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Diagnosed with VC-associated asthenospermia between June
2022 and December 2023 at the Third Affiliated Hospital of
Southern Medical University (n=76)
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1) The left VC was diagnosed by scrotal color Doppler ultrasound,
degree II or III;
2) Have underwent laparoscopic high ligation of the spermatic vein;
3) Have complete medical records;
4) Signed informed consent.
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Exclusion criteria:
1) lost to follow-up (n=8);
2) Received hormone therapy (n=0);
3) Have a history of assisted reproductive surgery (n=0);
4) Have a history of cancer (n=0);
5) Have a history of of metabolic disease, such as diabetes,
   hypertension, coronary heart disease (n=5);
6) Combined with secondary varicocele such as renal vein
   tumor thrombus and nutcracker syndrome, infectious
   diseases of urinary system, testis or epididymitis, congenital
   or acquired abnormalities and malformations of urinary and
   reproductive systems, etc (n=3).
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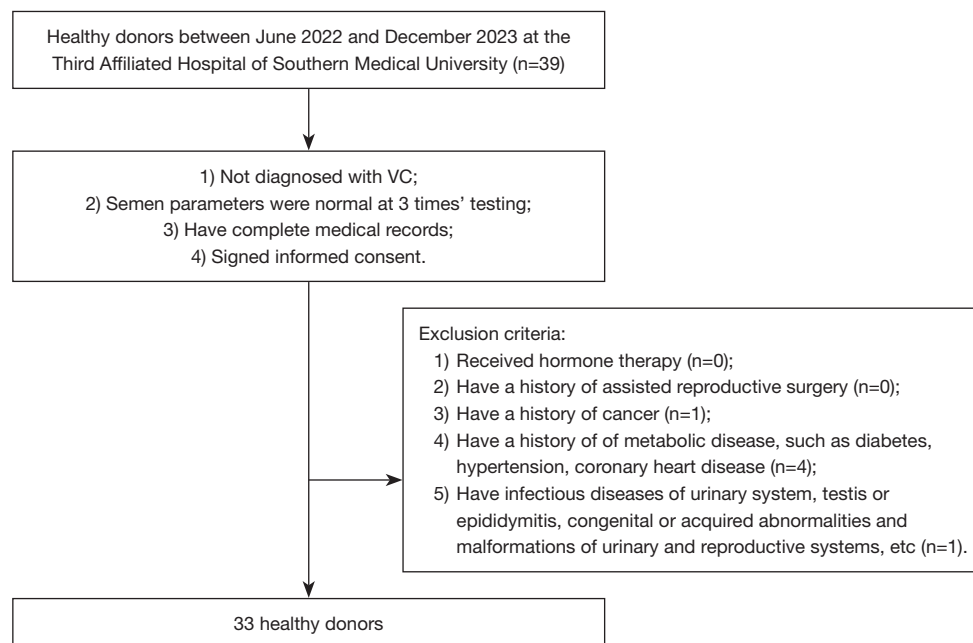
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60 VC subjects
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Figure S1 The inclusion and exclusion criteria of the varicocele (VC) individuals in this study.

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Healthy donors between June 2022 and December 2023 at the
Third Affiliated Hospital of Southern Medical University (n=39)
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1) Not diagnosed with VC;
2) Semen parameters were normal at 3 times' testing;
3) Have complete medical records;
4) Signed informed consent.
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Exclusion criteria:
1) Received hormone therapy (n=0);
2) Have a history of assisted reproductive surgery (n=0);
3) Have a history of cancer (n=1);
4) Have a history of of metabolic disease, such as diabetes,
   hypertension, coronary heart disease (n=4);
5) Have infectious diseases of urinary system, testis or
   epididymitis, congenital or acquired abnormalities and
   malformations of urinary and reproductive systems, etc (n=1).
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33 healthy donors
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Figure S2 The inclusion and exclusion criteria of the control individuals in this study.
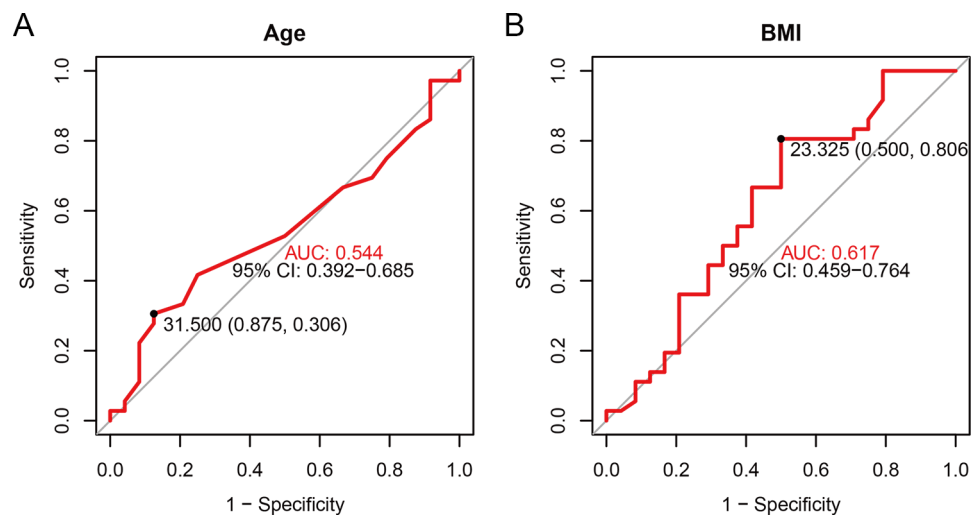
Figure S3 The optimal cut-off values for age (A) and BMI (B), which were detected by the ROC analyses. ROC, receiver operating characteristic; BMI, body mass index.
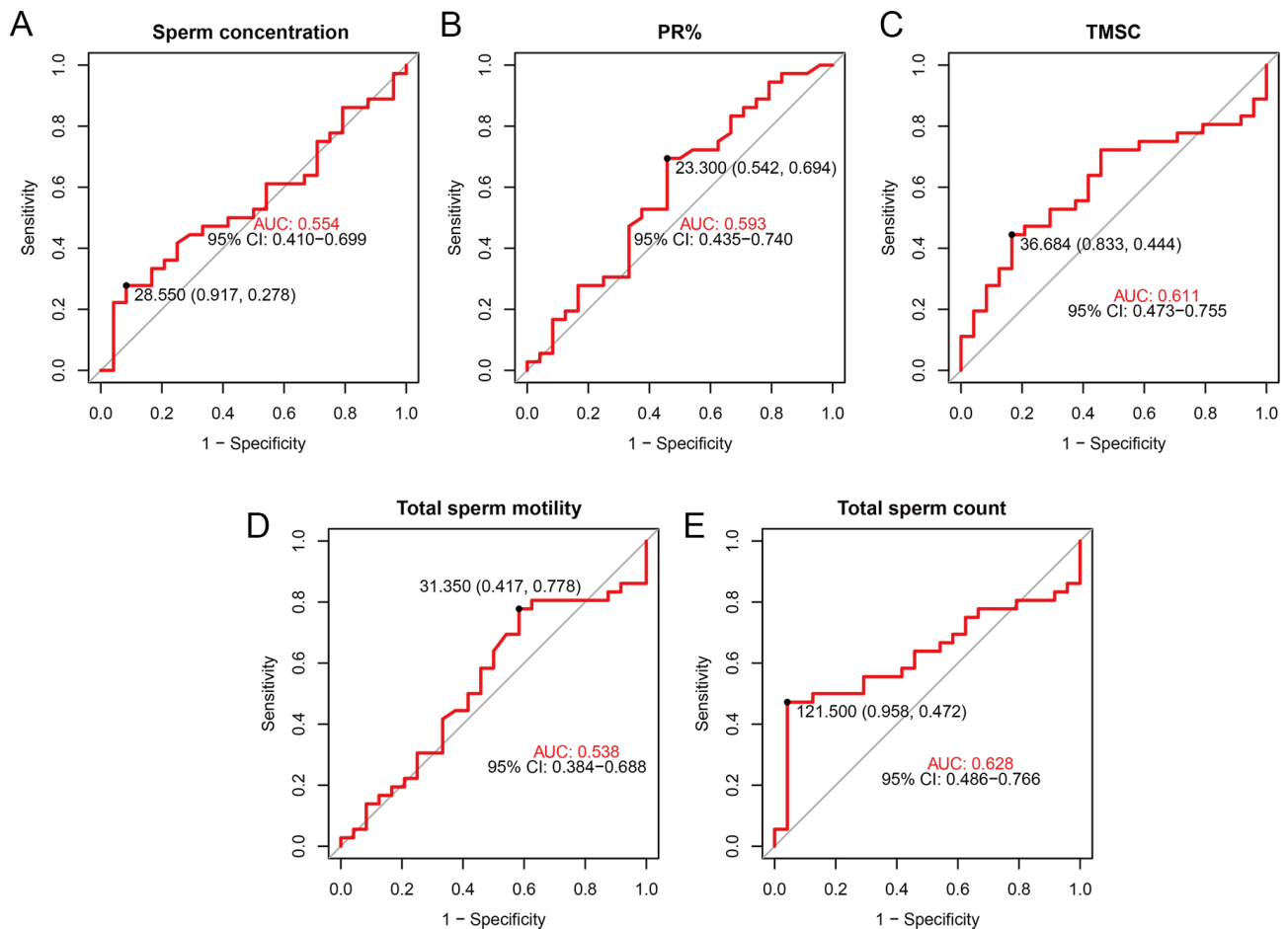

Figure S4 The optimal cut-off values for sperm concentration (A), PR% (B), TMSC (**C**), total sperm motility (D), and total sperm count (E), which were detected by the ROC analyses. TMSC, total motile sperm count; PR%, progressive motility rate.
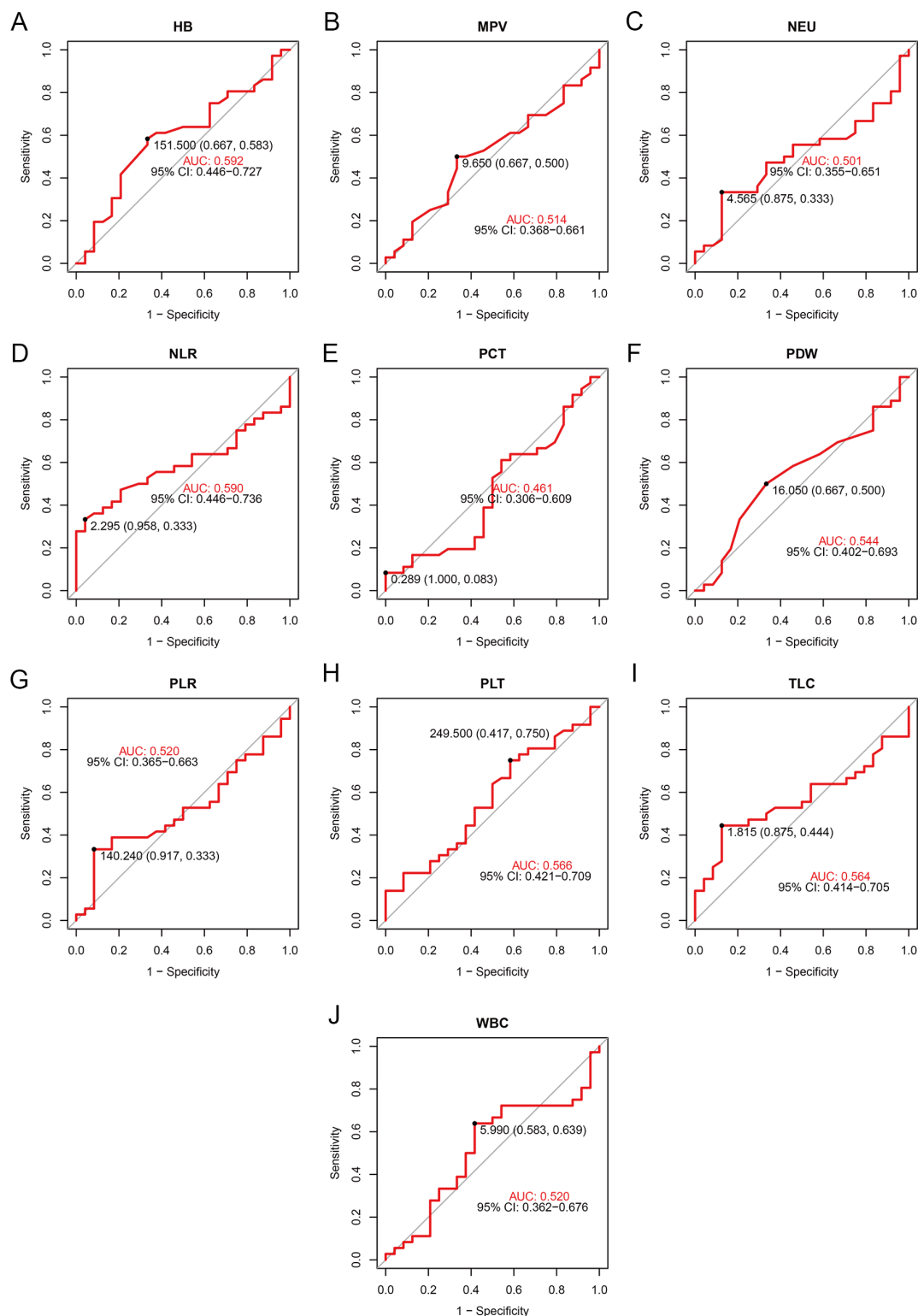
Figure S5 The optimal cut-off values for HB (**A**), MPV (**B**), NEU (**C**), NLR (**D**), PCT (**E**), PDW (**F**), PLR (**G**), PLT (**H**), TLC (**I**), and WBC (**J**), which were detected by the ROC analyses. HB, hemoglobin; MPV, mean platelet volume; NEU, neutrophils; NLR, neutrophil-to-lymphocyte ratio; PCT, plateletcrit; PDW, platelet distribution width; PLR, platelet-to-lymphocyte ratio; PLT, platelets; TLC, total leukocyte count; WBC, white blood cell count.
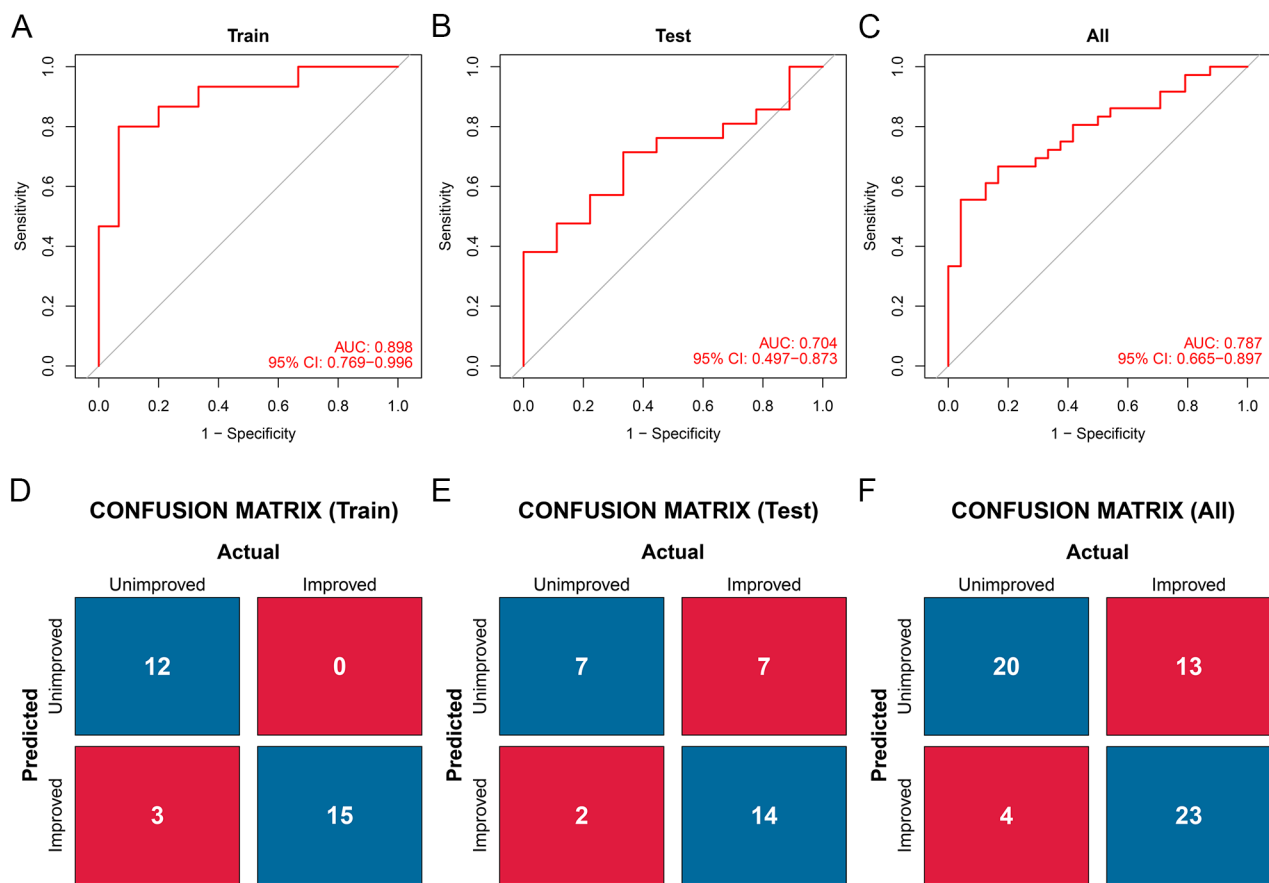
Figure S6 The predictive performance of the LDA model without PGK2. (A-C) The ROC analyses indicated the predictive performance of the LDA model without PGK2 in the training (A), testing (B), and all (C) cohorts. (D-F) The confusion matrixes indicated the predictive performance of the LDA model without PGK2 in the training (D), testing (E), and all (F) cohorts. ROC, receiver operating characteristics.
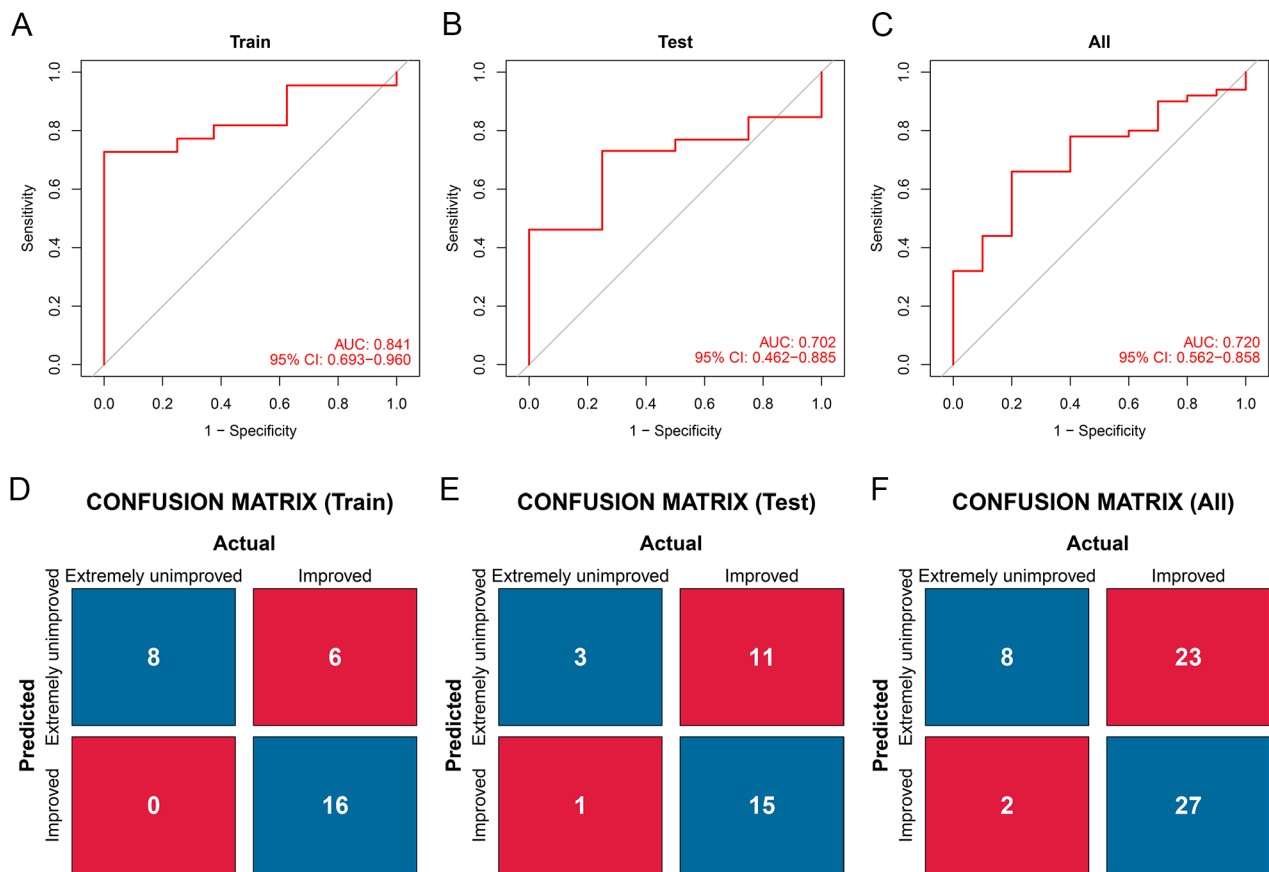
Figure S7 Predictive performance of the LDA model for the extremely unimproved condition following varicocelectomy. (A-C) ROC curve analyses demonstrating the predictive performance of the LDA model in the training (A), testing (B), and combined (C) cohorts. (D-F) Confusion matrices illustrating the predictive performance of the LDA model excluding PGK2 in the training (D), testing (E), and combined (F) cohorts.

Table S1 The correlation between PGK2 concentration in seminal plasma and age and hematological index in all subjects

Features	Spearman R	P value
Age	0.086	0.414
WBC	0.099	0.343
HB	−0.063	0.549
PLT	0.01	0.923
TLC	−0.068	0.517
NEU	0.054	0.609
PDW	−0.025	0.809
MPV	−0.19	0.068
PCT	−0.019	0.857
PLR	0.07	0.503
NLR	0.001	0.996

Table S2 The correlation between PGK2 concentration in seminal plasma and age and hematological index in VC subjects

Features	Spearman R	P value
Age	0.043	0.746
WBC	0.084	0.525
HB	−0.021	0.871
PLT	−0.147	0.262
TLC	−0.189	0.148
NEU	0.053	0.685
PDW	0.001	0.997
MPV	−0.092	0.485
PCT	−0.155	0.236
PLR	0.103	0.434
NLR	0.153	0.242