Following-up hemorheological consequences of gonadectomy in male and female rats

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Abstract

Growing number of clinical and experimental data reflect to the gender differences of hemorheological parameters. However, little is known about the potential hemorheological effect of gonadectomy and consequent changes in sex hormone concentration. Adult, same-aged male and female rats were involved in the study. In control male and female group no surgical intervention was performed. In gonadectomized (GoE) male and female groups bilateral orchidectomy or ovariectomy were completed. Body weight measurement and blood sampling were carried out in the 1st, 2nd and 3rd postoperative months. The GoE females had significant bodyweight augmentation and their plasma estrogen concentration decreased by 40-45% by the 1st postoperative month, while in males the testosterone level was not detectable after gonadectomy. Leukocyte and platelet counts moderately increased in GoE males. Elongation index values of erythrocytes slightly decreased in both genders after gonadectomy, showing converging values. Erythrocyte aggregation index values of GoE females significantly raised by the 2nd month. It can be concluded that gonadectomy in rats resulted in alteration (dominantly impairment) of blood micro-rheological parameters, by different manner in males and females. Supposedly decrease in estrogen can cause more expressed hemorheological changes than the cessation of testosterone.

Keywords: gonadectomy, gender differences, laboratory animals, red blood cell aggregation, red blood cell deformability

1. Introduction

Among the hemorheological parameters the factors representing the elongation, deformability of red blood cells or the reversible clamping of them, so aggregation are especially important because these factors have a major determinant role in the microcirculation [2,13,29,32,33,38]. These parameters show significant changes in various pathophysiological processes, diseases therefore the determination of them has a high value in surgical and microsurgical researches as well.

More and more clinical data reflect to the gender differences in the hemorheological parameters [4,12,15,22,41]. It is known, that in females blood viscosity, plasma viscosity, hematocrit and red blood cell aggregation have lower values and the red blood cell deformability parameters are better than in males [4,15]. However, in laboratory animals the rheological differences between females and males do not always correlate with the human pattern: the lower red blood cell aggregation values not obviously followed by better deformability parameters [17,28]. And all of this is additionally sophisticated with the inter-species differences of the laboratory animals [25,44]. In research work all these data, the gender and inter-species differences have a great importance according to the planning of experiments, analyzing, evaluating as well as extrapolating the results.

However, the question may arise that what could be the hemorheological effect and the magnitude of changes caused by gonadectomy and how well it could be followed-up. Its clinical relevance is high and not only because of the physiological decreasing in gonadal function. Surgical orchidectomy or ovariectomy can be needed due to traumatization, laesion or more frequently because of tumors.

Changes in gonadal function (caused by physiological or pathological reasons) may cause complex changes in many physiological parameters, including certain hematological variables (platelet- and leukocyte count) and affecting the coagulation parameters, the

complex neuroendocrin and immunological state, and even endothelial function [1,9,10,12,19,20,34]. However, little is known about the simultaneous changes of red blood cell aggregation and deformability after gonadectomy.

In pilot studies we could observe changes in red blood cell deformability (tested by bulk filtrometry) 1-3 months after surgical gonadectomy in dogs and in rats [24,27]. For examining better this issue with devices that requires very small amount of blood samples, we aimed to perform a follow-up study in rats, investigating the effect of ovariectomy and orchidectomy on the red blood cell deformability (tested by ektacytometry) and red blood cell aggregation, together with hematological parameters and sex hormones.

2. Materials and methods

2.1. Experimental animals

The experiments were approved and registered by the University of Debrecen Committee of Animal Research (permission Nr.: 37/2007), in accordance with the relevant Hungarian Animal Protection Act (Law XVIII/1998) and EU Directives (EEC 63/2010).

Twenty adult, same-aged, healthy male (bodyweight: 455.16 ± 38.23 g) and sixteen female (bodyweight: 286.63 ± 13.32 g) Sprague-Dawley rats (Janvier Co., France) were involved in the study.

In female animals the determination of actual estrus cycle phase was made by investigations of vaginal smear with Giemsa dying. The animals were in pro-estrus phase.

2.2. Experimental groups, operative techniques and sampling protocol

The following experimental groups were formed:

Control males (n=10) and Control females (n=6): No surgical procedure was made, only blood samplings were performed.

Gonadectomy (GoE) males (n=10): under general anesthesia (60 mg/kg Thiopenthal®, i.p.) the scrotum was incised, both testes together with the epididymes were gently elevated, and after careful ligation of the ductus deferens and the vasculature bilateral orchidectomy was carried out. The scrotum incision was sutured (4/0 Dexon®).

Gonadectomy (GoE) females (n=10): under general anesthesia (60 mg/kg Thiopenthal®, i.p.) a 1.5-2 cm long lower median laparotomy was carried out, the uterine tubes were identified till the ovariums. The ovaric arteries and veins were ligated together, as well as the upper third of the tuba, and the ovariectomy was made, bilaterally. The abdominal incision was sutured in two layers (4/0 Dexon®).

After the operation and on the 1st postoperative day the animals received analgesics (Flunixin®, 2.5 mg/bwkg, s.c.). In the Control animals the same dosage was used, in parallel.

Before operation (base) and on the 1st, 2nd and 3rd postoperative months –and in parallel in Control animals– body weight measurements and blood samplings were performed (0.6-0.8 ml per each) via puncture of the lateral tail vein. Sodium-EDTA (1.5 mg/ml) was used as anticoagulant.

2.3. Laboratory investigations

Hematological parameters were determined using a Sysmex F-800 microcell counter (TOA Medical Electronics Co., Ltd., Japan). In this study the white blood cell count (WBC [$x10^3/\mu l$]), monocyte-granulocyte and lymphocyte ratio (Mo-Gr%, Lymph%), red blood cell count (RBC [$x10^6/\mu l$]), hematocrit (Hct [%]), hemoglobin (Hgb [g/dl]), mean corpuscular volume (MCV [fl]), mean corpuscular hemoglobin (MCH [pg]), mean corpuscular hemoglobin concentration (MCHC [g/dl]) and platelet count (Plt [$x10^3/\mu l$]) were analyzed. A test requires approximately 70 µl of blood.

Red blood cell deformability was tested using a Rheoscan-D200 ektacytometer (Sewon Meditech Inc., Korea) [35]. For the measurements the red blood cell suspension was prepared in isotonic solution of polyvinylpyrrolidone (360 kDa, viscosity = 28.8 mPa.s; osmolarity = 305 MOsm/kg; pH = 7.36), taking 6 µl of native blood into 0.6 ml PVP solution. The device creates a shear stress profile (~ 0.5 – 20 Pa) by a special slit-flow system using vacuum, where the elongating red blood cells alter the laser diffraction pattern. The elongation index (EI) at a constant shear stress (SS [Pa]) is calculated from the length (L) and width (W) of the diffractogram: EI = (L-W)/(L+W). EI increases with cell deformability [2]. For comparative parameterization of individual EI-SS curves Lineweaver-Burke analysis was used and the maximal elongation index (EI_{max}) as well as the shear stress at half maximal deformation ($SS_{1/2}$ [Pa]) values were calculated [3].

For testing *red blood cell aggregation* a Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Germany) was used. The device determines M (at 0 shear rate) and M1 (at shear rate of 3 s^{-1}) indices, reflecting red blood cell aggregation according to the magnitude of light transmission at the 5^{th} or 10^{th} seconds of the aggregation process [33]. Both M and M1 indices increase with enhanced red blood cell aggregation. The measurements require approximately 20 µl of blood.

The hemorheological measurements (especially the aggregometry) were carried out within 1 hour after sampling, based upon the observation that rat red blood cell aggregation index may decrease by a highly significant manner over 2 hours after sampling [26].

The rest of the blood samples were centrifugated (at 2200G for 10 min). The determination of testosterone levels was based on electrochemiluminescence (ECL). 50 µl of plasma was incubated with Testosterone kit (ROCHE Diagnostics GMBH, Germany). For determining the estrogen levels, 35 µl plasma was required from each sample; Estradiol II kit

(ROCHE Diagnostics GMBH, Germany) was used for labeling. The measurements were performed by a Cobas E 411 analyzer (Hitachi High-Technologies Corporation, Japan).

2.4. Statistical analyses

Data are expressed as means and standard deviation (S.D.). Based on the normality of data distribution, for inter-group comparison Student t-test or Mann-Whitney RS test, for intra-group comparison (base and 1^{st}, 2^{nd}, 3^{rd} months) one-way ANOVA tests (Bonferroni-method) were used. The differences were accepted statistically significant at level of $p<0.05$.

3. Results

3.1. Bodyweight

Figure 1 A,B shows the absolute and relative changes (versus base) of bodyweight in Control and Gonadectomized male and female animals.

Comparing to the continuously increasing bodyweight of Control males, the Control females did not show remarkable changes over the 3-month follow-up period, keeping the well-visible gender differences ($p<0.001$). Bodyweight augmentation of GoE males was sustained over the 2^{nd} postoperative month, but still keeping the significant difference compared to females. By the 3^{rd} month the difference between Control and GoE males was significant ($p=0.036$). In GoE females obvious and significant augmentation in bodyweight was observed by the 1^{st} postoperative month (from 286.5 ± 16.2 to 358.6 ± 61.7 g; $p<0.001$), and showing further increase gradually. The increase significantly exceeded the data of Control females ($p=0.032$; $p=0.01$; $p=0.011$, respectively on 1^{st},2^{nd} and 3^{rd} month) (Fig. 1A).

The relative bodyweight data (relative value versus base) markedly showed the differences between groups. Control females did not expressed significant changes over the 3-month period, while Control males showed mild increase, and reaching the significance level

only by the 3rd month (1.25 ± 0.12; p=0.024). The largest augmentation was shown by the GoE female group (1st month: 1.22 ±0.19; 2nd month: 1.29 ± 0.23; 3rd month: 1.32 ± 0.21), which was significant versus base (p=0.001), versus Control females (p=0.011; p=0.016; p=0.011, respectively) and compared to GoE males, too (p=0.038; p=0.007; p=0.004, respectively). In GoE males the relative values were similar to Control females (Fig. 1B).

3.2. Estrogen and testosterone concentration

The base level of plasma estrogen concentration (E2) was 24.79 ± 15.7 pg/ml. By one month after ovariectomy the E2 concentration decreased by only about 40-45% (1st month: 13.9 ± 2.34 pg/ml; 2nd month: 16 ± 2.4 pg/ml; 3rd month: 14.8 ± 5.1 pg/ml, representing 56.4%, 64.6% and 59.7% of the base level, respectively) (Fig. 2A).

After orchidectomy, the initial 8.56 ± 3.67 nmol/l testosterone plasma concentration has been fallen under the level of detectability (<0.069 nmol/l) (Fig. 2B).

3.3. Hematological parameters

White blood cell (WBC) count significantly increased by the 1st postoperative month in both operated (GoE) groups. The increase was significant versus base (p=0.006) and compared to the Control animals (p<0.001). In the 2nd month the values of GoE males were similar to Controls, while GoE females expressed moderately elevated WBC count (p=0.049 vs Control). By the 3rd postoperative month in GoE males the WBC count increased again (p=0.002 versus control, p=0.012 versus GoE females) (Fig. 3A). The monocyte-granulocyte ratio was the highest in the 1st postoperative month (30-35%), then decreased in all groups, without marked difference.

The red blood cell (RBC) count was higher in males independently of the orchidectomy. However, a slight decrease in RBC count could have been observed in GoE

males during the 2nd and 3rd month compared to those increased values in the 1st postoperative month (Fig. 3B).

A hematocrit values have been changed in parallel with the RBC count. However, significant difference was seen only in GoE males versus GoE females in the 1st postoperative month and versus Control males in the 2nd month (p=0.021, p=0.019, respectively) (Fig. 3C).

The platelet (Plt) count was higher in males, which difference was significantly enlarged in GoE males in the 1st and 3rd postoperative months compared to base (p=0.022 and p=0.007, respectively) and versus GoE female group (p<0.001 and p=0.005, respectively) (Fig. 3D). The other hematological parameters did not show important differences.

3.4. Red blood cell deformability

Figure 4 (A-H) shows the elongation index (EI) – shear stress (SS) cumulative curves in all groups during the follow-up period. The comparative data and calculated parameters (EI_{max} and $SS_{1/2}$) are presented in Table I.

Before operations gender differences could be seen: in females the EI values were higher (at 5 Pa p=0.014, at 10 Pa: p=0.002 in Controls). In Control groups these differences were well observable over the follow-up period. In GoE groups the EI data of male and female animals slightly changed, converging to each other (Fig. 4).

In GoE males and females the EI at 3 Pa moderately increased by the 1st postoperative month (p=0.002 and p=0.004 versus base, respectively) and remained elevated (2nd month: in males p=0.002 versus base, in females 0.001 versus Control; 3rd month: in males p<0.001 versus base, in females p=0.019 versus base), while in Controls only the males showed an undulation in EI values (Table I).

At 5 or 10 Pa the control animals did not expressed important changes, while in GoE animals the increase in EI was significant: by the 1st postoperative month in GoE males and

females versus base values (at 5 Pa p=0.003 and p=0.002, at 10 Pa p=0.037 and p=0.0106, respectively). There was significant difference between GoE male and females groups, too: at 5 Pa p=0.009, at 10 Pa p=0.019.

By the 2nd postoperative month EI values of GoE groups slightly decreased and moderately augmented again by the 3rd month, showing significant difference compared to its base values (at 5 Pa: p<0.001 in GoE males and p=0.003 in GoE females). Remarkable, that in the 2nd postoperative month the comparative EI values of both GoE groups at 3, 5 or 10 Pa significantly were fallen short of Control values (at 3 Pa p=0.001 in males; at 5 Pa: p=0.007 and p=0.005, at 10 Pa: p=0.005 and p=0.002 in males and females respectively).

In Control and tendentiously in GoE males the EI_{max} values calculated from individual EI-SS curves slightly decreased in the 2nd and 3rd postoperative months. The initially visible gender difference in $SS_{1/2}$ disappeared over the follow-up period. In GoE males the decrease in $SS_{1/2}$ was marked by the 1st month (p<0.001 versus base) (Table I).

3.5. Red blood cell aggregation

Figure 5 shows M and M1 aggregation index values (at both 5 and 10 secundum) tested in samples taken in the 2nd and 3rd postoperative months.

In Control animals aggregation index values were higher in males. In 2nd postoperative month the GoE males showed slightly elevated index values but not as much as it could be seen in GoE female groups, where the rise in aggregation index values was remarkable. All index values showed significant difference compared to Control animals (5 sec M: p=0.003, 5 sec M1 and 10 sec M: p<0.001, 10 sec M1: p=0.002) and also versus GoE males at 10 sec values (M: p<0.001, M1: p=0.002) (Fig. 5A).

That marked rise in GoE females could not be observed in the 3rd postoperative month, rather a kind of converging of GoE group values was seen. However, the 10 sec M and M1

values of GoE female group were still significantly higher compared to Control females ($p=0.034$ and $p<0.001$, respectively) (Fig. 5B).

4. Discussion

Numerous population or epidemiological studies report hemorheological alterations in cardiovascular diseases with or without influencing by the gender [6,14,15,16,18,36,43,45]. However, in both clinical and experimental studies increasing data on hemorheological gender differences raise several questions regarding the background. The red blood cell deformability and red blood cell aggregation parameters show obvious gender differences in laboratory animals, too, complicated with inter-species differences [17,25,28,44]. While in human the men express higher aggregation index values and ’decreased’ red blood cell deformability [4,15], in rats the females show better red blood cell deformability values with higher aggregation index [28]. However, hemorheological consequences of decreased gonadal function (caused by physiological, pathological processes or exogen noxa) are still controversial and has not been completely clarified, yet [37,39,42].

In our study we aimed to follow-up the hemorheological effects of gonadectomy in male and female rats using same-aged control animals. The main findings were the followings: (1) The gonadectomised females had the most expressed augmentation in bodyweight, (2) their plasma estrogen concentration (E2) decreased only by 40-45% by the 1st postoperative month, while in males the testosterone level was not detectable after gonadectomy. (3) Hematological parameters did not show specific changes, however, moderately increased white blood cell and platelet counts were observed in gonadectomised males. (4) Elongation index values of red blood cells slightly decreased in both genders after gonadectomy, showing converging values by the 2nd and 3rd postoperative months. (5) Red

blood cell aggregation index values of gonadectomised females significantly raised by the 2nd postoperative month, which elevation was not observable in the 3rd month.

The hemorheological parameters can be altered by many factors *in vivo*, therefore the explanation of the effect of gender and sex hormones non red blood cell micro-rheological properties is quite complex and has not been clarified, yet.

Concerning the *estrus cycle*, in the current study all female animals were in the same cycle-phase: in pro-estrous (according to the investigations of vaginal smear with Giemsa dying). Since human data presented that hemorheological parameters can be varied during the menstrual cycle because of the sex-hormone level changes [11,30], it would be interesting to investigate this question in laboratory animals, too, following-up the variable through the other phases of the cycle (estrous, met-estrous, di-estrous). Machiedo et al. [21] investigated the trauma-hemorrhagic shock in female rats at different stages of the estrous cycle, besides male animals, and found significant differences of red blood cell morphological and deformability parameters according to the estrous cycle. Male rats and females in met-estrous or di-estrous had significantly higher percentage of abnormal red blood cells immediately after the shock and during the resuscitation period [21]. It is supposed that the difference we found between male and females rats in pro-estrous may differ during the estrous cycle.

In *previous animal experiments gonadectomy* as well as sex-hormone substitution had effect on hemorheological variables [24,27,31]. In rats ovariectomy was shown to increase hematocrit, fibrinogen concentration, red blood cell aggregation and blood viscosity [31]. However, the magnitude of these changes in laboratory animals is still not revealed completely, yet. Long-term follow-up studies are needed for investigating whether the hemorheological parameters can show various alterations during the postoperative months in gonadectomized male or female animals [27]. In the current study we used a 3-month period for follow-up, which is considered a relatively long-term period in case of the rat.

The question of *hormone substitution* is quite complex being still controversial concerning the potential effects of sex-hormones on hematological, hemostaseological and hemorheological variables. However, the administration of estrogen (as estrogen replacement) may have a protective effect against ischemic injury, it increases the risk of thrombotic complications both in women and even in men [10,19,20,39,41,42]. Spengler and co-workers demonstrated that in menopausal women hormone replacement therapy resulted in ameliorating hemorheological parameters over 6-month of therapy. They observed diminution in blood viscosity and erythrocyte rigidity, as well as decrease in fibrinogen concentration and erythrocyte aggregation [37].

In animal experiments this question has also been studied, however, the hemorheological data are diverse. In a previous mongrel dog study gonadectomy and reverse sex-hormone substitution protocols were used [7], where after gonadectomy estrogen for males and testosterone for females were administrated for a month. Besides cardiac effect [7], interesting hemorheological effects were observed: one month after gonadectomy filtration of red blood cell suspension worsened (RCTT increased) in both genders but expressed more in males. After a 1-month cross-sex hormone administration the RCTT improved in both genders, and in males hematocrit and blood viscosity decreased [24].

Growing number of studies show that various hormones (sex hormones, stress hormones) have direct or indirect effects on red blood cell aggregation or deformability [5,8,40]. However, the *direct effect of sex-hormones* on the red blood cells is still disputed. For evaluation and comparison of the results it is important to note that the investigative methods are different in the laboratories.

The *in vitro* preparations, type of buffers and vehicle molecules of the media in what the red blood cells are suspended, all may influence the results. Since sex-hormones are steroid-like molecules, their administration needs lipid-like carrier in the buffer that also

determines the results of the control investigations, too [23, unpublished observations]. Therefore this issue needs more studies to clarify the potential direct effect of sex hormones on red blood cells' rheological properties.

5. Conclusions

There are marked and sometimes controversially data available in the literature on gender differences in hemorheological parameters in human and in animals, that differences can be supposedly modulated by changes in gonadal function. In this study it could be observed that gonadectomy in rats resulted in alteration (dominantly impairment) in blood micro-rheological parameters during the first 3 postoperative months. The alterations were of different manner in males and females. Data suggested that a decrease in estrogen (E2) can cause more expressed increase in red blood cell aggregation and moderate impairment in red blood cell deformability than the cessation of testosterone.

Various effects of induced processes in male and female animals raise further aspects of comparisons and so the altered hormonal states need more studies to clarify better the complex hemorheological changes in follow-up studies. These observations may be interesting for veterinary medicine, too.

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8. Tables

Table 1. Changes of parameters describing red blood cell deformability in Control and Gonadectomized (GoE) male and female animals.

Variable	Group	*Gender*	Base	*Postoperative month*		
				1st	2nd	3rd
EI at 3 Pa	**Control**	*Male*	0.328 ± 0.018	0.340 ± 0.016	0.350 ± 0.011*#	0.331 ± 0.015 #
		Female	0.350 ± 0.007	0.345 ± 0.009	0.360 ± 0.012	0.345 ± 0.008
	GoE	*Male*	0.317 ± 0.02	0.336 ± 0.015 *	0.336 ± 0.021 *	0.339 ± 0.016 *
		Female	0.327 ± 0.02	0.345 ± 0.014 *	0.335 ± 0.021 +	0.342 ± 0.011 *
EI at 5 Pa	**Control**	*Male*	0.399 ± 0.001 #	0.401 ± 0.012	0.403 ± 0.015	0.398 ± 0.013 #
		Female	0.408 ± 0.005	0.404 ± 0.008	0.412 ± 0.009	0.410 ± 0.012
	GoE	*Male*	0.381 ± 0.015 #	0.396 ± 0.013*#	0.388 ± 0.019 +	0.403 ± 0.011 *
		Female	0.391 ± 0.016	0.407 ± 0.013 *	0.394 ± 0.018 +	0.407 ± 0.011 *
EI at 10 Pa	**Control**	*Male*	0.463 ± 0.01 #	0.462 ± 0.012	0.465 ± 0.011 #	0.459 ± 0.009 #
		Female	0.476 ± 0.006	0.470 ± 0.009	0.477 ± 0.012	0.470 ± 0.007
	GoE	*Male*	0.451 ± 0.014	0.461 ± 0.012*#	0.453 ± 0.014 +	0.460 ± 0.01 #
		Female	0.457 ± 0.015	0.470 ± 0.013 *	0.457 ± 0.017 +	0.467 ± 0.008
EI_{max}	**Control**	*Male*	0.595 ± 0.018	0.590 ± 0.022	0.566 ± 0.013*#	0.573 ± 0.011*#
		Female	0.602 ± 0.028	0.586 ± 0.015	0.586 ± 0.012	0.585 ± 0.012
	GoE	*Male*	0.589 ± 0.028	0.576 ± 0.018 +	0.561 ± 0.017*#	0.572 ± 0.02 *
		Female	0.578 ± 0.023	0.585 ± 0.015	0.579 ± 0.03 +	0.581± 0.023
$SS_{1/2}$ [Pa]	**Control**	*Male*	2.65 ± 0.32 #	2.48 ± 0.45	2.04 ± 0.18 *	2.32 ± 0.25 *
		Female	2.45 ± 0.49	2.29 ± 0.35	2.14 ± 0.24	2.24 ± 0.17
	GoE	*Male*	2.84 ± 0.54 #	2.33 ± 0.4 *	2.24 ± 0.48 *	2.22 ± 0.37 *
		Female	2.49 ± 0.4	2.25 ± 0.37	2.43 ± 0.6	2.23 ± 0.41

* $p<0.05$ vs Base, # vs Female; + vs Control

9. Figure captions

Figure 1.

Absolute (**A**) and relative bodyweight values (versus base) (**B**) in control and gonadectomized (GoE) male and female animals before operation and 1-3 months after gonadectomy.
means ± S.D., * $p<0.05$ vs Base, # vs Female; + vs Control

Figure 2.

Base values and postoperative changes in plasma concentration of estrogen [pg/ml] (**A**) and testosterone [nmol/l] (**B**) in gonadectomized female and male animals.
means ± S.D.

Figure 3.

White blood cell count (WBC [$x10^3/\mu l$]) (**A**), red blood cell count (RBC [$x10^6/\mu l$]) (**B**), hematocrit, (Hct [%]) (**C**) and platelet count (Plt [$x10^3/\mu l$]) (**D**) in control and gonadectomized (GoE) male and female animals before operation and 1-3 months after gonadectomy.
means ± S.D., * $p<0.05$ vs Base, # vs Female; + vs Control

Figure 4.

Elongation index (EI) values of base and postoperative samples (1^{st}, 2^{nd}, 3^{rd} month) in the function of shear stress (SS [Pa]) in control (**A-D**) and gonadectomized (GoE) male and female animals (**E-H**).
means ± S.D.

Figure 5.

Aggregation index M and M1 values at 5 and 10 secundum mode tested on 2^{nd} (**A**) and 3^{rd} postoperative month (**B**).
means ± S.D., # $p<0.05$ vs Female; + vs Control

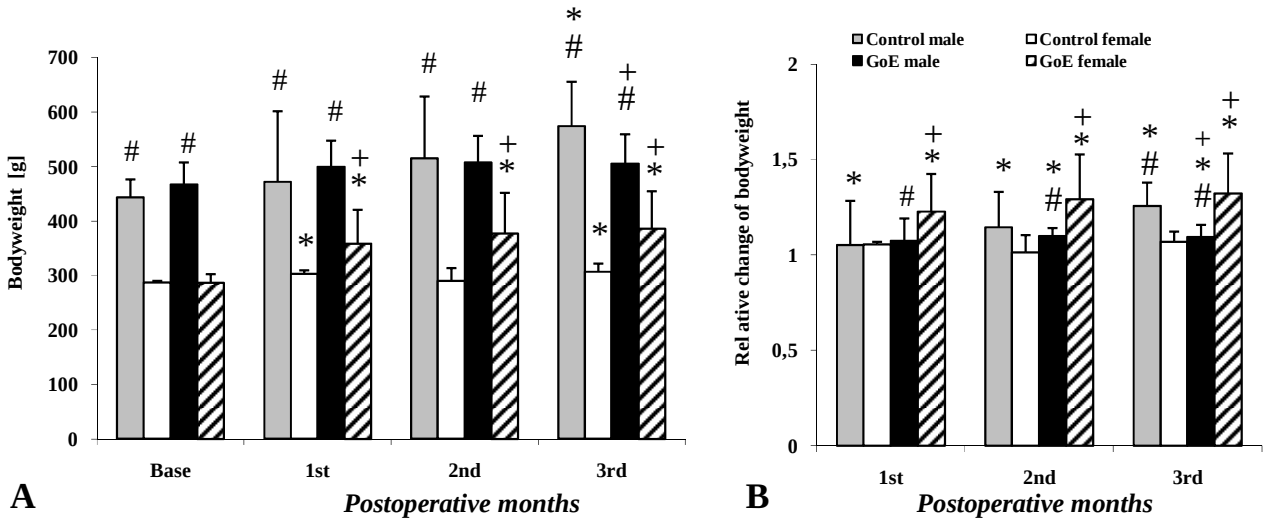

Figure 1.

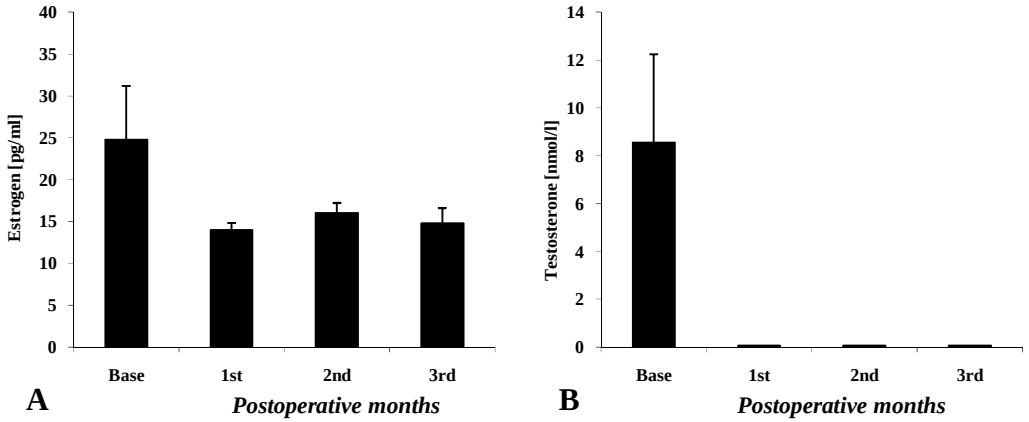

Figure 2.

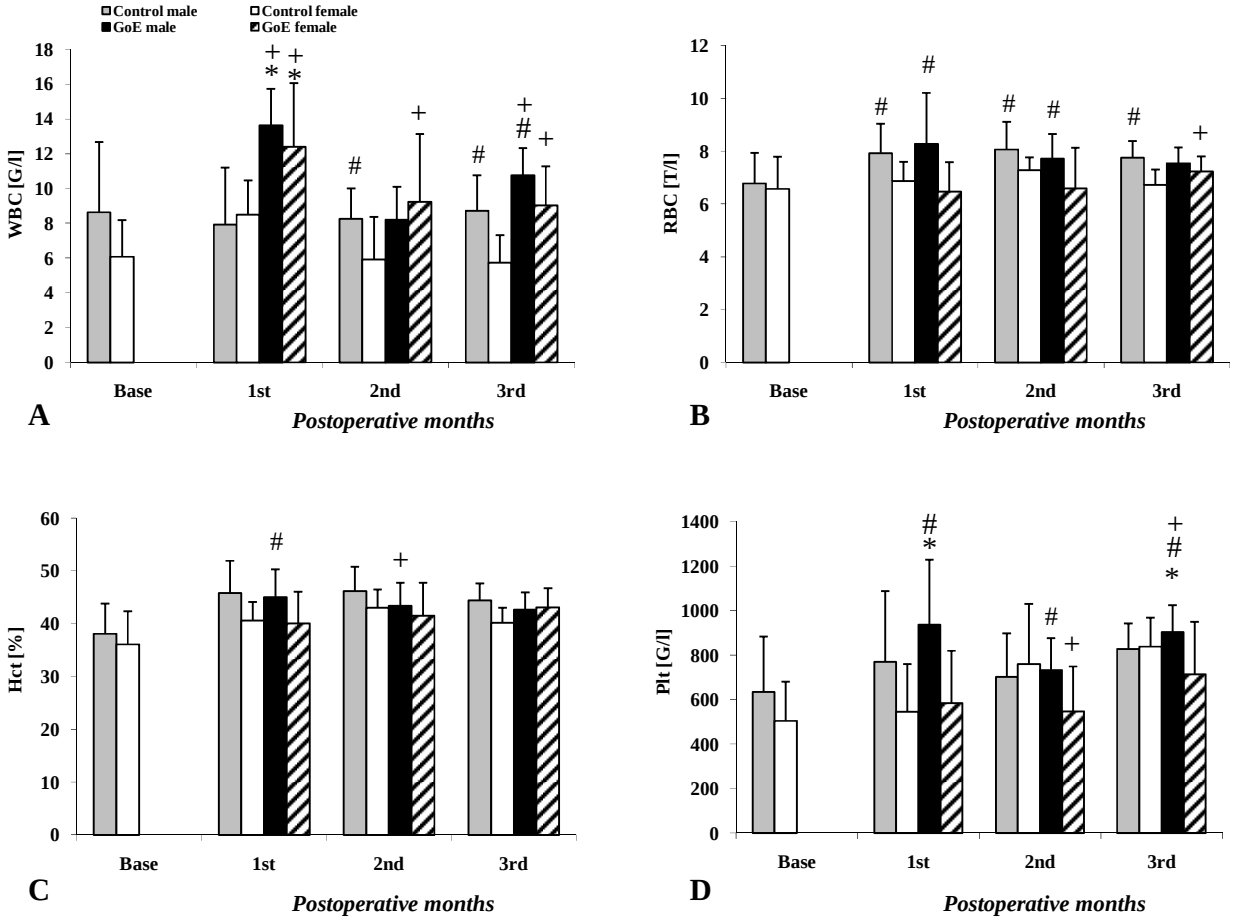
Control male
Control female
GoE male
GoE female
WBC [G/l]
RBC [T/l]
Hct [%]
Plt [G/l]
Base
1st
2nd
3rd
Postoperative months
A
B
C
D

Figure 3.

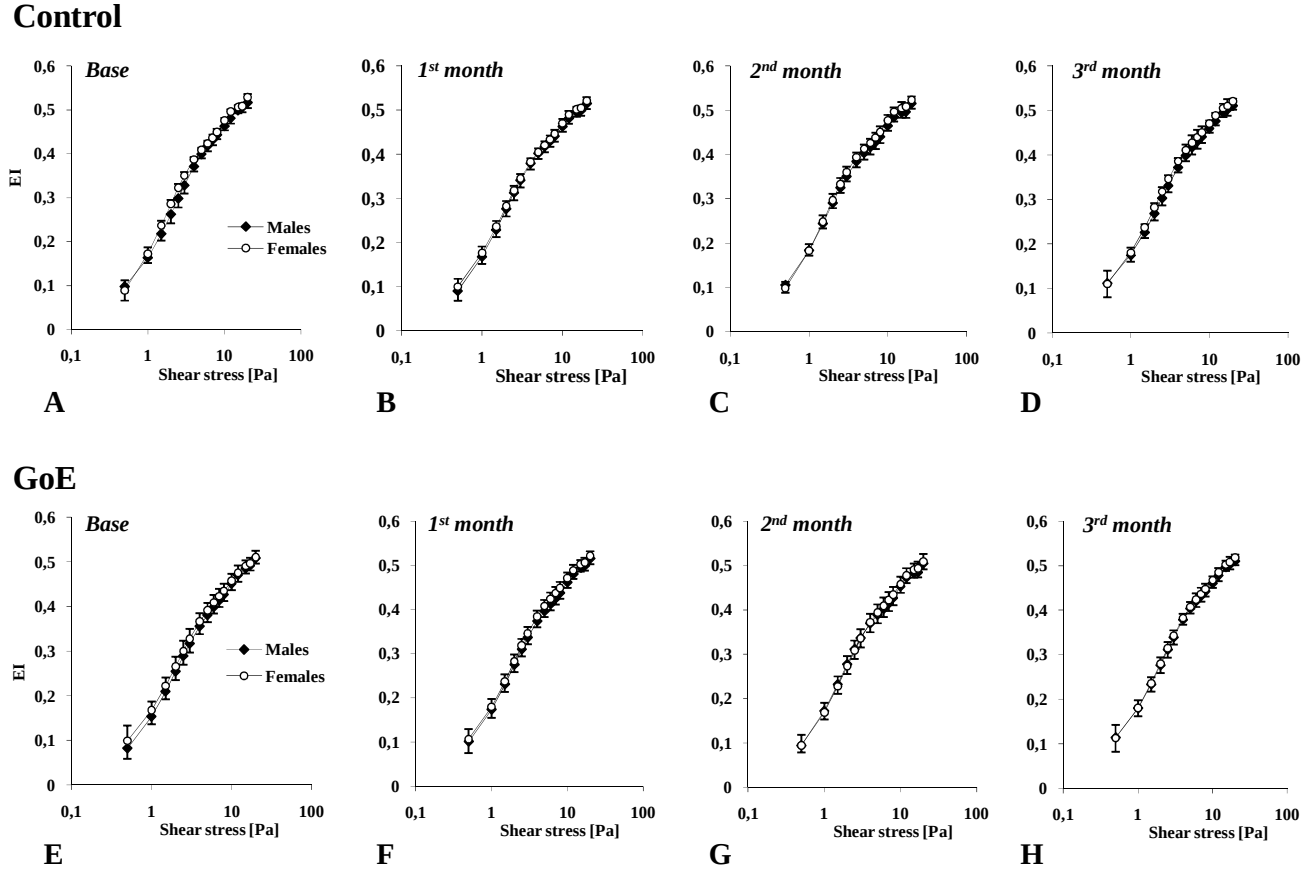

Figure 4.

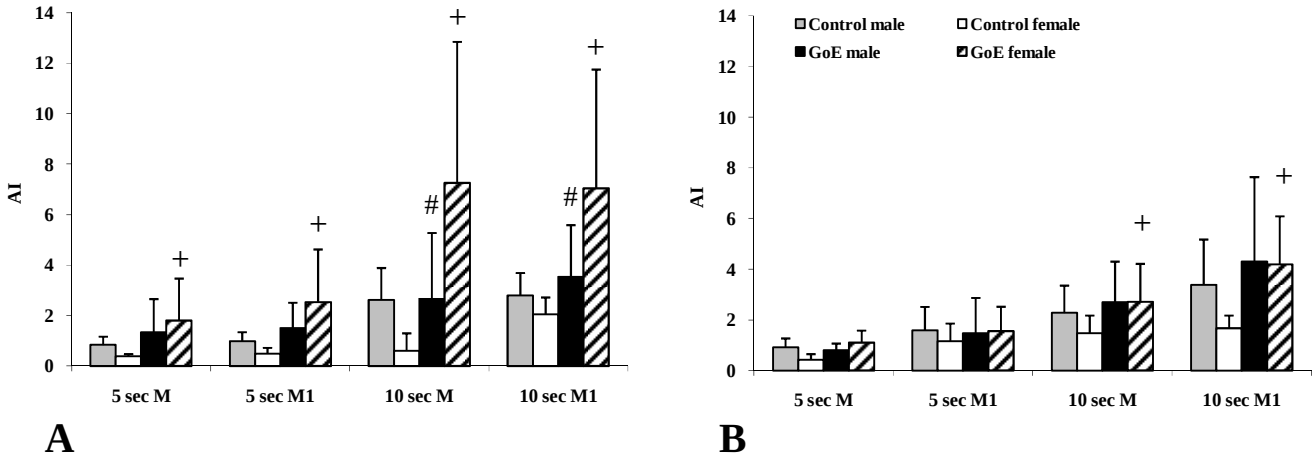

Figure 5.