The acute systemic toxicity study for normal catheter and cell-select catheter (CSC)

Ewa Florek[1], Grzegorz H. Bręborowicz[2], Klaus Lücke[3], Mateusz Madejczyk[2], Marek Chuchracki[4], Grzegorz Dworacki[5], Maciej Zabel[6], Michel Giersig[3]

Abstract

During early pregnancy foetal cells circulate in maternal blood. The frequency of trophoblast cells in maternal blood is enhanced in pregnancies complicated by preeclampsia. Trophoblast cells have been isolated from maternal blood by several different methods depending on surface antigen expression, e.g. HLA-G, and cell size. We suggest the use of trophoblast cells captured from the maternal blood stream as a source of DNA material for noninvasive prenatal diagnosis. Catheters covered with the antibody with and without the special gold nanometer sized structure for isolation of trophoblast from the maternal blood was constructed, and toxicological studies were performed. In experiment several groups of animals (non-pregnant and pregnant rats) were used. There were negative control groups, groups of animals for which the catheters with antibody were introduced in the groups after antibody injection. In biochemical tests in both non-pregnant and pregnant groups of rats, after the introduction of the catheter/catheter gold with the antibody and after the injection of the antibody, the concentration of IL-6 dropped below the limit of detection. In the case of non-pregnant rats, no other statistically significant differences in the level of IL-6, IL-10 or TNF have been observed. In the case of pregnant rats, the level of IL-10 was statistically significantly higher in the groups, which went through the introduction of the catheter in comparison with the rates that underwent only surgery. Haematological tests shown a small individual differences after the administration of the antibody were noticed in the case of mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red blood cell distribution width and mean platelet volume. It can be concluded that none of the performed measurements reflects the toxic effect of the examined devices on the physiological processes and health condition of the animals used in experiment. Observed deviations of some individual parameters from reference values do not have the grater biological importance.

Key words: prenatal diagnosis, trophoblast, catheter, cell-select catheter, rat

Introduction

During early pregnancy foetal cells circulate in maternal blood. Schmorl first observed foetal-maternal traffic of trophoblasts into maternal circulation in 1893 [1]. In focusing on the isolation of trophoblast cells from the maternal circulation during early pregnancy, however, different cell types have to be distinguished. These cells are syncytiotrophoblasts (STs), (extravillous) cytotrophoblasts (CTs) and anucleate throphoblasts (AT).

The frequency of trophoblast cells in maternal blood is enhanced in pregnancies complicated by preeclampsia. This includes the shedding of ST microvilli. CT or extravillous trophoblasts are regularely found in maternal peripheral blood. These originate from endovascular trophoblasts that migrate up the lumen of the spiral arteries to replace the endothelial cells during early pregnancy.

Because the ST fragments may be trapped in the capillary bed of the lungs as a result of their large size, and because the anucleate microvillus fragments are not useful for prenatal diagnosis, we have focused on the isolation of extravillous CT circulating in maternal blood. Extravillous trophoblast invasion into maternal tissue is a process that starts at implantation, rises to a peak in the mid second trimester, and declines rapidly thereafter.

Trophoblast cells vary in size from 20 to 200 μm and contain differing numbers of nuclei, depending on the trophoblast type. Hawes et al. [2] used antibodies specific for ST to identify large multinucleate fragments of trophoblasts in maternal blood and used these cells for prenatal diagnosis of β-thalassemia. Mueller et al. [3] used the same antibodies to detect STs and subsequently applied polymerase chain reaction (PCR).

By means of flow-activated cell sorting, Cacheux et al. [4] identified extravillous CTs in a woman known to carry a foetus with a 47, XYY karyotype. Sbracia et al. [5] used HLA-G antibodies in flow-activated cell sorting followed by PCR and correctly identified foetal sex in 9 patients. Durrant et al. [6] identified CT cells with a monoclonal antibody and correctly predicted foetal sex in 5 out of 6 male gestations, with no false-positive results in 7 female gestations.

According to Bianchi [7] in 90 samples of blood from women mainly in the second trimester of pregnancy a mean number of 19 male foetal cells were detected (1.2 cells per ml maternal blood). In contrast, in 109 samples (16 ml, maternal blood, 2nd trimester of pregnancy) the mean number of male cells obtained from female foetuses was 2 (0.1 cells per ml maternal blood are false positives). The cell number was determined using Y chromosomal PCR without cell separation. Trophoblast cells have been isolated from maternal blood by se-

[1] Laboratory of Environmental Research, Department of Toxicology, Medical University in Poznań, Poland

[2] Department of Perinatology and Gynecology, Medical University in Poznań, Poland

[3] GILUPI GmbH in Potsdam-Golm, Germany

[4] Department of Mother and Child Health, Medical University in Poznań, Poland

[5] Department of Clinical Immunology, Medical University in Poznań, Poland

veral different methods depending on surface antigen expression, e.g. HLA-G, and cell size. Proof of foetal origin as well as detection of aneuploidy and polyploidy may be accomplished with fluorescence in situ hybridization (FISH) and PCR-based methods [8-10].

During amniocentesis a sample of amniotic fluid is taken. Transabdominal amniocentisis is the most commonly used procedure to obtain foetal cells for cytogenetic analysis. The procedure is most commonly performed at 15 to 18 weeks of gestational age [11]. An accurate sample for analysis is provided in more than 99% of cases. Results are usually available within 1-2 weeks. Risks of this method include foetal losschorioamnionitis, foetal injury and maternal Rh sensitization, each of which is very uncommon. The largest risk is foetal loss at less than 0.5%. The rate of miscarriage after 15 weeks of gestation varies from unit to unit and is most commonly quoted to be 1% [12]. This risk increases in twin pregnancies to 2.73% up to four weeks after the procedure. Early amniocentesis, before week 15 of gestation increases the risk of foetal loss to between 3 and 5%. This also increases the risk of congenital foot deformities (mainly talipes equinovarus).

Chorionic villus sampling (CVS) has some disadvantages compared with amniocentesis. First, the procedure related pregnancy loss rate is somewhat higher than that of amniocentesis. Second, the incidence of limb deficiencies is greater in cases of CVS preformed before the completed ninth week of pregnancy. Third, there is a 2% false-mosaicism rate during the laboratory evaluation of the chorionic tissue [12]. The risks for CVS greatly depend on the time in pregnancy the procedure is carried out.

Evans and Wapner [13] conclude, that the procedure induced miscarriage rate following mid trimester (16-20 weeks) amniocentesis in experienced hands appears to be 1/300. In addition to this, the risk of "early amniocentesis" ($\leq$13 completed weeks) even with very experienced operators has a risk of foetal loss approaching 1/50 with a risk of Talipes equinovarus between 1% and 2%. For operators experienced in both first trimester CVS and mid trimester amniocentesis the two procedures have comparable safety outcomes. For parents desiring prenatal diagnosis before week 13 of gestation CVS is currently the safest procedure.

Further methods for prenatal diagnosis include the sampling of foetal blood and preimplantation genetic diagnosis (PGD) [11]. Foetal blood sampling is applied only in special circumstances and carries a risk of spontaneous abortion of between 1 and 2%, i.e. higher than amniocentesis or CVS. PGD is carried out in cases where the family carries a known genetic disorder. In this procedure a single cell is taken from the early embryo and analysed using PCR.

Non invasive prenatal diagnostic techniques include those using visualization techniques and maternal serum screening. Of the visualisation techniques ultrasonography is most commonly used. Other less common techniques are MRI (magnetic resonance imaging) and foetal echocardiography. All these techniques are not strictly considered to be diagnostic, but only give a strong suggestion of possible foetal developmental problems that are indicative for further testes such as amniocentesis or CVS. Maternal serum screening gives another indicator for foetal diagnosis called maternal serum α-fetoprotein (MSAFP) which is elevated in neural tube defects (NTDs) of the foetus. Maternal serum markers indicative of Down syndrome in the second trimester are MSAFP in combination with human chorionic gonadotropin and unconjugated estriol concentrations (these constitute the "triple screen" for foetal aneuploidy). This may also be an indicator for foetal trisomy 18. MSAFP screening for Down syndrome, however, only has a positive screening rate of approximately 5% and a positive predictive value of approximately between 3% and 5% so that the great majority of those who have a positive screening result have a normal outcome [11].

Aim of the study

The purpose of the study was the evaluation of acute systemic toxicity for the Cell-Select Catheter (CSC) and its bound antibody. All relevant procedures were performed in accordance with ISO 10993. Due to the fact that the medical product is to be applied to pregnant women, the animal trials was performed in two steps. In the first step the non-pregnant rats were used and in the second one, the same trial was carried out with the application of pregnant rats.

Materials and methods

Animals

Female Wistar rats, both pregnant and non-pregnant ones, were used in the trial. The trial was carried out in accordance with ISO 10993-2. The animals were bred at the Department of Toxicology, Medical University in Poznań.

All the tested rats were four months old at the start of the study. The pregnant animals were 4 months + 20 days old at the end of the experiment.

The minimum number of animals included in each studied group was 5. The control groups of non-pregnant and pregnant rats consist of 12 animals each.

All animals were kept under standardised husbandry at the Department of Toxicology. The rats were housed in Tecniplast (1291H001) stainless-steel cages, which were maintained at the temperature of 22 $\pm$ 2^{o}C, and relative humidity of 50 $\pm$ 10%. The 12/12 hours light/dark cycle was maintained throughout the study.

The animals were fed with standardised normal LABOFEED H (PN ISO 9001) produced by the Feeds and Concentrates Production Plant, Certificate of Quality System No 181/1/98, Kcynia, Poland. The animals were fed every day, at the same time, in the morning with a 10% surplus added to the amount consumed on the previous day. Water was available ad libitum.

To adapt the animals to the new environment, the rats were kept in husbandry for 3-5 days in groups of two to three animals before the start of the tests.

Studied groups

The animals were divided into several experimental groups.

Two negative control groups (Control, Pregnant-Control) without any surgical procedures were used. Another control groups constitute animals in the case of which the surgical procedures were performed in the same way as in the case of animals belonging to the experimental groups, however, the catheters were not introduced (Control/Catheter/Catheter Gold, Pregnant-Control/Catheter/Catheter Gold). In the case of rats from the other control groups, the catheters, which were not covered with an antibody were introduced (Catheter, Pregnant –Catheter, Catheter Gold, Pregnant-Catheter Gold).

The experimental groups (Table 1) of non-pregnant and pregnant animals were established in order to determine the systemic toxicity to small amounts of antibody, which the CSC is coated with. For this purpose, the antibody was injected into the peritoneum in three different doses and autopsy was done at a different time after administration of the antibody (Table 1). The applied antibody came from a monoclonal mouse – anti human antibody against HLA-G. The reasoning behind using an antibody from a rodent in another rodent is the fact that the final product contains a fully human antibody to be inserted into humans, the results obtained should thus be comparable to the real life situation. The antibody of rat species was not used, as this is not readily available.

The other experimental groups were established in order to test the systemic toxicity of the complete medical product or CSC. Catheters covered with the antibody with and without the special gold nanometer sized structure were introduced into a jugular vein for a period of 30 minutes. After this time, the autopsy was performed. The used CSC contained about 1 ng of the antibody, with and without gold nanostructures.

The animal trial was performed in accordance with the guidelines provided in the Ministry of High Education Report from 1959, and the UNESCO Declaration of Animal Rights from 1978 (Paris). An agreement was obtained from the Local Committee of Ethics for Animal Experiments (Wielkopolska district) (agreement – 16/2007) on February 19th, 2007.

The pregnancy was induced in 4-month-old rats and the experiments were performed (or terminated) on day 20 of the pregnancy.

Substances and devices

The anti-HLA-G antibody (EXBIO via BIOZOL, Germany, clone MEM-G/1) was used for injection into the peritoneum of the test animals. The stock solution of the antibody was obtained at a concentration of 1 mg/ml in PBS with 15 mM of sodium azide. The test substance was diluted to its final concentration (0.1 ng/ml; 0.2 ng/ml and 0.5 ng/ml) using 0.9% NaCl.

A stainless steel wire (EPflex GmbH, Germany) was used as the basis for the CSC construction. It was modified at a length of 2.5 cm with gold nanostructures by means of nanosphere lithography (NSL). The gold nanostructures were coated with a monoclonal antibody against the human cell surface antigen HLA-G (AbD Serotec, Germany, clone MEM-G/9). The antibody coating was established up to a constant value of 1.5 ± 0.75 ng.

The CSCs were coated under sterile conditions using a laminar flow hood with an additional application of UV light. The coated part of the CSC was stored before its application in a solution of 1% sodium azide. Immediately before the start of the experiment on animals, the wire was rinsed twice in sterile PBS, in order to remove the traces of sodium azide.

Antibody preparation and injection

The solutions of the anti HLA-G monoclonal antibody in concentrations of 0.05; 0.1 and 0.25 ng on 0.5 ml were prepared using 0.9% NaCl. The finished dilution was injected into the peritoneum of the rats from selected groups. Afterwards, the rats were observed for different time spans (from 2 hours to 6 weeks).

Application of the Cell-Select Catheter

The application of the CSC into the jugular vein was performed after intramuscular anesthesia (administration of xylazine + ketamine 40 mg/kg+5 mg/kg). In the first step of the procedure, the jugular vein was prepared and the catheter was introduced. The catheter stayed for 30 minutes in the circulatory system of the rat. Afterwards, it was removed from the jugular vein and autopsy was performed. The whole blood was collected from the heart of the rat, which caused the animal's death.

Laboratory testes

For all rats from each group the hematological tests, biochemical tests and urine analysis were preformed in Central Laboratory of the Gynaecology-Obstetrics University Hospital, Poznań, Poland – The International Certification Network – Certificate IQNet and PCBC, Registration Number PL – 779/5/2007; PN-EN ISO 9001:2001; validity date: 23.09. 2010; RIQAS – Certificate of Participation Immunoassay, Haematology, General Clinical Chemistry Programme.

Body and organs weight were recorded.

Biochemical testes contained determination of: interleukin 6 (IL-6), interleukin 10 (IL-10) and tumor necrosis factor (TNF alfa).

The following haematological testes were performed: hematocrit (HCT), hemoglobin (HGB), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), platelet hematocrit (PCT), platelet count (PLT), red blood cells (RBC), red blood cells distribution width (RDW), white blood cells (WBC).

In selected animals the standard urine analysis was performed: bilirubin, glucose, ketone, nitrate, pH, protein, red blood cells, specific gravity, urobilinogen and white blood cells.

Statistical analysis

The mean value and standard deviation were calculated for evaluated parameters. The differences in the distributions of the tested variables were evaluated by means of an analysis of variance (ANOVA). The mean values were compared applying the variance analysis and the Turkey post-hoc test. In the study, four control groups for pregnant and four for non-pregnant rats were established. The first one was the group described as *control,* which was a reference group for those groups where rats were treated with antibodies (peritoneum).

The rats from this group did not undergo any surgical procedure. The second control group described, as *control/catheter/catheter gold* was a reference for the groups described as catheter and catheter gold. The rats from this control group underwent a similar surgery as the rats from the catheter and catheter gold groups, but the catheter (catheter gold) was not placed in the vein. The third control group described, as *catheter* was the control group for the rats in whose case the catheter coated with antibodies was placed in a vein. The last one (fourth) control group described, as *catheter gold* was the control group for the rats where the catheter with gold nanostructures coated with antibodies was placed in the vein.

Results and discussion

Medical device

The Cell-Select Catheter (CSC) is based on a biofunctionalized wire which enables the capture of embryonic cells (trophoblasts) from the maternal blood stream. This CSC consists of a wire that contains a tip modified with gold nanostructures. An antibody against a special surface marker antigen of foetal trophoblasts is covalently bound to these nanostructures. By targeting these specific antigens, trophoblasts can be attached to the catheter. The antibody used for this purpose is targeted against the cell surface antigen HLA-G1 (human leukocyte antigen G1). It is a medical device for the one-time application

Rational for dose selection

The dose was calculated in relation to the amount of antibodies, which cover the nanostructure of the catheter and the weight of the rat. This was done purposely to test the reaction of the organism to the antibody, which could be shed in a theoretical situation from the surface of nanostructure of catheter, according to ISO-10993-11, within the framework of the acute systemic toxicity study.

For this purpose, the antibody was injected in three different doses (0.05 ng, 0.1 ng and 0.25 ng). The actual dose of antibody on the CSC is around 1.5 ± 0.75 ng. The corresponding total dose in humans (15.31 ng) is at least 10 times higher than that on the CSC.

The animals were observed during the whole time when the experiment was carried out. Depending of the studied group, the time frame ranged from 2 hours to 6 weeks (Table 1).

Table 1. Outline of the animal trial

Group symbol	Group description	
	Non-pregnant rats	Pregnant rats
Control	Control group; autopsy; non-pregnant	Control group; autopsy; pregnant
Catheter	Catheter introduction; autopsy after 30 min.; non-pregnant	Catheter introduction; autopsy after 30 min.; pregnant
Catheter Gold	Catheter Gold introduction; autopsy after 30 min.; non-pregnant	Catheter Gold introduction; autopsy after 30 min.; pregnant
Control/Catheter/ Catheter Gold	Control group; incision, prepare of carotid vein; autopsy after 30 min.; non-pregnant	Control group; incision, prepare of carotid vein; autopsy after 30 min.; pregnant
Catheter + Anti-HLA-G	Catheter cover with Anti-HLA-G introduction; autopsy after 30 min.; non-pregnant	Catheter cover with Anti-HLA-G introduction; autopsy after 30 min.; pregnant
Catheter Gold + Anti-HLA-G	Catheter Gold cover; with Anti-HLA-G introduction; autopsy after 30 min.; non-pregnant	Catheter Gold cover with Anti-HLA-G introduction; autopsy after 30 min.; pregnant
Anti-HLA-G 0.05 ng/2 hours	Antibody administration 0.05 ng/250 g; autopsy after 2 h; non-pregnant	Antibody administration 0.05 ng/250 g; autopsy after 2 h; pregnant
Anti-HLA-G 0.10 ng/2 hours	Antibody administration 0.10 ng/250 g; autopsy after 2 h; non-pregnant	Antibody administration 0.10 ng/250 g; autopsy after 2 h; pregnant
Anti-HLA-G 0.25 ng/2 hours	Antibody administration 0.25 ng/250 g; autopsy after 2 h; non-pregnant	Antibody administration 0.25 ng/250 g; autopsy after 2 h; pregnant
Anti-HLA-G 0.05 ng/24 hours	Antibody administration 0.05 ng/250 g; autopsy after 24 h; non-pregnant	Antibody administration 0.05 ng/250 g; autopsy after 24 h; pregnant
Anti-HLA-G 0.10 ng/24 hours	Antibody administration 0.10 ng/250 g; autopsy after 24 h; non-pregnant	Antibody administration 0.10 ng/250 g; autopsy after 24 h; pregnant
Anti-HLA-G 0.25 ng/24 hours	Antibody administration 0.25 ng/250 g; autopsy after 24 h; non-pregnant	Antibody administration 0.25 ng/250 g; autopsy after 24 h; pregnant
Anti-HLA-G 0.05 ng/6 or 3 weeks	Antibody administration 0.05 ng/250 g; autopsy after 6 weeks; non-pregnant	Antibody administration 0.05 ng/250 g; autopsy after 3 weeks; pregnant
Anti-HLA-G 0.10 ng/6 or 3 weeks	Antibody administration 0.10 ng/250 g; autopsy after 6 weeks; non-pregnant	Antibody administration 0.10 ng/250 g; autopsy after 3 weeks; pregnant
Anti-HLA-G 0.25 ng/6 or 3 weeks	Antibody administration 0.25 ng/250 g; autopsy after 6 weeks; non-pregnant	Antibody administration 0.25 ng/250 g; autopsy after 3 weeks; pregnant

The animals in some groups were not observed for at least three days, as was mentioned in ISO 10993-11 Section 5.2.5, because the main part of rates in the applied models was scarified after 2 or 24 hours.

During the observation, no changes in the behaviour and motoricity of animals were observed. The condition of skin, mucosa and fur was not altered.

The organs prepared during autopsy (kidney, liver, lung, spleen) were evaluated macroscopically, and no visible changes were observed.

In the groups of pregnant rats the condition of foetuses were evaluated. The appearance of foetuses from experimental groups did not differ from the ones belonging to the control groups (control group, control /catheter /catheter gold group, catheter group, catheter gold group).

The mean values of body weight non-pregnant and pregnant rats were collected in Table 2.

During the autopsy, the selected organs (kidney, lung, liver, spleen) were collected, and after drying them in the filter paper, they were weighed. Additionally, in the case of the pregnant rats the placentas and foetuses were weighed.

The mean values of organs, placentas and foetuses from all the studied groups are shown in Table 3 and 4.

Table 2. The mean of the body weight of non-pregnant and pregnant rats

Group symbol	Body weight [g]	
	Non-pregnant	Pregnant
	Mean ± SD (*n*)	Mean ± SD (*n*)
Control	251.0 ± 3.6 (12)	286.5 ± 12.5 (11)
Catheter	252.8 ± 3.7 (11)	280.0 ± 5.7 (6)
Catheter Gold	248.8 ± 4.5 (15)	288.2 ± 6.1 (6)
Control/Catheter/ Catheter Gold	249.5 ± 6.3 (6)	302.8 ± 16.8 (6)
Catheter + Anti-HLA-G	243.2 ± 3.1 (5)	298.8 ± 23.6 (6)
Catheter Gold + Anti-HLA-G	253.7 ± 7.2 (7)	279.0 ± 16.3 (6)
Anti-HLA-G 0.05 ng/2 hours	249.5 ± 4.2 (6)	290.2 ± 10.4 (6)
Anti-HLA-G 0.10 ng/2 hours	247.7 ± 6.4 (6)	286.8 ± 13.9 (6)
Anti-HLA-G 0.25 ng/2 hours	248.0 ± 4.8 (6)	272.8 ± 9.9 (6)
Anti-HLA-G 0.05 ng/24 hours	244.8 ± 4.6 (6)	288.3 ± 8.9 (6)
Anti-HLA-G 0.10 ng/24 hours	242.8 ± 4.1 (6)	289.2 ± 6.2 (6)
Anti-HLA-G 0.25 ng/24 hours	242.3 ± 0.4 (6)	282.5 ± 7.1 (6)
Anti-HLA-G 0.05 ng/6 weeks*	241.7 ± 3.6 (6)	255.7 ± 6.9 (6)
Anti-HLA-G 0.10 ng/6 weeks*	241.5 ± 1.5 (6)	254.8 ± 8.3 (5)
Anti-HLA-G 0.25 ng/6 weeks*	244.7 ± 4.9 (6)	252.2 ± 3.8 (5)

* In pregnant rats time was 3 weeks

Table 3. The mean weight of lungs, liver, kidneys, spleen of non-pregnant and pregnant rats

Group symbol	Organ							
	Lungs [g]		Liver [g]		Kidneys [g]		Spleen [g]	
	Mean ± SD (*n*)		Mean ± SD (*n*)		Mean ± SD (*n*)		Mean ± SD (*n*)	
	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant
Control	ND	1.43 ± 0.21 (12)	ND	8.56 ± 0.66 (12)	ND	1.71 ± 0.20 (12)	ND	0.53 ± 0.09 (12)
Catheter	1.65 ± 0.19 (6)	1.88 ± 0.13 (6)	9.40 ± 0.32 (6)	9.40 ± 0.58 (6)	2.17 ± 0.16 (6)	2.24 ± 0.26 (6)	0.68 ± 0.10 (6)	0.82 ± 0.11 (6)
Catheter Gold	1.53 ± 0,10 (6)	1.67 ± 0.28 (6)	8.67 ± 1.3 (6)	9.67 ± 0.63 (6)	1.88 ± 0.19 (6)	2.23 ± 0.41 (6)	0.73 ± 0.08 (6)	0.85 ± 0.05 (6)
Control/Catheter/ Catheter Gold	1.55 ± 0.18 (6)	1.58 ± 0.13 (6)	7.37 ± 1.33 (6)	8.93 ± 0.49 (6)	1.65 ± 0.26 (6)	1.77 ± 0.14 (6)	0.62 ± 0.10 (6)	0.68 ± 0.10 (6)
Catheter + Anti-HLA-G	1.54 ± 0.15 (5)	1.65 ± 0.23 (6)	7.86 ± 0.67 (5)	8.48 ± 0.74 (6)	1.78 ± 0,18 (5)	1.62 ± 0.15 (6)	0.76 ± 0.10 (7)	0.70 ± 0.06 (6)
Catheter Gold + Anti-HLA-G	1.53 ± 0.08 (6)	1.62 ± 0.19 (6)	7.79 ± 0.64 (6)	8.60 ± 0.47 (6)	1.77 ± 0.20 (6)	1.87 ± 0.20 (6)	0.74 ± 0.18 (5)	0.78 ± 0.12 (6)
Anti-HLA-G 0.05 ng/2 hours	1.28 ± 0.21 (6)	1.60 ± 0.09 (6)	7.58 ± 0.85	8.43 ± 0.35 (6)	1.87 ± 0.14 (6)	1.68 ± 0.10 (6)	0.62 ± 0.08	0.78 ± 0.04 (6)
Anti-HLA-G 0.10 ng/2 hours	1.38 ± 0.21 (6)	1.67 ± 0.30 (6)	7.12 ± 0.19 (6)	8.60 ± 0.55 (6)	1.55 ± 0.08 (6)	1.75 ± 0.18 (6)	0.50 ± 0.00 (6)	0.72 ± 0.08 (6)
Anti-HLA-G 0.25 ng/2 hours	1.48 ± 0.04 (6)	1.57 ± 0.27 (6)	7.70 ± 0.73 (6)	8.18 ± 0.35 (6)	1.75 ± 0.23 (6)	1.73 ± 0.14 (6)	0.65 ± 0.18 (6)	0.70 ± 0.09 (6)
Anti-HLA-G 0.05 ng/24 hours	1.33 ± 0.23 (6)	1.42 ± 0.08 (6)	5.90 (6)	7.65 ± 0.84 (6)	1.53 ± 0.14 (6)	1.70 ± 0.19 (6)	0.52 ± 0.04 (6)	0.58 ± 0.10 (6)
Anti-HLA-G 0.10 ng/24 hours	1.38 ± 0.12 (6)	1.28 ± 0.21 (6)	5.97 ± 0.70 (6)	6.98 ± 0.96 (6)	1.55 ± 0.05 (6)	1.73 ± 0.12 (6)	0.55 ± 0.08 (6)	0.50 ± 0.09 (6)
Anti-HLA-G 0.25 ng/24 hours	1.28 ± 0,08 (6)	1.18 ± 0.16 (6)	6.00 ± 0.30 (6)	6.98 ± 0.89 (6)	1.52 ± 0.04 (6)	1.72 ± 0.12 (6)	0.52 ± 0.04 (6)	0.57 ± 0.08 (6)
Anti-HLA-G 0.05 ng/6 weeks*	1.50 ± 0.17 (6)	1.57 ± 0.29 (6)	6.17 ± 0.68 (6)	8.10 ± 1.03 (6)	1.70 ± 0.22 (6)	1.75 ± 0.16 (6)	0.55 ± 0.05 (6)	0.52 ± 0.04 (6)
Anti-HLA-G 0.10 ng/6 weeks*	1.50 ± 0.11 (6)	1.54 ± 0.11 (6)	6.20 ± 0.20 (6)	8.88 ± 0.54 (6)	1.78 ± 0.26 (6)	1.78 ± 0.16 (6)	0.57	0.58 ± 0.08 (6)
Anti-HLA-G 0.25 ng/6 weeks*	1.43 ± 0.08 (6)	1.38 ± 0.13 (6)	6.35 ± 0.36 (6)	8.76 ± 0.08 (6)	1.63 ± 0.08 (6)	1.62 ± 0.18 (6)	0.50	0.50 ± 0.00 (6)

* In pregnant rats time was 3 weeks; NO – no detected

The biochemical tests were performed using blood samples collected from all animals. The concentration of interleukin 6 (IL-6), and interleukin 10 (IL-10) as well as the tumour necrosis factor (TNF) were measured within the framework of these studies (Table 5). In both non-pregnant and pregnant groups of rats, after the introduction of the catheter/catheter gold with the antibody and after the injection of the antibody, the concentration of IL-6 dropped below the limit of detection.

In the case of non-pregnant rats, no other statistically significant differences in the level of IL-6, IL-10 or TNF have been observed.

Table 4. The mean weight of placenta and foetus in pregnant rats

Group symbol	Organ	
	Placenta [g]	Foetus [g]
	Mean ± SD (*n*)	Mean ± SD (*n*)
Control	2.32 ± 0.38 (12)	9.27 ± 2.83 (12)
Catheter	1.72 ± 0.23 (6)	11.00 ± 1.71 (6)
Catheter Gold	1.73 ± 0.23 (6)	10.87 ± 2.13 (6)
Control/Catheter/ Catheter Gold	1.57 ± 0.15 (6)	8.52 ± 2.13 (6)
Catheter + Anti-HLA-G	1.78 ± 0.39 (6)	11.10 ± 4.05 (6)
Catheter Gold + Anti-HLA-G	1.70 ± 0.35 (6)	9.48 ± 4.46 (6)
Anti-HLA-G 0.05 ng/2 hours	1.68 ± 0.16 (6)	8.10 ± 0.91 (6)
Anti-HLA-G 0.10 ng/2 hours	1.820.20 (6)	9.20 ± 2.01 (6)
Anti-HLA-G 0.25 ng/2 hours	1.77 ± 0.24 (6)	8.03 ± 2.01 (6)
Anti-HLA-G 0.05 ng/24 hours	1.85 ± 0.15 (6)	9.42 ± 1.34 (6)
Anti-HLA-G 0.10 ng/24 hours	1.72 ± 0.17 (6)	8.55 ± 2.52 (6)
Anti-HLA-G 0.25 ng/24 hours	1.77 ± 0.26 (6)	8.70 ± 1.30 (6)
Anti-HLA-G 0.05 ng/6 weeks*	2.07 ± 0.26 (6)	10.67 ± 1.41 (6)
Anti-HLA-G 0.10 ng/6 weeks*	2.30 ± 0.46 (6)	15.02 ± 2.66 6
Anti-HLA-G 0.25 ng/6 weeks*	1.62 ± 0.22 (5)	11.38 ± 1.68 (5)

* In pregnant rats time was 3 weeks

In the case of pregnant rats, the level of IL-10 was statistically significantly higher in the groups, which went through the introduction of the catheter (Pregnant-catheter and Pregnant catheter gold) in comparison with the rates that underwent only surgery (Pregnant-control/catheter/catheter gold).

These increases caused the concentration of IL-10 to belower among the rats to whom catheter and catheter gold coated with antibodies was introduced, however, they were not different from the concentration in the control group (without surgery).

The mean values of haematological parameters for non-pregnant and pregnant rats are shown in Tables 6, 7 and 8.

In the group of non-pregnant rats, the values of mean corpuscular haemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width and mean platelet volume were higher after the introduction of catheter and catheter gold coated with the antibody than after the introduction of these devices into the vein without antibodies. However, the differences were statistically significant, from the biological point of view. An increase below 20% has no great importance.

Table 5. The mean values of biochemical parameters for non-pregnant and pregnant rats

Group symbol	Parameter					
	IL-6 [pg/ml]		IL-10 [pg/ml]		TNF alfa [pg/ml]	
	Mean ± SD (*n*)		Mean ± SD (*n*)		Mean ± SD (*n*)	
	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant
Control	83.54 ± 32.83 (12)	16.92 ± 15.57 (8)	6.12 ± 2.53 (12)	5.44 ± 2,70 (10)	6.01 ± 3.43 (7)	1.60 ± 1.03 (12)
Catheter	64.12 ± 33.99 (8)	30.17 ± 2.83 (5)	9.82 ± 9.42 (8)	14.59 ± 0.82[2] (5)	8.78 ± 11.30 (10)	6.26 ± 0.53[2] (5)
Catheter Gold	146.88 ± 107.9 (9)	30.42 ± 0.98 (6)	9.79 ± 6.43 (8)	13.76 ± 1.04[2] (6)	16.87 ± 17.92 (14)	7.00 ± 0.54[2] (6)
Control/Catheter/ Catheter Gold	bld (6)	24.00 ± 20.50 (5)	bld (6)	7.76 ± 2.92 (6)	1.54 ± 0.58 (6)	2.87 ± 0.56 (6)
Catheter + Anti-HLA-G	bld (5)	15.29 (2)	bld (5)	3.81 ± 5.20 (3)	1.78 ± 0.77 (5)	1.53 ± 0.85[3] (5)
Catheter Gold + Anti-HLA-G	bld (7)	14.58 (2)	bld (7)	6.25 ± 0.92 (3)	1.50 ± 0.87 (7)	1.33 ± 0.28[4] (5)
Anti-HLA-G 0,05 ng/2 hours	10.35 (2)	26.29 (2)	8.96 ± 1.00 (6)	6.35 ± 6.35 (6)	0.35 ± 0.19 (4)	2.62 ± 0.25 (6)
Anti-HLA-G 0,10 ng/2 hours	bld (6)	19.70 (1)	9.87 ± 3.83 (6)	4.67 ± 1.62 (6)	1.49 ± 0.95 (5)	2.12 ± 0.88 (6)
Anti-HLA-G 0,25 ng/2 hours	bld (6)	Bld (6)	9.64 ± 1.96 (5)	6.06 ± 2.68 (6)	0.40 (2)	0,95 ± 0.28 (6)
Anti-HLA-G 0,05 ng/24 hours	bld (6)	Bld (6)	8.41 ± 2.08 (6)	5.14 ± 2.44 (6)	0.31 ± 0.20 (3)	0.60 ± 0.44 (6)
Anti-HLA-G 0,10 ng/24 hours	bld (6)	Bld (6)	9.11 ± 2.36 (6)	12.2417.31 (4)	0.32 (2)	0.37 ± 0.31 (5)
Anti-HLA-G 0,25 ng/24 hours	bld (6)	15.92 (2)	7.53 ± 4.44 (5)	6.25 ± 3.83 (6)	5.48 ± 7.14 (5)	0.76 ± 0.47 (6)
Anti-HLA-G 0,05 ng/6 weeks*	bld (6)	10.57 (2)	bld (6)	5.20 ± 3.16 (4)	1.67 ± 0.99 (6)	1.97 ± 1.57 (6)
Anti-HLA-G 0,10 ng/6 weeks*	bld (6)	20.07 ± 9.29 (4)	bld (6)	7.92 ± 3.49 (5)	1.21 ± 0.67 (5)	3.49 ± 2.12 (6)
Anti-HLA-G 0,25 ng/6 weeks*	bld (6)	8.65 ± 4.54 (3)	bld (6)	6.38 ± 2.36 (3)	1.40 ± 0.26 (6)	2.60 ± 1.68 (4)

* In pregnant rats time was 3 weeks; bld – below limit of detection

1 – Differences statistically significant from control group; $p < 0.05$

2 – Differences statistically significant from control/catheter/catheter gold group; $p < 0.05$

3 – Differences statistically significant from catheter group; $p < 0.05$

4 – Differences statistically significant from catheter gold group; $p < 0.05$

The observed decreases in hematocrit after the injection of antibodies were relatively small and are of no biological importance.

The statistically significant differences were caused by high stability of this parameter in the control group (very low standard deviation). A small individual differences after the administration of the antibody were noticed in the case of mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red blood cell distribution width and mean platelet volume. There were much less differences in the studied parameters among different experimental groups with regard to the pregnant rats. The individual statistical differences were observed in the number of white blood cells (2 hours after the injection of antibody in a dose of 0.1 ng/rat) and concentration of haemoglobin (24 hours the injection of the antibody in a dose of 0.1 ng/rat). The introduction of the catheter coated with antibodies decreased the mean platelet volume by about 10%. Also these changes did not have any biological importance.

Table 6. The mean values of WBC, RBC, HGB and HCT for non-pregnant and pregnant rats

Group symbol	Parameter							
	WBC [10^9/l]		RBC [10^{12}/l]		HGB [mmol/l]		HCT [l/l]	
	Mean ± SD (n)		Mean ± SD (*n*)		Mean ± SD (*n*)		Mean ± SD (*n*)	
	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant
Control	3.11 ± 0.91 (11)	4.09 ± 0.66 (12)	8.35 ± 0.33 (11)	6.57 ± 0.43 (!2)	9.18 ± 0.32 (11)	8.05 ± 0.34 (12)	0.44 ± 0.01 (11)	0.35 ± 0.03 (12)
Catheter	2.57 ± 1.18 (5)	5.38 ± 1.38 (6)	7.55 ± 0.96 (5)	6,75 ± 0.40 (6)	8.58 ± 0.90 (5)	8.70 ± 0.64 (6)	0.40 ± 0.04 (5)	0.35 ± 0.02 (6)
Catheter Gold	3.26 ± 1.39 (7)	6,33 ± 3.35 (6)	7.59 ± 0.98 (7)	6.18 ± 0.32 (6)	8.74 ± 1.13 (7)	8.12 ± 0.26 (6)	0.40 ± 0.05 (7)	0.33 ± 0.01 (6)
Control/Catheter/ Catheter Gold	3.170.96 (6)	5.58 ± 1.65 (6)	7.16 ± 0.61 (6)	6.41 ± 0.51 (6)	8.87 ± 0.59 (6)	8.25 ± 0.57 (6)	0.36 ± 0.04 (6)	0.33 ± 0.02 (6)
Catheter + Anti-HLA-G	3.88 ± 0.62 (5)	4.220.80 (6)	6.99 ± 0.72 (5)	6.07 ± 0.68 (6)	9.10 ± 0.70 (7)	7.73 ± 0.81 (6)	0.37 ± 0.03 (5)	0.33 ± 0.05 (6)
Catheter Gold + Anti-HLA-G	4.06 ± 1.47 (7)	3.50 ± 1.15 (6)	6.78 ± 0.66 (7)	6.37 ± 0.98 (6)	8.41 ± 0.71 (7)	7.78 ± 1.38 (6)	0.35 ± 0.03 (7)	0.32 ± 0.02 (6)
Anti-HLA-G 0.05 ng/2 hours	4.00 ± 0.71 (6)	6.40 ± 2.17 (6)	6.64 ± 1.96[1] (6)	6.49 ± 0.18 (6)	9.02 ± 0.38 (6)	8.45 ± 0.29 (6)	0.36 ± 0.03[1] (6)	0.34 ± 0.02 (6)
Anti-HLA-G 0.10 ng/2 hours	5.35 ± 2.30 (6)	8,07 ± 189[1] (6)	7.31 ± 0.99 (6)	6,37 ± 0.23 (6)	9.02 ± 0.12 (6)	8,30 ± 0.57 (6)	0.36 ± 0.04[1] (6)	0.34 ± 0.02 (6)
Anti-HLA-G 0.25 ng/2 hours	4.72 ± 0.86 (5)	5.75 ± 1.27 (6)	7.52 ± 0.66 (5)	6.76 ± 0.58 (6)	9.44 ± 0.38 (5)	8.67 ± 0.33 (6)	0.37 ± 0.02 (5)	0.35 ± 0.02 (6)
Anti-HLA-G 0.05 ng/24 hours	4.57 ± 2.35 (6)	3.70 ± 1.11 (5)	7.02 ± 0.51 (6)	6.86 ± 0.09 (5)	9.05 ± 0.37 (6)	8.40 ± 0.25 (5)	0.36 ± 0.02[1] (6)	0.35 ± 0.02 (5)
Anti-HLA-G 0.10 ng/24 hours	4.16 ± 0.48 (5)	3.05 ± 0.63 (6)	6.92 ± 0.38 (5)	7.41 ± 0.27 (6)	8.90 ± 0.47 (5)	9.50 ± 0.26[1] (6)	0.35 ± 0.01[1] (5)	0.37 ± 0.01 (6)
Anti-HLA-G 0.25 ng/24 hours	3.67 ± 0.94 (6)	3.23 ± 0.87 (6)	6.85 ± 0.31 (6)	7.45 ± 0.40 (6)	9.28 ± 0.45 (6)	8.73 ± 0.45 (6)	0.35 ± 0.02[1] (6)	0.38 ± 0.02 (6)
Anti-HLA-G 0.05 ng/6 weeks*	4.22 ± 0.57 (6)	9.75 (2)	7.37 ± 0.51 (6)	6.16 (2)	9.52 ± 0.55 (6)	7.45 (2)	0.38 ± 0.03 (6)	0.32 (2)
Anti-HLA-G 0.10 ng/6 weeks*	3.85 ± 1.13 (6)	6.32 ± 2.28 (5)	7.19 ± 0.55 (6)	6.58 ± 0.55 (5)	9.70 ± 0.35 (6)	8.18 ± 0.65 (5)	0.36 ± 0.09[1] (6)	0.34 ± 0.03 (5)
Anti-HLA-G 0.25 ng/6 weeks*	2.80 ± 0.68 (6)	7.60 (2)	8.08 ± 0.80 (6)	6.83 (2)	9.50 ± 0.34 (6)	8.35 (2)	0.42 ± 0.05 (6)	0.35 (2)

* In pregnant rats time was 3 weeks

1 – Differences statistically significant from control group; $p < 0.05$

2 – Differences statistically significant from control/catheter/catheter gold group; $p < 0.05$

3 – Differences statistically significant from catheter group; $p < 0.05$

4 – Differences statistically significant from catheter gold group; $p < 0.05$

For technical (no possibility to collect enough urine after two hours) and factual reasons (no justification for collection of urine after 3 or 6 weeks since the introduction of the catheter or administration of antibodies), the analysis of was performed only in animals, which had been subjected to autopsy after 24h and in the control group. The obtained results do not indicate the impact of pregnancy on the specific weight of urine. Within the non-pregnant group of rats, it ranged from 1.01 to 1.03 g/ml, and within the group of the pregnant ones – 1.02-1.03 gl. A similar lack of differences concerned pH of urine, which amounted to 6.0-6.58 in the group of pregnant females, and 6.0-7.0 in the group of non-pregnant rats. Thevalue of these parameters did not depend on the earlier administration of antibodies.

In the case of the remaining determined parameters (bilirubin, glucose, ketone, nitrate, protein, red blood cells, specific gravity, urobilinogen and white blood cells), neither the impact of the administration of antibodies nor pregnancy on

Table 7. The mean values of MCV, MCH, MCHC and RDW for non-pregnant and pregnant rats

Group symbol	Parameter							
	MCV [fl]		MCH [fmol]		MCHC [mmol/l]		RDW [%]	
	Mean ± SD (*n*)		Mean ± SD (*n*)		Mean ± SD (*n*)		Mean ±S D (*n*)	
	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant
Control	52.36 ± 1.12 (11)	52.37 ± 1.43 (12)	1.09 ± 0.04 (11)	1.25 ± 0.06 (9)	21.01 ± 0.36 (11)	23.88 ± 0.89 (9)	11.17 ± 0.45 (11)	13.59 ± 0.65 (12)
Catheter	53.12 ± 2.25 (5)	52.21 ± 1.49 (6)	1.14 ± 0.03 (5)	1.29 ± 0.05 (6)	21.36 ± 0.47 (5)	24.76 ± 0.50 (6)	19.48 ± 1.85 (5)	13.35 ± 0.44 (6)
Catheter Gold	53.09 ± 3.01 (7)	52.87 ± 0.93 (6)	1.15 ± 0.04 (7)	1.32 ± 0.05 (6)	21.67 ± 0.56 (7)	24.92 ± 0.72 (6)	19.30 ± 2.61 (7)	14.12 ± 2.32 (6)
Control/Catheter/ Catheter Gold	ND	52.37 ± 1.57 (6)	ND	1.29 ± 0.03 (6)	ND	23.28 ± 0.73 (4)	ND	13.12 ± 0.34 (6)
Catheter + Anti-HLA-G	53.82 ± 1.66 (5)	52.00 ± 0.97 (6)	1.33 ± 0.05[3] (3)	1.20 ± 0.04 (4)	24.23 ± 0.71[3] (3)	23.87 ± 1.01 (6)	12.74 ± 0.44[3] (5)	13.48 ± 1.9 (6)
Catheter Gold + Anti-HLA-G	52.69 ± 2.03 (7)	53.43 ± 3.44 (6)	1.25 ± 0.03[4] (5)	1.28 ± 0.05 (6)	23.84 ± 0.80[4] (5)	24.60 ± 0,50 (6)	12.81 ± 0.69[4] (7)	13.47 ± 0.73 (6)
Anti-HLA-G 0.05 ng/2 hours	50.55 ± 0.64 (6)	52.82 ± 2.16 (6)	ND	1.30 ± 0.04 (6)	ND	24.65 ± 0.39 (6)	12.77 ± 0.4 (6)8	13.68 ± 0.75 (6)
Anti-HLA-G 0.10 ng/2 hours	50.93 ± 1.44 (6)	52.83 ± 1.67 (6)	1.29	1.30 ± 0.05 (6)	24.40	24.67 ± 0.74 (6)	12.80 ± 0.55 (6)	13.38 ± 0.54 (6)
Anti-HLA-G 0.25 ng/2 hours	50.34 ± 0.79 (5)	52.82 ± .29 (6)	ND	1.31 ± 0.02 (5)	ND	24.78 ± 0.40 (5)	13.42 ± 0.46 (5)	14.00 ± 0.60 (6)
Anti-HLA-G 0.05 ng/24 hours	51.53 ± 1.60 (6)	51.62 ± 1.86 (5)	1.32 ± 0.05[1] (4)	1.22 ± 0.02 (5)	25.35 ± 0.24[1] (4)	23.72 ± 0.7 (5)	13.40 ± 0.65[1] (6)	13.56 ± 0.39 5
Anti-HLA-G 0.10 ng/24 hours	51.52 ± 1.38 (5)	51.48 ± 1.42 (6)	1.29 ± 0.05[1] (3)	ND	25.03 ± 040[1] (3)	ND	12.96 ± 0.54 (5)	13.30 ± 0.70 (6)
Anti-HLA-G 0.25 ng/24 hours	50.95 ± 1.46 (6)	51.48 ± 1.06 (6)	1.25	1.17	25.60	22.80 (1)	13.30 ± 0.72 (6)	13.10 ± 0.72 (6)
Anti-HLA-G 0.05 ng/6 weeks*	51,73 ± 1.11 (6)	51.60 (2)	1.29	1.21	25.05	23.35 (2)	12.93 ± 0.44 (6)	14.25 2
Anti-HLA-G 0.10 ng/6 weeks*	52.12 ± 0.75 (6)	51.02 ± 0.41 (5)	ND	1.24 ± 0.01 (4)	ND	24.40 ± 0.12 (4)	13.12 ± 0.52 (6)	13.52 ± 0.91 (5)
Anti-HLA-G 0.25 ng/6 weeks*	52.92 ± 0.75 (6)	50.75 (2)	ND	1.24	ND	24.45 (2)	14.32 ± 1.45[1] (6)	13.95 (2)

* In pregnant rats time was 3 weeks

1 – Differences statistically significant from control group; $p < 0.05$

2 – Differences statistically significant from control/catheter/catheter gold group; $p < 0.05$

3 – Differences statistically significant from catheter group; $p < 0.05$

4 – Differences statistically significant from catheter gold group; $p < 0.05$

ND – no detected

their size were observed. Only in the case of the pregnant rats, the increased concentration of protein and number of white blood cells was observed in 50% of animals. These changes concerned both the animals from the control group and the animals to which antibodies were administered, and the deviations from the normal values were not significant.

In general, the main part of the results regarding the urine analysis were within the range of the reference values. The observed values above the recommended values did not point to any important disturbances in homeostasis caused by the antibodies or the catheters.

Table 8. The mean values of PLT, MPV and PCT for non-pregnant and pregnant rats

Group symbol	Parameter					
	PLT [10^9/l]		MPV [fl]		PCT [10^2/l]	
	Mean ± SD (*n*)		Mean ± SD (*n*)		Mean ± SD (*n*)	
	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant
Control	805 ± 71.9 (11)	1040 ± 132.1 (12)	5.94 ± 0.19 (11)	6.67 ± 0.48 (12)	0.4 (1)	6.90 (1)
Catheter	687 ± 77.1 (3)	760 ± 161.1 (6)	7.80 ± 0.10 (3)	7.32 ± 0.55 (6)	0.53 ± 0.05 (3)	5.61 ± 1.00 (6)
Catheter Gold	643 ± 107.1 (7)	601 ± 17.9 (6)	7.83 ± 0.42 (7)	7.28 ± 0.77 (6)	0.49 ± 0.08 (7)	4.73 ± 1.16 (6)
Control/Catheter/ Catheter Gold	758 ± 99.3 (5)	877 ± 135.2 (6)	ND	6.65 ± 0.32 (6)	ND	6.23 ± 0.70 (4)
Catheter + Anti-HLA-G	755 ± 177.0 (5)	939 ± 118.6 (6)	6.88 ± 0.24[3] (5)	6.23 ± 0.37[3] (6)	5.13 ± 1.50[3] (3)	5.90 ± 0.85 (3)
Catheter Gold + Anti-HLA-G	742 ± 55.3 (6)	931 ± 60.5 (4)	6.98 ± 0.23[4] (5)	6.37 ± 0.30 (6)	5.45 ± 0.26[4] (4)	5.43 ± 1.33 (3)
Anti-HLA-G 0.05 ng/2 hours	704 ± 205.7 (6)	940 (2)	6.77 ± 0.31[1] (6)	6.32 ± 0.21 (6)	5.60 (1)	6.00 (2)
Anti-HLA-G 0.10 ng/2 hours	823 ± 176.2 (6)	890 (2)	6.52 ± 0.25 (6)	6.22 ± 0.22 (6)	6.30 (1)	5.55 (2)
Anti-HLA-G 0.25 ng/2 hours	718 ± 54.7 (5)	754 ± 155.0 (3)	6.78 ± 0.29[1] (4)	6.28 ± 0.21 (6)	ND	4.10 (2)
Anti-HLA-G 0.05 ng/24 hours	887 ± 91.7 (6)	971 ± 118.3 (5)	7.05 ± 0.52[1] (6)	6.60 ± 0.16 (5)	6.33 ± 0.29 (3)	ND
Anti-HLA-G 0.10 ng/24 hours	871 ± 69.1 (5)	853 ± 85.3 (6)	7.28 ± 026[1] (5)	6.85 ± 0.28 (6)	6.85 (2)	ND
Anti-HLA-G 0.25 ng/24 hours	838 ± 104.1 (6)	835 ± 108.5 (6)	6.90 ± 0.14[1] (6)	6.60 ± 0.34 (6)	6.50 (10)	ND
Anti-HLA-G 0.05 ng/6 weeks*	916 ± 46.1 (6)	903 (2)	6.83 ± 0.31[1] (6)	6.60 (1)	ND	6.50 (1)
Anti-HLA-G 0.10 ng/6 weeks*	839 ± 117.7 (6)	966 ± 134.7 (5)	6.73 ± 0.27[1] (6)	6.44 ± 0.30 (5)	ND	5.07 (1)
Anti-HLA-G 0.25 ng/6 weeks*	805 ± 69.0 (6)	988 (2)	7.60 ± 0.19[1] (5)	6.50 (2)	ND	5.20 (1)

* In pregnant rats time was 3 weeks

1 – Differences statistically significant from control group; $p < 0.05$

2 – Differences statistically significant from control/catheter/catheter gold group; $p < 0.05$

3 – Differences statistically significant from catheter group; $p < 0.05$

4 – Differences statistically significant from catheter gold group; $p < 0.05$

NO – no detected

Conclusions

The toxicological studies of the Cell-Selected Catheter provided by GILUPI Nanomedizine GmbH, Potsdam-Golm, were carried out on the basis of observation of animals, macroscopic evaluation of the selected organs, biochemical and haematological tests and urine analysis.

None of the performed measurements reflects the toxic effect of the examined devices on the physiological processes and health condition of the animals used in experiment. Observed deviations of some individual parameters from reference values do not have the grater biological importance.

References

[1] Schmorl, G. (1893) *Pathologisch-anatomische Untersuchungen über Puerperal-Eklampsie*. Leipzig: Verlag FCW Vogel.

[2] Hawes C.S., Suskin H.A., Kalionis B. et al. (1994*) Detection of paternally inherited mutations for beta-thalassemia in trophoblast isolated from peripheral maternal blood*. Ann. N. Y. Acad. Sci. 731:181-185.

[3] Mueller U.W., Hawes C.S., Wright A.E. et al. (1990) *Isolation of fetal trophoblast cells from peripheral blood of pregnant women.* Lancet 33:197-200.

[4] Cacheux V., Milesi-Fluet C., Tachdjian G. (1992) *Detection of 47,XYY trophoblast fetal cells in maternal blood by fluorescence in situ hybridization after using immunomagnetic lymphocyte depletion and flow cytometry sorting*. Fetal. Diagn.Ther. 7:190-194.

[5] Sbracia M., Scarpellini F., Lalwani S. et al. (1994) *Possible use of an unusual HLA antigen to select trophoblast cells from the maternal circulation to perform early prenatal diagnosis.* Ann. N. Y. Acad. Sci. 731:170-174.

[6] Durrant L., McDowall K., Holmes R., Liu D. (1996) *Non-invasive prenatal diagnosis by isolation of both trophoblasts and fetal nucleated red blood cells from the peripheral blood of pregnant women.* Br. J. Obstet. Gynaecol. 103:219-222.

[7] Bianchi D.W. (1998) *Current knowledge about fetal blood cells in the maternal circulation.* J. Perinat. Med. 26:175-185.

[8] van Wijk I.J., Tjoa M.L., Griffioen S. et al. (2001) *Circulating Trophoblasts in Maternal Peripheral Blood*, in Hahn, S., Holzgreve, W. (Eds.), Fetal Cells and Fetal DNA in Maternal Blood, New Developments for a New Millennium, 11th Fetal Cell Workshop, Basel, 2000, Basel, Krager, 111-119.

[9] van Wijk I.J., van Vugt J.M., Mulders M.A. et al. (1996*) Enrichment of fetal trophoblast cells from the maternal peripheral blood followed by detection of fetal deoxyribonucleic acid with a nested X/Y polymerase chain reaction*. Am. J. Obstet. Gynecol. 174: 871-878.

[10] Vona G., Beroud C., Benachi A. et al. (2002) *Enrichment, immunomorphological, and genetic characterization of fetal cells circulating in maternal blood*. Am. J. Pathol. 160: 51-58.

[11] Cunniff C. (2004) *Prenatel Screening and Diagnosis for Pediatricians*. Pediatrics 114: 889-894.

[12] Papp C., Papp Z. (2003) *Chorionic villus sampling and amniocentesis: what are the risks in current practice?* Curr. Opin. Obstet. Gynecol. 15:159-165.

[13] Evans M.I., Wapner R.J. (2005) *Invasive prenatal diagnostic procedures 2005.* Semin. Perinatol. 29: 215-218.

Ewa Florek
Laboratory of Environmental Research
Department of Toxicology
Medical University in Poznań, Poland
Dojazd 30, 60-631 Poznań
e-mail: eflorek@ump.edu.pl