Scand. J. Lab. Anim. Sci. No. 4. 1997. Vol. 24

Clinical pathology values in pregnant and non-pregnant rabbits

by *Maud Palm*
Safety Assessment, Astra AB, S-151 85 Södertälje, Sweden
Correspondence to Dr. Maud Palm, Safety Assessment, Astra AB, S-151 85 Södertälje, Sweden

Introduction

Reproduction studies are an important part of the toxicological evaluation of new drugs. According to regulatory guidelines, it is not necessary to include clinical pathology in these studies. However, it has been found at our laboratory that changes occurring in hematology and biochemistry in the dose-finding studies have been helpful in determining dosages in the main studies. To be able to evaluate the results obtained in drug-treated pregnant animals, it is an advantage to have reference values for pregnant animals to compare with. The aim of this study was to establish reference values for pregnant rabbits and defines differences noted in the clinical pathology variables between pregnant and nonpregnant animals of similar age.

Materials and Methods

Nonpregnant New Zealand White rabbits were obtained from a commercial breeder in Sweden (ESF-Produkter Estuna AB, Norrtälje). The rabbits were mated at the laboratory after an acclimatization period. The rabbits were kept individually in polypropylene cages with perforated floor, each with a separate dung tray. Target values for temperature and humidity were 14-20°C and 40-70%, respectively. The air was changed 15 times per hour and the rooms were illuminated on a 16/8-hour light/dark cycle. The animals had free access to tap water and the commercial laboratory animal food K5 (Lactamin AB, Stockholm, Sweden). They were not fasted before blood collection.

Blood samples were collected from 46 pregnant rabbits on day 15 or 16 of gestation and from 46 non-pregnant female rabbits. The age of the rabbits was 6-8 months with a bodyweight of 2.5-5.0 kg. Blood was collected from the central ear artery of non-sedated animals. Two tubes were taken, one containing EDTA for the hematology variables and one without any additive for the clinical chemistry analysis. All blood samples were taken between 8 and 10 a.m. and analysed the same day.

The hematolgy variables, erythrocyte count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), total leukocyte count (WBC), platelet count (PLT) and erythrocyte indices (MCV, MCH, MCHC) were measured using a Sysmex F-800 (Toa Medical Electronics Co., LTD. Kobe, Japan). The differential count was determined by a manual count of 100 white blood cells after May-Grünewald Giemsa staining of blood smear.

The clinical chemistry analyses listed in table 1 were performed on a Cobas Mira (Hoffman-La Roche & Co., Basle, Switzerland). The reagent kits were from Roche (Hoffman-La Roche & Co., Basle, Switzerland) except the albumin reagents, which were from Boehringer Mannheim (Mannheim, Germany). The analyses were performed according to the manufacturer´s methods. The enzymes were analysed at 37°C.

Data were tested for statistical differences using Wilcoxon-Mann-Whitney test, and probabilities (p) less than 0.01 (**) were considered statistically significant.

Results

There were statistically significant differences in several variables between pregnant and non-pregnant animals. However, these differences were in general small (Tables 2, 3 and Figures 1, 2 and 3). The most pronounced difference was noticed inthe mean serum concentration of cholesterol that was 30 % lower in the pregnant animals than in the non-pregnant animals. In addition, slightly lower values were also noticed in

pregnant animals .for glucose, total protein, albumin, sodium, chloride, calcium, phosphate, creatinine and MCHC. The erythrocyte indici MCV and MCH were slightly higher in pregnant than in the non-pregnant animals.

No differences of statistic significance were observed in the other variables measured.

Table 1 **The serum biochemistry parameters measured**

Variable	Unit	Method
Glucose	mmol/l	Enzymatic colorimetric, GOD/PAP
Urea	mmol/l	Enzymatic UV, urease/GLDH
Creatinine	µmol/l	Colorimetric,Jaffé, buffered, kinetic
Total protein	g/l	Biuret reaction
Albumin	g/l	Bromocresol-green
Cholesterol	mmol/l	Enzymatic colorimetric
Alkaline phosphatase, ALP	mkat/l	Kinetic UV, IFCC/37°C
Alanine amino transferase ALT	mkat/l	Kinetic UV, IFCC/37°C
Sodium	mmol/l	Ion selective electrode
Potassium	mmol/l	Ion selective electrode
Chloride	mmol/l	Ion selective electrode
Calcium	mmol/l	Methylthymol blue
Phosphate	mmol/l	Phosphomolybdate,UV

Table 2 **Means ± standard deviation and ranges of hematology variables in nonpregnant and pregnant rabbits**

Analysis	Unit	Nonpregnant		Pregnant	
		Mean±SD	Ranges	Mean±SD	Ranges
Red blood cell count	$x10^{12}/l$	**6.1**±0.6	4.7-7.4	**5.9**±0.5	4.8-7.2
Hemoglobin	g/L	**121**±11	101-152	**123**±9	100-141
Hematocrit	%	**39**±3	32-48	**40**±3	33-49
MCV	fl	**63.7**±2.7	58.6-69.8	**68.1**±3.2***	61.4-76.3
MCH	pg	**20.0**±0.8	17.1-21.6	**20.8**±1.2***	18.0-23.6
MCHC	g/L	**313**±6	300-327	**306**±10***	279-325
Platelet count	$x10^{12}/L$	**336**±70	163-503	**356**±118	129-727
White blood cell count	$x\ 10^{9}/L$	**11.4**±3.2	5.7-18.7	**10.0**±2.0	6.0-14.2
Segmented neutrophils	%	**36**±14	14-68	**35**±16	5-70
Lymphocytes	%	**60**±15	25-84	**61**±18	25-95
Monocytes	%	**1**±1	0-3	**2**±2	0-6
Eosinophils	%	**1**±1	0-6	**1**±1	0-4
Basophils	%	**1**±1	0-4	**1**±2	0-6

** = $p<0.01$
*** = $p<0.001$
The table presents values from 46 nonpregnant animals and 46 pregnant animals.

Table 3. **Means ± standard deviation and ranges of serum biochemistry variables in nonpregnant and pregnant rabbits**

Analysis	Unit	Nonpregnant		Pregnant	
		Mean±SD	Range	Mean±SD	Range
Glucose	mmol/L	**8.4**±0.6	7.1-9.9	**7.6**±0.9***	4.5-10.3
Urea	mmol/L	**6.3**±1.2	4.2-10.3	**6.9**±1.5	4.5-11.4
Creatinine	µmol/L	**121**±20	93-194	**108**±19**	74-164
Total protein	g/L	**60**±3	52-69	**58**±4***	51-71
Albumin	g/L	**42**±2	37-46	**40**±3*	35-46
A/G		**2.2**±0.2	1.9-2.7	**2.3**±0.4	1.8-3.4
Cholesterol	mmol/L	**1.2**±0.3 [a]	0.7-2.1	**0.8**±0.5***	0.2-2.1
Alkaline phosphatase, ALP	µkat/L	**0.9**±0.4	0.3-2.0	**0.9**±0.3	0.3-1.7
Alanine aminotransferase ALT	µkat/L	**0.60**±0.24	0.23-1.23	**0.59**±0.30	0.21-1.39
Sodium	mmol/L	**138**±2	134-144	**136**±2**	133-140
Potassium	mmol/L	**4.4**±0.3	3.5-5.2	**4.2**±0.4	3.1-4.9
Chloride	mmol/L	**105**±2	99-109	**101**±3***	96-107
Calcium	mmol/L	**3.7**±0.2	3.4-4.0	**3.6**±0.2**	3.2-4.0
Phosphate	mmol/L	**1.6**±0.3	0.6-2.1	**1.5**±0.2**	1.1-2.1

** = $p<0.01$
*** = $p<0.001$
Values from 46 nonpregnant animals (chloride n=37) and 46 pregnant animals (phosphate n=42, creatinine n=41 and chloride n=34)
a) n=45 since one value of 6.0 mmol/L has been excluded

Discussion

The mean values for the nonpregnant rabbits were of the same order of magnitude as those found in other studies (*Wolford et al.*, 1986; *Hewitt et al.*, 1989; *Hall*, 1992; *Jensen et al.*, 1992). Pregnant animals had slightly higher MCV (Fig. 1) and MCH values than nonpregnant animals, indicating an influence of pregnancy on the red cell variables. *Kriesten* (1987) reported, in a study in which blood samples were analysed every week during pregnancy, a rise in the blood volume from day 10 to day 19 of pregnancy, associated with a higher oxygen transport to the organs. Small fluctuations in the hemoglobin concentration, erythrocyte count, MCV and hematocrite value were also noticed. In another study *(Bortolotti et al.*, 1989) in which blood samples were taken on day 29 of gestation, MCV was higher in the pregnant than in the non-pregnant animals although no statistically significant differences were reported.

Hemodynamic changes in the rabbit during pregnancy have been studied by *Nuwayhid* (1979) who reported an increased total blood cell volume, plasma volume and red cell volume. The lower serum concentrations of total protein and albumin seen in the pregnant rabbits in the present study and in earlier studies *(Fischer,* 1980, *Viard-Drouet et al.* 1980, *Staiger,* 1986; *Bortolotti et al.*, 1989) might be related to these hemodynamic changes. The decreased levels of sodium, chloride, calcium and phosphate may also be related to the increased plasma volume. However, the differences were small and only about 3 % for protein and the ranges were similar in the two groups (Fig. 2). The serum concentration of cholesterol was about 30 % lower in the pregnant than in non-pregnant rabbits (Fig. 3). Similar findings have been reported before *(Fischer,* 1980; *Viard-Drouet et al.* 1980). Drastic changes in the cholesterol levels were shown in two rabbits investigated before

pregnancy and at day 28, since the values obtained day 28 were only 10% of those observed before pregnancy *(Quig & Zilversmit* 1986). A decrease in the serum concentration of glucose was noted in the pregnant animals in the present study which is in contrast to the findings of *Fischer* (1980) and *Staiger* (1986). Furthermore, the serum concentration of creatinine was lower in pregnant than in non-pregnant animals.

Figure 1. **Serum concentration of total protein in 46 nonpregnant and 46 pregnant rabbits. The lines inserted represent the median values.**

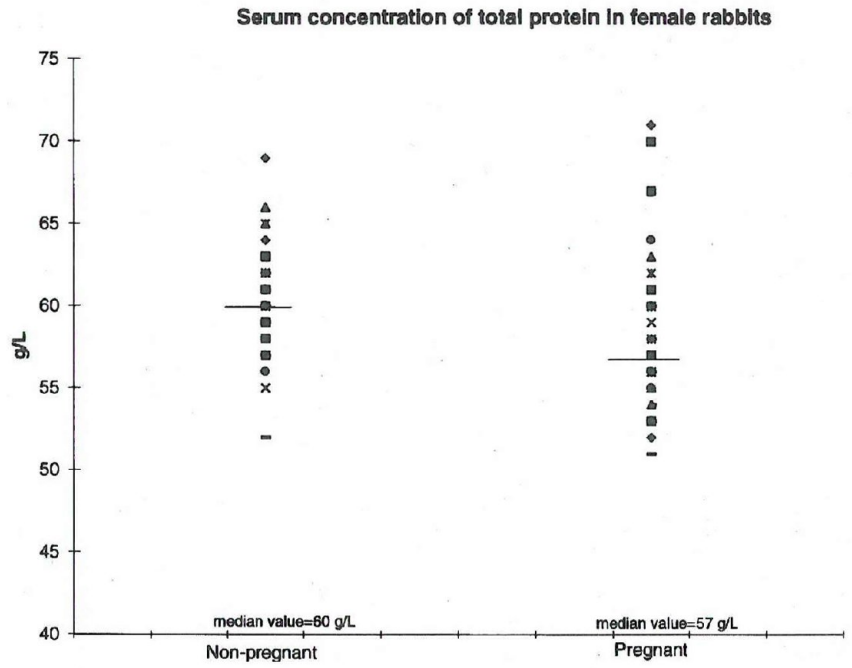


Figure 2. **MCV in 46 nonpregnant and 46 pregnant rabbits. The lines inserted represent the median values.**

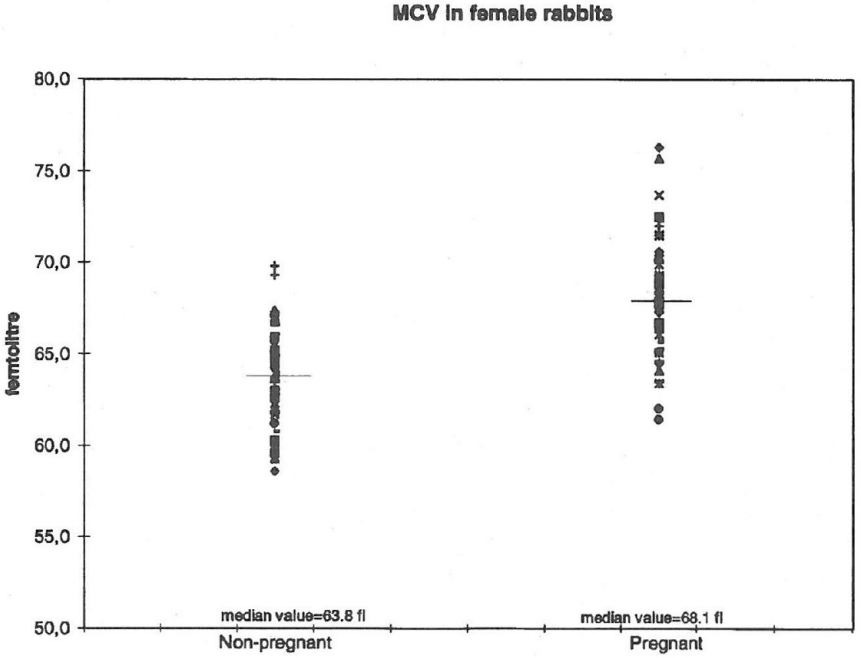
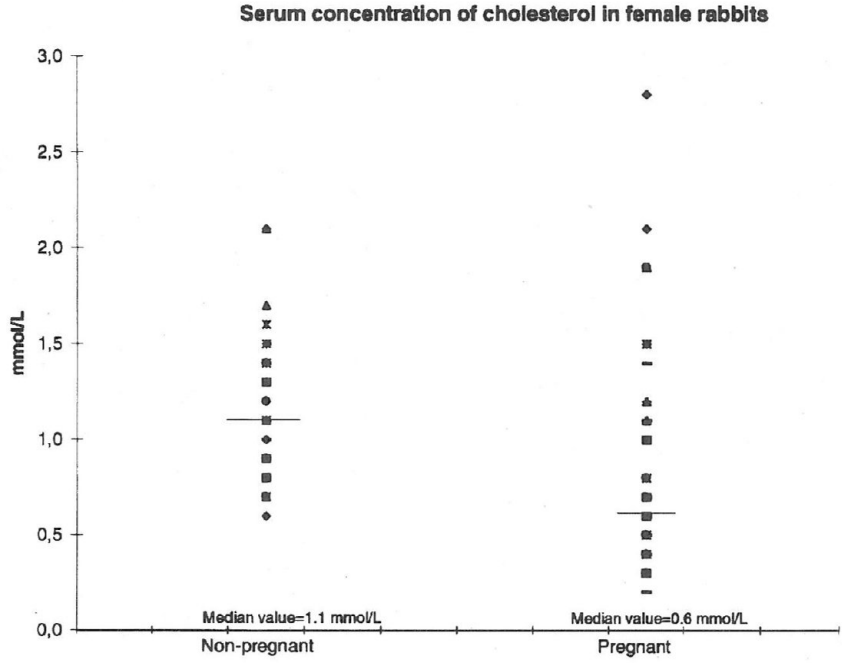


Figure 3. **Serum concentration of cholesterol in 45 nonpregnant and 46 pregnant rabbits. The lines inserted represent the median values.**



ALP is present in the placenta of domestic and laboratory animals and in humans. In humans, the ALP activity in serum is markedly increased during pregnancy *(Clubb et al.,* 1965). However, in the present study no increased serum activity of ALP was observed in the pregnant rabbits. This is in agreement with a study with pregnant rabbits done by *Boyne and Fell* (1972). Despite a twofold increase in the concentration of ALP in the rabbit placenta, these authors observed no increased activity of ALP in serum and *Viard-Drouet et al.* (1980) reported lower serum activity of ALP in pregnant rabbits.

In conclusion, this study presents hematology and clinical chemistry variables in pregnant and nonpregnant rabbits. The data define certain statistically significant differences between nonpregnant rabbits and pregnant rabbits in the middle of the gestation period, indicating an influence of pregnancy on the clinical pathology variables. Most differences, however, are small and the reference ranges for most analysis are similar in the two groups.

Summary
Blood samples were collected from pregnant and nonpregnant rabbits for hematology and serum biochemistry analyses. Comparison of the results revealed statistically significant differences between pregnant and nonpregnant animals in several variables. The most pronounced difference was noticed in the mean serum concentration of cholesterol with a 30% lower mean value in the pregnant than in the non-pregnant animals. Other findings were increased mean values of MCV and MCH and decreased mean serum concentrations of total protein, albumin, electrolytes, glucose and creatinine in the pregnant does. Although the differences were of statistic significance, most changes were small and the ranges obtained were similar in the two groups.

Sammanfattning
Blodprov togs från dräktiga och icke dräktiga kaniner och blodet analyserades med avseende på hematologiska och klinisk kemiska variabler. En jämförelse av värdena mellan de båda grupperna visade statistiskt signifikanta skillnader för flera av variablerna. Den största skillnaden sågs i kolesterolhalten, där dräktiga kaniner hade 30% lägre medelvärde än icke dräktiga djur. Andra skillnader var ökad MCV och MVH samt lägre serumnivåer av totalprotein, albumin, elektrolyter, glukos och kreatinin hos de dräktiga djuren. Trots att skillnadrna var statistiskt signifikanta var de flesta förändringarna små, och de gränser inom vilka värdena varierade var lika för de två grupperna.

Acknowledgement
I want to thank the staff at the Department of Clinical Pathology for skillful technical assistance and S Gidlund for help with the manuscript.

References
Bortolotti A, D Castelli & M Bonati: Hematology and serum chemistry values of adult, pregnant and newborn New Zealand rabbits (*Oryctolagus cuniculus*). Lab. Anim. Sci. 1989, *39*, 437-439.

Boyne R & BF Fell: Observation on the alkaline phosphatase content of the neutrophil granulocytes of some laboratory and farm animals, with particular reference to the effects of intensive management and reproduction. Res. Vet. Sci. 1972, *13*, 347-355.

Clubb JS, FC Neale & S Posen: The behavior of infused human placental alkaline phosphatase in human subjects. J. Lab. Med. 1965, *66*, 443-507

Fischer Von B: Referenzbereiche von 18 blutwerten und die bedeutung einiger einflußgrößen auf Meßwerte bei weiblichen kaninchen. Z. Versuchstierk. 1980, *22*, 234-248.

Hall RL: Clinical pathology of laboratory animals. In: Animal models in toxicology (Shayne CG, Christopher PC eds). Marcel Dekker, Inc., New York 1992, pp 765-811.

Hewitt CD, DJ Innes, J Savory & MR Wills: Normal biochemical and hematological values in New Zeeland white rabbits. Clin. Chem. 1989, *35*, 1777-1779.

Jensen AL, E Wedo & M Bantz: Critical differences of clinical chemical components in blood from laboratory rabbits. Scand. J. Lab. Anim. Sci. 1992, *19*, 179-184.

Kriesten K, U Murawski & W Schmidtmann: Haematological values during normal reproduction of the maternal and fetal rabbit. Comp. Biochem. Physiol. 1987, *87a*, 479-485.

Nuwayhid B: Hemodynamic changes during pregnancy in the rabbit. Am. J. Obstet. Gynecol. 1979, *135*, 590-96.

Quig D & D Zilversmit: Parallel changes in plasma cholesterol and lipid transfer activity in pregnant rabbits. Proc. Soc. Exp. Biol. Med. 1986, *182*, 386-392.

Staiger D: Blood values of rabbits with and without noise stress at different stages of the reproductive cycle. Isr. J. Vet. Med. 1986, *42*, 333-336.

Viard-Drouet F, F Provot & P Coudert: Évolution de paramètres plasmatiques chez des lapines reproductrices en fonction de l'état physiologique et du rationnement alimentaire. Ann. Rech. Vét., 1984, *15*, 417-424.

Wolford ST, RA Schroer, FX Gohs, PP Gallo, M Brodeck, HB Falk & R Ruhren: Reference range data base for serum chemistry and hematology values in laboratory animals. J. Tox. Envir. Health. 1985, *18*, 161-188.