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Reference Values for Selected Blood Parameters in Rabbits: Effects of Age and Physiological Status

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ABSTRACT

Rabbit is widely used as an experimental animal model in infectious and non-infectious diseases. The haematologic data can be helpful in evaluating the health status of animals over time. The aim of this study was to determine the levels of red blood cells (RBC), white blood cells (WBC) and differential leukocyte counts in 5 nulliparous and 5 multiparous females, i.e. in young and older animals, at mating and at delivery. The values of RBC did not change with age, but WBC and lymphocytes decreased with age, a -33% and a -60% less in multiparous females than nulliparous ones. Monocytes count was double at delivery than at mating. In conclusion, aging on the immune system is manifested as reduction in production of mature lymphocytes and as a result, older females would not respond to immune challenge as robustly as the young ones. Physiological status is only related to production of monocytes.

Key words: Lymphocyte, Multiparous, Neutrophils, Nulliparous, Rabbits.

INTRODUCTION

Rabbits have significant economic importance, both in livestock and pet industry (DG Health and Food Safety, 2017). Moreover, this species is also used frequently as an animal model for studying infectious (Bansal *et al.,* 2014; Peng *et al.,* 2015) and non-infectious diseases (Anjum *et al.,* 2019; Fan *et al.,* 2014), due to several advantages such as easier housing and handling over larger species of animals. Blood parameters represent a great diagnostic tool for animal health status and disease evaluation (Barkakati *et al.,* 2015; Etim *et al.,* 2013; Kumar *et al.,* 2010). Hence, establishing their standard reference ranges become imperative for any disease diagnosis, prevention and controlling program as it forms the very basis for clinical interpretation of laboratory data (Moore *et al.,* 2015). It is well known in rabbits that various factors as breed (Abdel-Azeem *et al.,* 2010; Martinec *et al*., 2012), gender (Özkan *et al.,* 2012; Tokarz-Deptuła *et al.,* 2014), physiological condition (Archetti *et al., 2008*; ÇetÝn *et al*., 2009) and age (Jeklová *et al.,* 2009; Tůmová *et al.,* 2017) affect the cellular hemodynamic. However, the information is really scarce about differential leukocyte counts in adult animals, with more than one year old.

The aim of this study was to determine the levels of red blood cells (RBC), white blood cells (WBC) and differential leukocyte counts at mating and delivery in nulliparous and multiparous females, i.e. in young and older animals.

MATERIALS AND METHODS

Animals: Ten female rabbits from a synthetic line were used in this study, which were divided according to age in young (5 nulliparous females with no parturition) and older females (5 multiparous females with more than four parturitions, i.e., being one year old). This line was selected during twelve generations for decreasing litter size variability. The animals were housed individually in breeding cages (37.5 x 33 x 90 cm) at the farm of the Universidad Miguel Hernández of Elche (Spain). They were under a constant photoperiod of 16 h continuous light: 8h continuous darkness and controlled ventilation throughout the experiment. The animals were allowed to adapt to the feed and environment for a month before collection of the samples. The rabbits were given *ad libitum* commercial pelleted rabbit food (Cunilactal, Nutreco) and drinking water. Commercial pelleted food contained 16.5% CP, 15.8% fibre, 4% fat, 36% NDF, 18.5% ADF, 12% indigestible fibre and 2.400 kcal digestible energy. The female rabbits were first mated at 18 weeks of age and at 10 days after parturition thereafter. The animals were housed and handled with the agreement of the Miguel Hernández University of Elche Research Ethics Committee (Reference number 2019/VSC/PEA/0077) according to European Council Directives 98/58/EC and 2010/63/EU.

Blood sampling and analysis: Blood sampling in nulliparous and multiparous females was performed at 2 different time points, at mating and immediately after delivery. Blood samples were collected from the marginal ear vein to 2 ml tube of K3-EDTA (Deltalab, Madrid, Spain). The animals were restrained manually. Diurnal variations in haematological parameters were avoided by sampling the animals during the same time of the day (09:00-10:00). Blood

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samples were delivered to the laboratory within 0.5 h of collection and immediately analysed. The total RBC and WBC counts and the absolute number of each type of leukocytes were assessed using the Abacus Junior 5 hematology analyzer (*Diatron* Messtechnik, Budapest, Hungary).

Reproductive traits: At delivery, it was recorded the age of female (AF), the litter size at birth (LSB) and the kit's individual weight at birth (IWB) in both nulliparous and multiparous females.

Data analysis: The differences between nulliparous and multiparous females and between mating and delivery status for total RBC and WBC counts and the different populations of leukocytes were analysed using the following model:

$$y_{ijk} = \mu + T_i + PS_j + e_{ijk}$$

Where y_{ijk} is the observed trait, μ is the general mean, T_i is the effect of female's type (with two levels: nulliparous and multiparous female), PS_k is the effect of female's physiological status (with two levels: at mating and at delivery) and e_{ijk} is the residual term. The model for AF and LSB only included the effect of female's type.

The used model for IWB was:

$$y_{ijklm} = \mu + T_i + S_j + SEX_k + p_{ijkl} + e_{ijklm}$$

Where y_{ijklm} is the observed trait, μ is the general mean, T_i is the effect of female's type, S_j is the effect of suckling in the first 24 hours after delivery (with two levels: the kit sucked and no sucked), SEX_k is the effect of sex (with two levels: male and female), p_{ijkl} is the random effect of the female and e_{ijklm} is the residual term. IWB was also analysed with LSB as covariate. All statistical analyses were performed with SAS program (SAS Institute, 2019).

RESULTS AND DISCUSSION

Effect of age on reproductive traits: The minimum and maximum values, the least squared mean and its standard error of female's age (AF), litter size at birth (LSB), kit's individual weight at birth (IWB) and statistical comparisons between nulliparous and multiparous female rabbits are presented in Table 1. We found that the nulliparous female rabbits (young females) had their first delivery around 6 month of age and the multiparous female rabbits (older females) at more than one year old at their fifth delivery ($P < 0.001$). Besides, litter size was greater in young females

Table 1: Reference intervals, least square mean (LSM) and standard error (SE) for age of female (AF), litter size at birth (LSB) and individual weight of kits birth (IWB) in nulliparous and multiparous female rabbits.

	Nulliparous (n=5)[a]			Multiparous (n=5)[b]			
	N	LSM ± SE	Range	N	LSM ± SE	Range	Sig.[c]
AF, months	4	6.1 ± 0.2	5.9-6.8	4	16.1 ± 1.7	13.1-21.0	<0.001
LSB, kits	4	9.2 ± 0.9	9.0-10.0	4	7.5 ± 0.5	7.0-8.0	0.04
IWB, gr	37	50.3 ± 1.6	35.5-76.4	30	62.0 ± 1.7	38.9-80.9	<0.001

n: number of records. a: data in the first delivery. b: data in the fifth delivery. c: significance in difference between nulliparous and multiparous females.

Table 2: Reference intervals, least square mean (LSM) and standard error (SE) for red blood cells (RBC), white blood cells (WBC) and differential count in nulliparous and multiparous female rabbits.

	Nulliparous (n=10)		Multiparous (n=10)		
	LSM ± SE	Range	LSM ± SE	Range	Sig. [a]
RBC (10^{12}/l)	5.69 ± 0.24	4.78 - 6.53	5.32 ± 0.24	3.87 – 6.46	ns
WBC (10^9/l)	10.50 ± 1.09	5.92 - 16.61	7.01 ± 1.09	3.64 - 13.9	0.039
Lymphotyces	6.99 ± 0.74	3.37 -11.93	2.78 ± 0.74	1.44 – 5.54	0.001
Neutrophils	2.36 ± 0.64	0.64 – 5.90	3.41 ± 0.64	1.77 - 8.93	ns
Monophils	0.50 ± 0.06	0.09 - 0.94	0.38 ± 0.06	0.17 - 0.66	ns
Eosinophils	0.56 ± 0.09	0.23 - 1.19	0.37 ± 0.09	0.17 - 0.73	ns
Basophils	0.10 ± 0.02	0.01 - 0.25	0.08 ± 0.02	0.02 - 0.21	ns
LWR	0.67 ± 0.05	0.40 - 0.80	0.41 ± 0.05	0.24 - 0.65	0.001
NWR	0.22 ± 0.04	0.09 - 0.45	0.48 ± 0.05	0.42 - 0.66	0.001
MWR	0.05 ± 0.01	0.01 - 0.07	0.05 ± 0.01	0.03 - 0.08	ns
EWR	0.05 ± 0.01	0.02 - 0.10	0.05 ± 0.01	0.03 - 0.10	ns
BWR	0.01 ± 0.01	0.00 - 0.01	0.01 ± 0.01	0.00 - 0.01	ns
NLR	0.41 ± 0.21	0.11 - 0.28	1.36 ± 0.21	0.34 -2.64	0.006
MLR	0.08 ± 0.01	0.02 - 0.16	0.13 ± 0.01	0.08 – 0.19	0.009

n: number of records. LWR: lymphocytes to total white blood cell count ratio. NWR: neutrophils to total white blood cell count ratio. MWR: monocytes to total white blood cell count ratio. EWR: eosinophils and total white blood cell count ratio. BWR: basophils and total white blood cell count ratio. NLR: neutrophils to lymphocytes ratio. MLR: monocytes to lymphocytes ratio. a: significance in difference between nulliparous and multiparous females.

than older ones (+1.7 kits, $P < 0.05$). However, kits of young females weighed lesser at birth compared to that of those multiparous female rabbits (-11.7 g, $P < 0.001$). This difference disappeared when the litter size was included in the model as covariate (-0.5 g, $P > 0.10$). Therefore, larger litters in young female are related to a lower weight of their kits ($P < 0.001$).

In commercial rabbit breeding, females are normally inseminated at 10-12 days post-delivery; thus, in multiparous females, there is an overlap between lactation of their current litter and gestation of their future litter. The high body energy deficit in multiparous females and the negative interactions between lactation and fertility are well known (Fortun-Lamothe *et al.*, 1999; Theau-Clement and Roustan, 1992; Xiccato *et al.*, 2004), which would explain a lower litter size in multiparous females than nulliparous ones. In this sense, literature in rabbits shows that reproductive performance is age dependent and the litter size at birth is increased as parity increased reaching the peak at the 3rd parity (Das and Yadav, 2007; Xiccato *et al.*, 2004) then decreased gradually thereafter (Mahmoud, 2013). As weight of kits at birth in nulliparous females is concerned, it is well established that female's body growth has not finished during the first pregnancy because of the feed intake is lower than thereafter (Rommers *et al.*, 1999) and that a greater embryonic uterine overcrowding decreases available uterine surface area for the development of each placenta and nutrient supply to embryo (Argente *et al.*, 2006 and 2008), which would explain kits born from nulliparous females are lighter than those multiparous ones.

Effect of age on haematological parameters: Even though rabbit is widely used as experimental animal model for monitoring evolution in infectious and non-infectious diseases (Fan *et al.*, 2014; Peng *et al.*, 2015), there is scarce information about evolution in haematological reference values with age in this species. Using appropriate reference values are vital in these studies. Table 2 shows the minimum and maximum values, the least square mean and its standard error of red blood cells (RBC), white blood cells (WBC) and differential count for lymphocytes, neutrophils, monocytes, eosinophils and basophils both in nulliparous and multiparous female rabbits, i.e. young and older animals. Nulliparous and multiparous females had similar RBC count ($P > 0.10$). This finding showed that the values of RBC do not change with age and they are consistent with reference data of RBC in other populations (Hewitt *et al.*, 1989; Jain, 1993; Jeklová *et al.*, 2009). In contrast to our observation for RBC, WBC and lymphocytes decrease substantial with age (10.50 10^9/L in nulliparous female vs 7.01 10^9/L in multiparous females, $P \leq 0.05$, e.g. -33% for WBC; 6.99 10^9/L in nulliparous females vs 2.78 10^9/L in multiparous females, $P \leq 0.001$, e.g. -60% for lymphocytes). Accordingly, the percentage of lymphocytes to WBC count was lesser in multiparous females than nulliparous ones (-26% for LWR, $P \leq 0.001$) and the percentage of neutrophils to WBC ratio was higher in multiparous females than in nulliparous ones (+26% for NWR, $P \leq 0.001$). A reduction in number of lymphocytes may related to higher ratios of both neutrophils to lymphocytes (NLR) and monocytes to lymphocytes (MLR) in multiparous females than in nulliparous ones (+95% for NLR and +5% for MLR, $P \leq 0.01$). For the rest of haematological parameters, there were no differences between young and older females.

Table 3: Reference intervals, least square mean (LSM) and standard error (SE) for red blood cells (RBC), white blood cells (WBC) and differential count at mating and at delivery in female rabbits.

	Mating (n=10)		**Delivery (n=10)**		
	LSM ± SE	**Range**	**LSM ± SE**	**Range**	**Sig. [a]**
RBC (10^{12}/l)	5.34 ± 0.23	3.87 - 6.53	5.68 ± 0.25	4.78 – 6.46	ns
WBC (10^9/l)	8.05 ± 1.03	3.64 - 13.90	9.46 ± 1.16	5.53 - 16.61	ns
Lymphotyces	4.58 ± 0.70	1.44 - 9.73	5.19 ± 0.78	1.57 - 11.93	ns
Neutrophils	2.69 ± 0.61	0.64 - 8.93	3.07 ± 0.68	1.91 - 5.90	ns
Monophils	0.28 ± 0.06	0.09 - 0.65	0.59 ± 0.07	0.28 - 0.94	0.003
Eosinophils	0.43 ± 0.09	0.21 - 0.73	0.49 ± 0.10	0.17 - 1.19	ns
Basophils	0.08 ± 0.02	0.01 - 0.21	0.09 ± 0.02	0.02 - 0.25	ns
LWR	0.56 ± 0.04	0.24 - 0.80	0.51 ± 0.05	0.26 - 0.72	ns
NWR	0.33 ± 0.04	0.09 - 0.64	0.36 ± 0.05	0.15 - 0.66	ns
MWR	0.03 ± 0.01	0.01 - 0.06	0.06 ± 0.0	0.05 - 0.08	0.007
EWR	0.06 ± 0.01	0.03 - 0.10	0.05 ± 0.01	0.02 - 0.10	ns
BWR	0.01 ± 0.01	0.00 - 0.01	0.01 ± 0.01	0.00 - 0.01	ns
NLR	0.88 ± 0.20	0.11 - 2.64	0.89 ± 0.22	0.21 – 2.49	ns
MLR	0.08 ± 0.01	0.02 - 0.19	0.13 ± 0.01	0.08 -0.18	0.008

n: number of records. LWR: lymphocytes to total white blood cell count ratio. NWR: neutrophils to total white blood cell count ratio. MWR: monocytes to total white blood cell count ratio. EWR: eosinophils and total white blood cell count ratio. BWR: basophils and total white blood cell count ratio. NLR: neutrophils to lymphocytes ratio. MLR: monocytes to lymphocytes ratio. a: significance in difference between nulliparous and multiparous females.

This strong decrease in WBC and lymphocytes would be related to impairment of the adaptive immune function primarily because of the decline in production of naïve lymphocytes in the bone marrow and thymus as well as the expansion of incompetent memory lymphocytes (Weng, 2006). Monocyte to lymphocyte ratio (MLR) has been recently proposed to a simple biomarker of immune system; moderate and low values are related to a good combative power against disease in the host, by contrary high value is associated to impair immune system (Zelmer *et al.*, 2018). On the other hand, neutrophil to lymphocyte ratio (NLR) has been proposed as an indirect biomarker of stress (Jain, 1993). Stress stimulates release of glucocorticoids from the adrenal glands that induces neutrophilia or lymphopenia, or both, increasing NLR (Davis *et al.*, 2008). We stress that, in healthy rabbits, NLR increases from 1:2 at 2 months of age until 1:1 at 1 year of age (Washington and Van Hoosier, 2012), as a consequence of maturation of the hypothalamic-pituitary-adrenal axis over time (Hillmann *et al.*, 2008). Chronic stress is associated with immune dysfunction. In this regard, the highest values in NLR and MLR of multiparous females would agree with a higher adrenal activity and in consequence an increase in the susceptibility to infections in older animals.

Effect of physiological status on haematological parameters: Table 3 shows the minimum and maximum values, the least square mean and its standard error of red blood cells (RBC), white blood cells (WBC) and differential count for lymphocytes, neutrophils, monocytes, eosinophils and basophils at mating and at delivery. There was difference only in monocytes count. In this sense, monocytes count doubled at delivery compared to mating (0.28 10^9/L at mating vs. 0.59 10^9/L at delivery, $P \leq 0.01$). Accordingly, its percentages increased in WBC count (+3%, $P \leq 0.01$) and number of lymphocytes (+3% for MWR and +5% for MLR, $P \leq 0.01$).

CONCLUSION

Aging on the immune system is manifested as reduction in production of mature lymphocytes and as a result, older females would not respond to immune challenge as robustly as the young ones. Physiological status is only related to production of monocytes.

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Declaration of interest

The authors declare that they have no competing interests.

REFERENCES

Abdel-Azeem, A.S., Abdel-Azim, A.M., Darwish, A.A., Omar, E.M. (2010). Haematological and biochemical observations in four pure breeds of rabbits and their crosses under Egyptian environmental conditions. *World Rabbit Science*. **18**: 103-110.

Anjum, K.M., Rasool, F., Bhatti, E.M., Liaquat, S., Yousaf, M.Z., Zahid, S., Khan, N., Yameen, M. (2019). Comparative hypolipidemic efficacy of homeopathic mother tincture Allium Sativa Q, Curcuma Longa Q and statin in normal and cholesterol fed rabbits. *Indian Journal of Animal Research*. **53**: 1029-1032.

Archetti, I., Tittarelli, C., Cerioli, M., Brivio, R., Grilli, G., Lavazza A. (2008). Serum chemistry and hematology values in commercial rabbits: preliminary data from industrial farms in northern Italy. *Proceedings 9th World Rabbit Congress, Verona, Italy.* 1147-1151.

Argente, M.J., Santacreu, M.A., Climent, A., Blasco, A. (2006). Influence of available uterine space per fetus on fetal development and prenatal survival in rabbits selected for uterine capacity. *Livestock Science*. **102**:83-91.

Argente, M.J., Santacreu, M.A., Climent, A. and Blasco, A. (2008). Effects of intrauterine crowding on available uterine space per fetus in rabbits. *Livestock Science*. **114**: 211-219.

Bansal, K., Singh, C.K., Sandhu, B.S. Sood, N.K. and Dandale, M. (2014). Antemortem diagnosis of rabies from skin by TaqMan Real Time PCR. *Indian Journal of Animal Research*. **48**: 597-600.

Barkakati, J., Sarma, S. and Dhruba, D.J. (2015). Effect of foot and mouth disease on haematological and biochemical profile of cattle. *Indian Journal of Animal Research*. **49**: 713-716.

Çetİn, N., Bekyürek, T. and Çetİn, E. (2009). Effects of sex, pregnancy and season on some haematological and biochemical blood values in Angora rabbits. *Scandinavian Journal of Laboratory Animal Science*. **36**: 155-162.

Das, S.K. and Yadav, B.P.S. (2007). Effect of mating system, parity and breed on the reproductive performances of broiler rabbits under the agro-climatic condition of Meghalaya. *Livestock Research for Rural Development*. **19**: 25-29.

Davis, A., Maney, D., Maerz, J. (2008). The use of leukocyte profiles to measure stress in vertebrates: *A Review for Ecologists*. *Functional Ecologists*. **22**: 760-772.

DG Health and Food Safety. (2007). Overview report Commercial Rabbit Farming in the European Union.

Etim, N.N., Enyenihi, G.E., Williams, M.E., Udo, M.D., Offiong, E.E.A. (2013). Haematological Parameters: Indicators of the Physiological Status of Farm Animals. *British of Journal Science*. **10**: 33-45.

Fan, J., Kitajima, S., Watanabe, T., Xu, J., Zhang, J., Liu, E., Chen, Y. E. (2014). Rabbit models for the study of human atherosclerosis: from pathophysiological mechanisms to translational medicine. *Pharmacology & Therapeutics*. **146**: 104-19.

Fortun-Lamothe, L., Prunier, A., Bolet, G., Lebas, F. (1999). Physiological mechanisms involved in the effects of concurrent pregnancy and lactation on foetal growth and mortality in the rabbit. *Livestock Production Science*. **60**: 229-241.

Hewitt, C.D., Innes, D.J., Savory, J., Wills, M.R. (1989). Normal biochemical and hematological values in New Zealand white rabbits. *Clinical Chemistry*. **35**: 1777-1779.

Hillmann, E., Schrader, L., Mayer, C., Gygax, L. (2008). Effects of weight, temperature and behaviour on the circadian rhythm of salivary cortisol in growing pigs. *Animal*. **2**: 405-409.

Jain, N.C. (1993). Comparative hematologic features of some avian and mammalian species. In: Essentials of Veterinary Hematology. Jain, N.C. (Ed.), Lea and Febiger, Philadelphia, pp. 54-71.

Jeklová, E., Leva, L., Knotigová, P., Faldyna, M. (2009). Age-related changes in selected haematology parameters in rabbits. *Research Veterinary Science*. **86**: 525-528.

Kumar, M.C.A., Udupa, G., Kumar, S.P. (2010). Comparative study of haematological and biochemical parameters in healthy and ascariosis affected buffalo calves before and after treatment with levamisole. *Indian Journal of Animal Research*. **44**: 123-126.

Mahmoud, E.A.A. (2013). Studies on some factors affecting doe, buck and litter performance in White New Zealand rabbit under Egyptian condition. *Benha Veterinary Medical Journal*. **25**: 1-12.

Martinec, M., Härtlová, H., Chodová, D., Tůmová, E., Fučíková, A. (2012). Selected haematological and biochemical indicators in different breeds of rabbits. *Acta Veterinaria Brno*. **81**: 371-375.

Moore, D. M., Zimmerman, K., Smith, S.A. (2015). Hematological Assessment in Pet Rabbits: Blood Sample Collection and Blood Cell Identification. *Veterinary Clinics of North America: Exotic Animal Practice*. **18**: 9-19.

Özkan, C., Kaya, A., Akgúl, Y. (2012). Normal values of haematological and some biochemical parameters in serum and urine of New Zealand White rabbits. *World Rabbit Science*. **20:** 253-259.

Peng, X., Knouse, J. A., Hernon, K. M. (2015). Rabbit models for studying human infectious diseases. *Comparative Medicine*. **65**: 499-507.

Rommers, J.M., Kemp, B., Meijerhof, R., Noordhuizen, J.P.T.M. (1999). Rearing management of rabbit does. A review. *World Rabbit Science*. **7**: 125-138.

SAS Institute. (2019). SAS Version 9.4. SAS Institute Inc, Care, NC.

Theau-Clement, M. and Roustan, A. (1992). A study on relationships between receptivity and lactation in the doe and their influence on reproductive performances. *Journal of Applied Rabbit Research*. **15**: 412-421.

Tokarz-Deptuła, B., Niedźwiedzka-Rystwej, P., Adamiak, M., *et al* (2014). Values of white and red blood cell parameters in Polish mixed breed rabbits in the annual cycle. *Polish Journal of Veterinary Sciencies*. **17**:643-55.

Tůmová, E., Fučíková, A., Volek, Z., Vlčková, J. (2017). Changes of haematological and biochemical indices with age in rabbits with ad libitum and limited feed intake. *Acta Veterinaria Brno*. **86**: 29-35.

Washington, I. and Van Hoosier, G. (2012). Clinical biochemistry and hematology. In: The Laboratory Rabbit, Guinea Pig, Hamster and Other Rodents. Suckow M, Stevens K, Wilson R, eds. San Diego, CA. Academic Press Elsevier. pp. 59-116.

Weng, N. P. (2006). Aging of the immune system: how much can the adaptive immune system adapt?. *Immunity*. **24**: 495-499.

Xiccato, G., Trocino, A., Sartori, A., Queaque, P.I. (2004). Effect of parity order and litter weaning age on the performance and body energy balance of rabbit does. *Livestock Production Science*. **85**: 239-251.

Zelmer, A., Stockdale, L., Prabowo, S.A., Cia, F., Spink, N., Gibb, M., *et al* (2018). High monocyte to lymphocyte ratio is associated with impaired protection after subcutaneous administration of BCG in a mouse model of tuberculosis. *F1000Rearch*. **7**: 296-314.