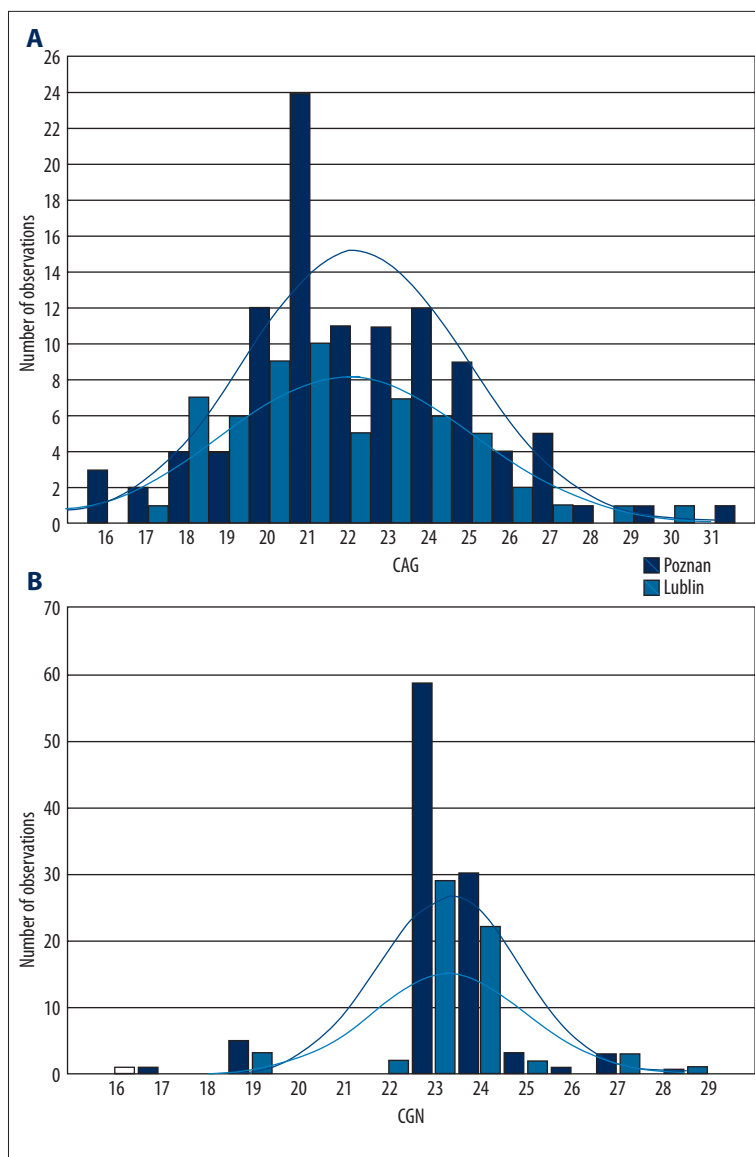
group ( $p=0.01$ ), in which sperm concentration (the main semi-nological parameter) was significant higher than for the Lublin group. Testosterone levels were again statistically higher in Poznan than for the Lublin group ( $p=0.0000001$ ). The levels of PRL found for the Poznan group were slightly higher than for the Lublin males, but the difference was statistically insignificant. The calculated means for LH differed in a statistically significant way between the Lublin and Poznan groups ( $p=0.000001$ ). (Lower levels of LH were found in males from the Lublin region.) High means were observed in the Lublin group for IGF-1 and estradiol levels; these were significantly different from the means obtained for the Poznan group.

**Numerous significant correlations between seminological parameters, hormones, and proteins were found in the whole**

tested population and in the two subgroups analyzed separately (see Table 3).

In the whole tested population of Polish males, significant correlations between spermatozoa concentration per ml of ejaculate and motility type A ( $R=0.58$ ;  $p=0.0000001$ ), and between spermatozoa concentration/ml and inhibin B ( $R=0.37$ ;  $p=0.0001$ ), were found. The concentration of spermatozoa/ejaculate and motility type A ( $R=0.55$ ), as well as the concentration of spermatozoa/ejaculate and inhibin B ( $R=0.40$ ;  $p=0.00003$ ), correlated with similar powers. Inhibin B correlated negatively with the FSH level ( $R=-0.38$ ;  $p=0.00008$ ).

In the males from the Poznan group, similar correlations were observed as in the whole tested population of Polish males.



**Figure 2. (A, B)** Allelic frequency distribution of CAG and GGN in a population of healthy men from Poznan and Lublin regions, analyzed separately.

The positive correlations were seen between the mean level of inhibin B and the total spermatozoa concentration in the ejaculate ( $R=0.37$ ,  $p=0.00006$ ), as well as in the concentration of spermatozoa/ml of ejaculate ( $R=0.35$ ;  $p=0.0001$ ). Negative correlations were observed between FSH and inhibin B concentrations ( $R=-0.34$ ;  $p=0.0003$ ) and sperm A motility ( $R=-0.34$ ;  $p=0.0003$ ).

In males of the Lublin group, positive correlations between spermatozoa concentration per ml of ejaculate and motility type A ( $R=0.57$ ;  $p=0.0000001$ ), and between total concentration of spermatozoa and sperm motility type A ( $R=0.54$ ;  $p=0.000001$ ) were observed. Sperm morphology ( $R=0.67$ ;  $p=0.000001$ ) and vitality ( $R=0.49$ ;  $p=0.000003$ ) correlated positively with motility type A. A negative correlation was observed between sperm viability and prolactin levels ( $R=-0.38$ ;  $p=0.0004$ ).

**Studies of the polymorphic regions of the AR gene** were performed on the samples of 180 men from the Poznan ( $n=103$ ) and Lublin regions ( $n=77$ ). The number of trinucleotide CAG and GGN repeats within the first exon of the AR was investigated using PCR. The mean number of CAG repeats calculated for the whole male Polish population ( $n=180$ ) was  $21.93\pm 2.79$ . The assessed lengths of CAG repeats for the particular groups were  $22.12\pm 2.70$  for Poznan and  $21.69\pm 2.92$  for Lublin. The length of CAG repeats did not differ significantly between the groups.

The number of GGN repeats ( $23.20\pm 1.66$ ) was calculated for the whole group of investigated men. The mean number of GGN repeats was then assessed for each group separately, showing  $23.30\pm 1.54$  for Poznan and  $23.06\pm 1.81$  for Lublin. The length of GGN repeats between Poznan and Lublin did not differ significantly.

**Table 4.** Spearman's rank correlations between sperm parameters, hormones, and CAG and GGN trinucleotide repeats in the studied groups.

	R (Spearman)	p
<b>Variables (whole tested populations)</b>		
CAG & FSH	0.244859	0.000445
CAG & LH	0.257292	0.000219
CAG & IGF1	-0.207105	0.003103
CAG & SHGB	-0.227199	0.001181
GGN & PRL	0.209922	0.002713
<b>Variables in Lublin group</b>		
CAG & vitality	-0.258180	0.018445
CAG & PRL	0.302915	0.003909
CAG & FSH	0.371753	0.000335
CAG & LH	0.392016	0.000145
CAG & IGF1	-0.449996	0.000010
CAG & estradiol	-0.386892	0.000180
CAG & testosterone	-0.381866	0.000241
CAG & DHEA	-0.374665	0.000350
CAG & DHEAS	-0.393374	0.000137
CAG & SHGB	-0.438549	0.000019
GGN & vitality	-0.224410	0.041394
GGN & PRL	0.283790	0.007037
GGN & FSH	0.319527	0.002271
GGN & LH	0.316182	0.002540
GGN & IGF1	-0.345185	0.000923
GGN & estradiol	-0.252766	0.016856
GGN & testosterone	-0.289164	0.006287
GGN & DHEA	-0.289250	0.006582
GGN & DHEAS	-0.297610	0.004619
GGN & SHGB	-0.360627	0.000557

The correlations between CAG length repeats and somatic features of the individuals failed to reach statistical significance. Physical examinations consisted of: anatomy of the penis, location of the testes, testicular volume, testicular density, structure of the epididymis, structure of the vas deferens, surgical scars, the occurrence of varicocele, hydrocele, or testicular cancer, and the development of pubic hair.

The range of AR CAG and GGN repeats in the total studied population of males (from Poznan and Lublin) is displayed in Figures 1 and 2.

In Table 4, the Spearman rank correlations between the sperm parameters, hormones and CAG and GGN trinucleotide repeats have been summarized.

To our surprise, the Spearman's rank correlations for all the men had rather weak coefficients. Low-power but statistically significant negative correlations were found between CAG repeats and SHGB ( $R=-0.23$ ,  $p=0.001$ ) as well as for IGF-1 levels ( $R=-0.21$ ,  $p=0.003$ ). Positive correlations were identified between CAG repeats and FSH ( $R=0.24$ ,  $p=0.0004$ ), and LH levels



( $R=0.26$ ,  $p=0.0002$ ). A positive correlation was also found between GGN repeats and prolactin ( $R=0.21$ ,  $p=0.003$ ).

Surprisingly, no statistically significant correlations were found in the Poznan group between CAG or GGN and sperm parameters or hormones.

Among the men from the Lublin region, there is an unusually high number of statistically significant correlations (Table 4).

## Discussion

### Semen analysis

Systematic epidemiological studies of human semen quality have to date mostly been carried out in Scandinavian countries. A study of sperm count in young Finnish men of reproductive age revealed that the mean concentration of spermatozoa/ml of ejaculate was 54 million. The value in Estonian men was 57 million. In Denmark and Norway, the mean number of spermatozoa/ml of ejaculate was calculated to be similar, though lower, at 41 million [15]. In our study, the mean number of spermatozoa per milliliter of ejaculate was found to be lower (49.6 mln/ml) in the Poznan group than for the Estonian and Finnish men. However, the concentration of spermatozoa per milliliter of ejaculate found in the Lublin population, at 40.9 mln/ml, was similar to the mean values calculated for the Danish and Norwegian male populations. Comparisons of total sperm counts among the tested male groups, including countries from the Baltic region, showed that the mean number of spermatozoa was the highest in population of Poznan, at 209.0 million. The total number of spermatozoa in ejaculates from Finland was 185.17 million, in Estonia 174 million, in Norway 133 million, and in Denmark 144 million [15]. The calculated mean for the Poznan group was (209 million), statistically higher than the mean calculated for Lublin (121.4 million). Furthermore, the Lublin group, exhibited lower total sperm counts than those obtained for Scandinavian populations. In the investigation of sperm morphology, it was found that the percentage of normal spermatozoa was higher in Finland (8.9%) and Estonia (9.2%) than in Norway (6.9%) and Denmark (6.4%) [15]. In our study, the percentage of sperm with good morphology for the Poznan group was 32.1%. However, the relatively high values revealed in the groups of Polish males may rather indicate the low sensitivity of sperm morphology assessment than truly objective values. This suggestion is based on the fact that quality controls are carried out only occasionally at Polish andrological evaluation centers. Studies of men from the Baltic region revealed that, in populations of young males from Northern Europe (Finns and Estonians), the number of spermatozoa/ml of ejaculate and the number of spermatozoa in ejaculate (and the number with good morphology) was found to be higher than in populations

from Western Europe. A rather positive East-West gradient was also noted in the earlier European epidemiological studies [15]. Our study groups may be surprising, since the Poznan population was more similar to Eastern population data, while the Lublin group revealed similar values to the male populations of Western Europe. Not all the tested seminological parameters showed this pattern. Many other studies have demonstrated regional differences in the quality of semen, even within the same country [16]. Unexpected statistically significant differences were shown during sperm parameters analysis of volunteers from different regions of Denmark, and were explained by differences in sampling procedures, rather than in constitution, geography, lifestyle or environmental differences. However, significant regional differences in FSH and inhibin B levels were found [16], and this finding, in our view, argues for the great hormonal sensitivity of the spermatogenesis process. Better-focused epidemiological surveys could explain more precisely the occasionally indicated associations between spermatogenesis and sex hormone levels, regulatory circulating proteins and so on, taking into account examples of previously reported differences in FSH and inhibin B levels.

Our study recruited young men between 18–35 years of age. This age range may also explain the observed unexpected differences between sperm parameters among the study groups of young Polish men. The period of sexual abstinence required prior to the first ejaculation may result in statistical differences in the analysis of the biological quality of the semen [17]. For example, when further subdividing the obtained sperm concentration data between two Polish subgroups of men – those under 30 years of age and those above 30 years of age – we found that, in the group over 30, the mean value of spermatozoa/ml was 81.2, and the total number of spermatozoa was 360 million. However, in the group of younger men, the mean number of spermatozoa/ml was only 55.9, and the mean of the total number of spermatozoa in the ejaculate was 252.2 million. These differences, although not statistically significant, reveal a high heterogeneity among the studied individuals in respect to their age.

On the other hand, Carlsen et al. [18] also investigated young males, who provided quarterly semen samples for up to 4.5 years; they found that sperm concentration, total sperm count, and morphology did not change significantly during the 4 years of follow-up.

### Hormones and circulating protein levels

The comparisons obtained between the mean levels of the hormones and the proteins revealed statistically significant differences among the groups of men from Poznan and Lublin. These differences may reflect the analysis of seminological parameters. In our view, unpredictable error may be a reason for the differences. For example, the time of blood collection

may have an impact on hormonal measurements, as the level of testosterone differs markedly depending on the time of the day. However, the measurement of FSH does not seem to be time-dependent. The calculated mean FSH level for the Poznan group was 6.23 ng/ml – statistically higher than the FSH levels in the Lublin group. However, the means of the hormones and proteins for the two tested Polish groups did not exceed the accepted reference limits, with exception of DHEA. The mean level of LH in the Poznan group (6.88 mIU/ml) approached the upper limit of the reference value (8.34 mIU/ml).

In many epidemiological studies of semen quality, a positive relationship has been seen between inhibin B level and the number of spermatozoa. This was also generally confirmed in our study. Apart from sperm concentration, we also observed a positive correlation between sperm A motility and inhibin B. We have also shown – as in the study of the Danish population – a negative correlation between inhibin B and FSH levels. Inhibin B is considered to be a better marker of Sertoli cell function and a more direct factor for estimating spermatogenetic efficiency than FSH [19].

A potential limitation of our studies was that only a single blood sample was collected to assess hormone and protein levels. However, it was found [20] that, despite the variables (pulsed or cyclic hormonal secretion), a single measurement seems to be sufficient; this was employed in most of the epidemiological studies that have been performed so far [20]. Brambilla et al. found that one sample is generally not sufficient to characterize an individual's hormone levels (testosterone and other reproductive adrenal hormones), but collecting more than three samples is probably not a realistic expectation [21].

#### Androgen receptor gene: CAG and GGN nucleotide repeats

The number of CAG repeats can be related to androgen receptor function. The revealed distribution of CAG repeats differed significantly between the studied ethnic groups [22]. Irvine et al. observed a significant difference in the prevalence of short CAG alleles among the three ethnic groups studied in respect of prostate cancer [23]. Ethnic differences were also revealed by Ackerman and coworkers [24]. The calculated means for CAG repeats of different ethnic groups were as follows: 19.6±3.2 for Afro-Caribbeans; 21.9±2.9 for Caucasoids; 22.6±3.1 for Hispanics; and 23.1±3.3 for Thais [23]. A study in China revealed that CAG repeats ranged from 10 to 34, and the mean number of repeats was 23 in randomly selected men [4]. Similar observations for Chinese populations (mean 23.0±3.1) were confirmed by Tse [25].

To our knowledge, Polish investigations of CAG repeats have previously been performed by Filus et al. [26] and Trzmiel-Bira et al. [27] for men of 25–65 years of age randomly selected from the population of Wrocław. The average number of CAG repeats was 24±7.68, ranging from 1 to 57. The average number of CAG

repeats in our tested groups (Poznan, Lublin) was 21.93±2.79, ranging from 16 to 31. The means calculated for the particular groups were 22.12±2.70 for Poznan and 21.69±2.92 for Lublin. Among a fertile control population of Indian men, the mean number of CAG repeats was 22.4±0.19 with a predominant repeat number of 23 and an observed range of 12 to 32 [28]. The calculated means of CAG (length) repeats were thus comparable to Europeans. For comparison, in French normal fertile male populations, the mean number of CAG was 22.2, with a range of 17–27 [5]. In a German population of healthy men, the CAG range was 13–31 and the mean was 21.4±3.5; in a Belgian population, the mean number of repeats was 21 and the range was from 15 to 31. However, in a population from Greenland, the mean number of CAG repeats was 24. In a Japanese population of healthy men, the range was from 17 to 30, with a mean of 23.9±2.9, which differed from the range and mean obtained for infertile men – 20–34 with a mean of 26.6±3.5. In North American, populations the range of repeats in healthy fertile men was 8–37 and the mean number of repeats was 22±3. The range and means of repeats in our studied population have been thus similar to West European and North American healthy male populations. The ethnic differences might, however, explain the differences in the number of CAG repeats between the Polish groups studied here and, for example, males from Wrocław, who show rather an Eastern profile – it is possible to recognize this phenomenon as the result of demographical drift (immigration) from Ukraine to Wrocław after the Second World War. There is some evidence that the differences found between the mean and the range of CAG repeats in healthy fertile or infertile men may originate from recruitment to the studies of heterogeneous population samples that are, nonetheless, inadequate in number. We assumed a value of great interindividual differences despite the relatively homogenous nature of the Polish population.

The initial analysis with Spearman rank correlations between CAG and GGN, and the hormonal or regulatory proteins levels, was carried out by summing up all the tested males and their groups separately (Poznan, Lublin). Some correlations were found: for all the tested Polish males, we have found positive correlations between CAG length repeats and LH and FSH levels. Negative correlations were found between CAG length repeat and IGF-1 and SHGB levels. A positive correlation between the number of GGN repeats and PRL level was also observed. In the published literature, only a few, random correlations were noted between the number of repeats and the hormonal levels. Similarly, von Eckardstain et al. [7] have published data on the positive correlation between CAG and LH and FSH levels in a group of healthy volunteers with normal sperm parameters. It is known that testosterone is produced by Leydig cells under the influence of LH, and LH thus regulates testosterone level. High FSH level and low inhibin B levels are rather connected with anomalies in gonadal function. In patients with a

normal hypothalamic-pituitary-gonadal axis and with abnormal function of Sertoli cells, inhibin B secretion is reduced and FSH levels are increased. The increase in the FSH level therefore indirectly marks abnormal gonadal function. Elevated LH and FSH levels can be further observed in patients with oligozoospermia. Despite this, statistically significant differences between the number of CAG repeats observed in infertile patients versus healthy fertile population have not always been identified [6–8]. It may be that even subtle genetic changes in the biochemical properties of AR can disturb the development of sperm cells through changes in androgenic regulation.

## Conclusions

This report has attempted to determine the quality of semen samples and of sex hormones and regulatory proteins in a Polish population of young males. The seminological values were found to be better than expected; however, regional differences were

clearly identified, and the age factor unexpectedly appeared to play a role within individuals recruited to the study. However, we have examined a total of only 202 serum and semen samples, and this is the main limitation of the study. Future surveys should be undertaken to provide more information regarding the epidemiological condition and fertility potential of young Polish men.

The results shown here indicate that the calculated means and the range of CAG repeats of the AR gene in the studied population of Polish young men were comparable to the West European data, while both CAG and GGN correlations with hormonal and other proteins levels need to be further confirmed in a larger population sample.

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