رقم البحث (39)

COMPARATIVE KARYOLOGICAL STUDIES ON SOME RABBIT BREEDS

BY

Abd El-fattah A. H., El-Bayomi Kh. M. and Iman E. El-Araby

Animal Wealth Development Department, Faculty of Veterinary Medicine, Zagazig University, Egypt.

ABSTRACT

Cytogenetic studies were carried out on three breeds of rabbit, namely ***New Zealand white, Flanders (Flemish Giant), and Californian*** to compare Karyotyping and some chromosomal studies. The result of the present study revealed that the diploid number of the three rabbit breeds (New Zealand White, Californian and Flander) was 2n=44, and the karyotype of these breeds nearly the same. There were significant differences ($P \leq 0.05$) between chromosomes number 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 17, 19, X and Y. while no significant differences ($P \geq 0.05$) among chromosomes number 2, 13, 14, 15, 18, 20, and 21. These results concluded that cytogenetic studies could be used as tool to compare between rabbit breeds.

Key words, Chromosomes, Karyotype, Rabbit

INTRODUCTION

The domestic rabbit is characterized by small body size, early age of sexual maturity (4-5 months), high prolificacy, relatively short gestation period, short generation interval, high productive potential, rapid growth, good ability to utilize forages and fibrous plant materials and agricultural by-products, more efficient feed conversion, low cost per breeding female and by its profitability for small-scale system of production and in backyards ***(Finzi and Amici, 1991).***

The rabbit meat is nearly white, fine grained, palatable, mild flavored, nearly of the same nutritive value as beef meat and comparable to that of broiler chicken ***(Lukefahr et al., 1989),*** also rabbit meat shown to be very high in protein, low in fat, triglyceride and

cholesterol, low in energy value and have a mineral percentage higher than other meat. ***(Schlolaut, 1992***; ***Lebas et al., 1997***).

The karyotype as described by ***Battaglia, (1952)*** is the particular chromosome complement of an individual; it is determined by chromosome size, morphology and number in the somatic cells. Although, in all the somatic cells of all individuals of species, the numbers of chromosomes were constant, it may be used as an indicator of relatedness of species interrelationships within genera.

Analysis of chromosomes can be useful in breed's identification and also can be useful for addressing a variety of evolutionary relationships and genetic questions among species. Karyotype analysis help to predict with considerable certainly the fertility or sterility of hybrids by comparing the number and morphology of the chromosomes of parental species ***(Serebryakova, 1972)***.

Both wild and domestic rabbit belong to *Oryctolagus cuniculus*. Although rabbits provide value through meat and fur production, and they serve as an important biomedical animal model for research. Rabbits have been hampered because a lack of genomic resources. With the recent development of a rabbit linkage map containing cytogenetically anchored linkage data, large insert Bacterial Artificial Chromosomes (BAC) libraries, and some sequencing data, it is anticipated that genomic based studies using rabbits will increase over the next decade ***(Gaillard et al., 2009)***. This study was conducted to compare Karyotyping and some chromosomal studies between three rabbit breeds; ***New Zealand white rabbit, Californian rabbit and Flanders (Flemish Giant) rabbit.***

MATERIAL AND METHODS

Three breeds of rabbit, namely ***New Zealand white, Flanders (Flemish Giant), and Californian*** were used in this study. Twenty rabbits (ten male and ten female) were randomly chosen from each breed and taken from rabbit project of Animal Wealth Development Department, weight about (300-400gm) with age about (10-15 days). The chosen rabbits transferred to Genetics and Genetic Engineering Laboratory, to investigate the chromosome complement of each breed.

Karyotype preparations were obtained from the bone marrow of the colchicined animal according to ***Nichols et al., (1964)*** with some modifications. After these preparations,

conventional Giemsa-staining was carried out. From each specimen, 10 to 20 slides were prepared, and at least 20 well-spread metaphase plates were analysed. The previously stained slides were investigated by Leica microscope and the best metaphase chromosome figures were selected and photographed by Leica Monochrome Digital Camera for each rabbit breeds under study.

The photographed chromosomes were magnified and divided into four groups according to the location of their centromeres and then arranged according to size within each group by using Image Processing Analysis System (***Mona et al., 2009)***.

Statistical analysis:

The means of the total lengths of chromosomes (10 from each breed) in the three rabbit breeds were analyzed by ***SPSS (1995)***, to compare between them.

RESULT AND DISCUSSION

I. The Chromosome Complement of the Rabbit Breeds:

The karyotype of ***New Zealand white, Flanders (Flemish Giant), and Californian*** rabbit breeds were investigated. The model chromosome number of each species was determined from a preliminary study of the most frequent diploid number observed in the metaphase spreads analyzed.

I.A. Chromosome Complement of New Zealand White rabbit:

In this study the diploid chromosome number of ***New Zealand White rabbit*** was 2n=44, (Figure, 1). All chromosomes have been arranged in homologous pairs, descendingly according to size from 1 to 21 (Figure, 2) then in to four groups metacentric, submetacentric, subtelocentric, and telocentric (Figure, 3).

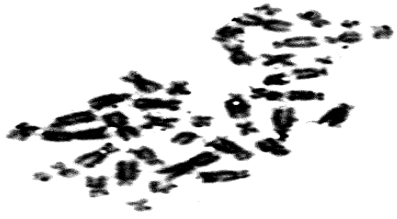

Figure (1): The Mitotic Metaphase Chromosomes Obtained from Bone Marrow of Male New Zealand White Rabbit. 2n=44, XY.

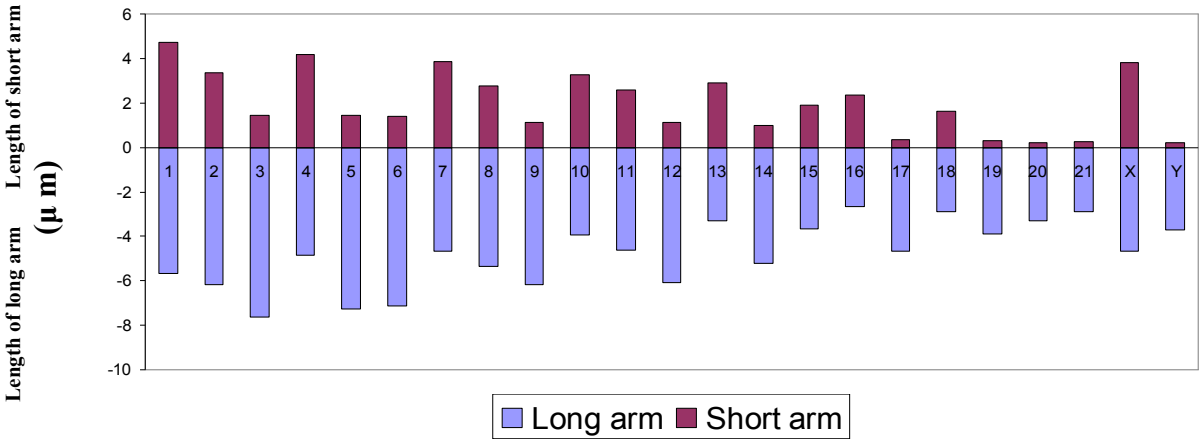

Figure (2): The Histogram of the New Zealand White Rabbit Chromosomes.

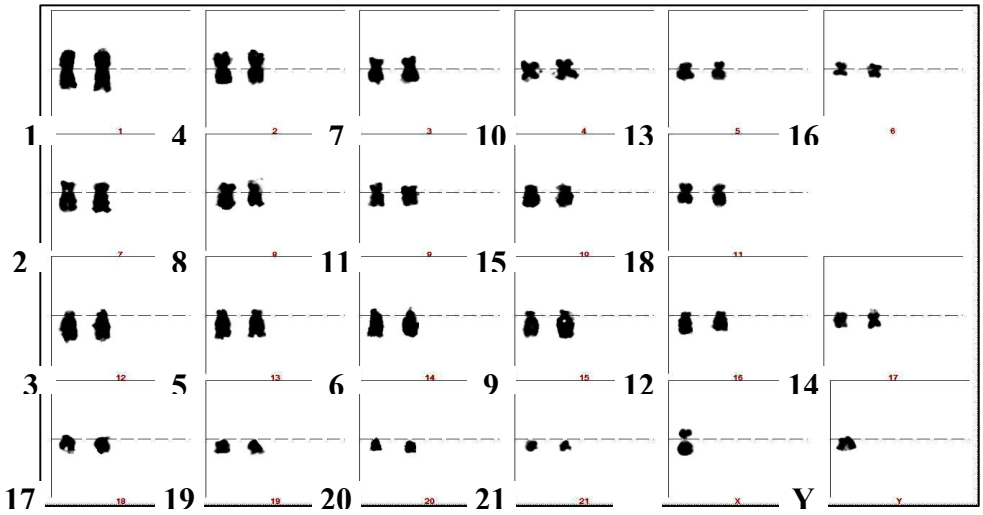

Figure (3): Chromosomal Analysis of Male New Zealand White Rabbit (Giemsa stain, vedio test-karyo program).2n = 44, XY. Metacentric group (m), 6 pairs (1, 4, 7, 10, 13, and 16), submetacentric group (sm), 5 pairs (2, 8, 11, 15, and 18), subtelocentric group (st), 6 pairs (3, 5, 6, 9, 12, and 14), and telocentric group (t), 4 pairs (17, 19, 20, and 21). X (m) and Y (t).

The arrangement of the autosomes in falling size from (1 – 21) agree with ***Melander, (1965).*** In this study the X chromosome of ***New Zealand White rabbit*** was metacentric and this agree with ***Ray and Williams, (1966)*** but disagree with ***Paul et al., (2004)*** as described the X- chromosome as third largest submetacentric. On the other hand, the Y-chromosome in our study was telocentric that disagree with ***Paul et al., (2004)*** as described it as smallest submetacentric. Also disagree with ***Ray and Williams, (1966)*** where Y- chromosome was subtelocentric**.**

I.B. Chromosome Complement of Californian Rabbit:

In this study the diploid chromosome number of ***Californian was*** 2n=44, (Figure, 4). All chromosomes have been arranged in homologous pairs, descendingly according to size from 1 to 21 (Figure, 5) then in to four groups metacentric, submetacentric, subtelocentric, and telocentric (Figure, 6).

Figure (4): The Mitotic Metaphase Chromosomes Obtained from Bone Marrow of Male Californian Rabbit. 2n=44, XY.

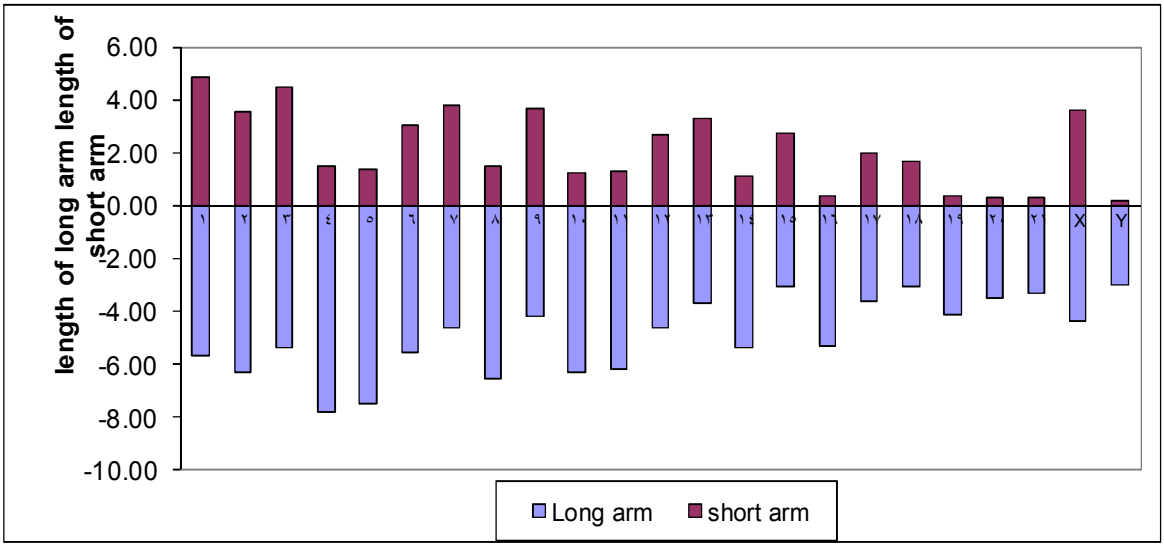

Figure (5): The Histogram of the Californian Rabbit Chromosomes.

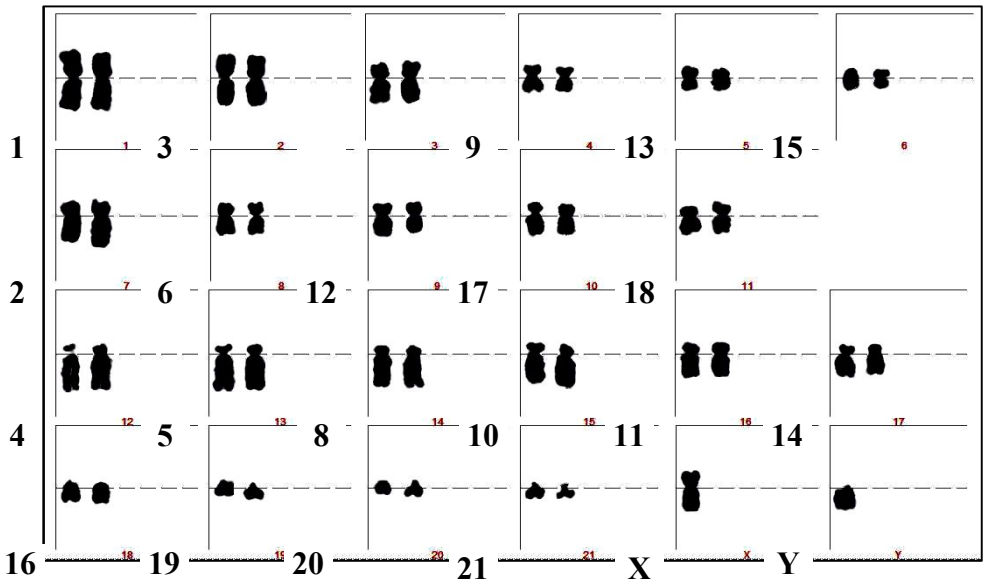

Figure (6): Chromosomal Analysis of male Californian Rabbit (Giemsa stain, vedio test-karyo program).2n = 44, XY. Metacentric group (m), 6 pairs (1, 3, 7, 9, 13, and 15), submetacentric group (sm), 5 pairs (2, 6, 12, 17, and 18), subtelocentric group (st), 6 pairs (4, 5, 8, 10, 11, and 14), and telocentric group (t), 4 pairs (16, 19, 20, and 21). X (m), and Y (t).

This result agree with ***Ortaya and Agulogyu, (1990)*** which described the Y-chromosome as acrocentric but disagree with them in describing the X- chromosome as they described it as submedian but in this study was metacentric.

I.C. Chromosome Complement of Flander (Flemish Giant) rabbit:

In this study the diploid chromosome number of **Flander (Flemish Giant) rabbit** was 2n=44, (Figure, 7). All chromosomes have been arranged in homologous pairs, descendingly according to size from 1 to 21 (Figure, 8) then in to four groups metacentric, submetacentric, subtelocentric, and telocentric (Figure, 9).

Figure (7): The Mitotic Metaphase Chromosomes Obtained From Bone Marrow of Male Flander Rabbit (Giemsa stain, lieca monochrome microscope, X=100). 2n=44, XY.

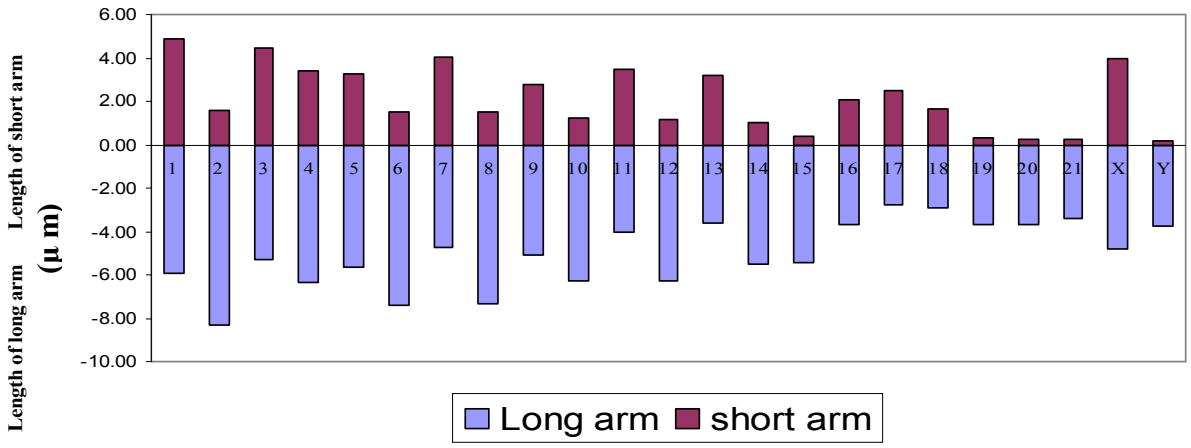

Figure (8): The Histogram of the Flander (Flemish Giant) Rabbit Chromosomes.

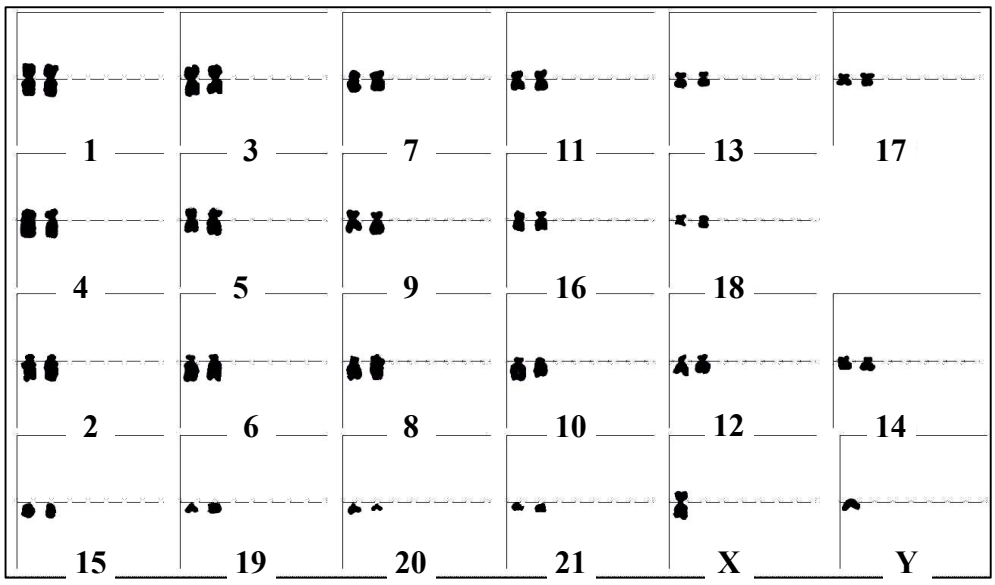

Figure (9): Chromosomal Analysis of Male Flander Rabbit (Giemsa stain, vedio test-karyo program). 2n = 44, XY. Metacentric group (m), 6 pairs (1, 3, 7, 11, 13, and 17), submetacentric group (sm), 5 pairs (4, 5, 9, 16, and 18), subtelocentric group (st), 6 pairs (2, 6, 8, 10, 12, and 14), and telocentric group (t), 4 pairs (15, 19, 20, and 21). X (m), and Y (t).

This result disagrees with (***Yerle et al., 1987; Poulsen et al., 1988)***, as describe X-chromosome as one of the larger submetacentric chromosomes and the Y- chromosome as one of the smaller submetacentric chromosomes.

Finally the comparison of the important karyotype data of the Rabbit Breed (New Zealand White, Californian and Flander) was summarized in Table (1) and Figures (10, 11).

Table (1): Comparison of the Important Karyotype Data of the Rabbit Breed.

Breeds	2n	Metacentric group	Submetacentric group	Subtelocentric group	Telocentric group	Total haploid length
N	**44**	1, 4, 7, 10, 13, 16.	2, 8, 11, 15, 18.	3, 5, 6, 9, 12, 14.	17, 19, 20, 21.	156.61μm
C	**44**	1, 3, 7, 9, 13, 15.	2, 6, 12, 17, 18	4, 5, 8, 10, 11, 14.	16, 19, 20, 21.	162.18 μm
F	**44**	1, 3, 7, 11, 13, 17.	4, 5, 9, 16, 18.	2, 6, 8, 10, 12, 14	15, 19, 20, 21.	165.06 μm

N= New Zealand White, C= Californian breed, and F = Flander.

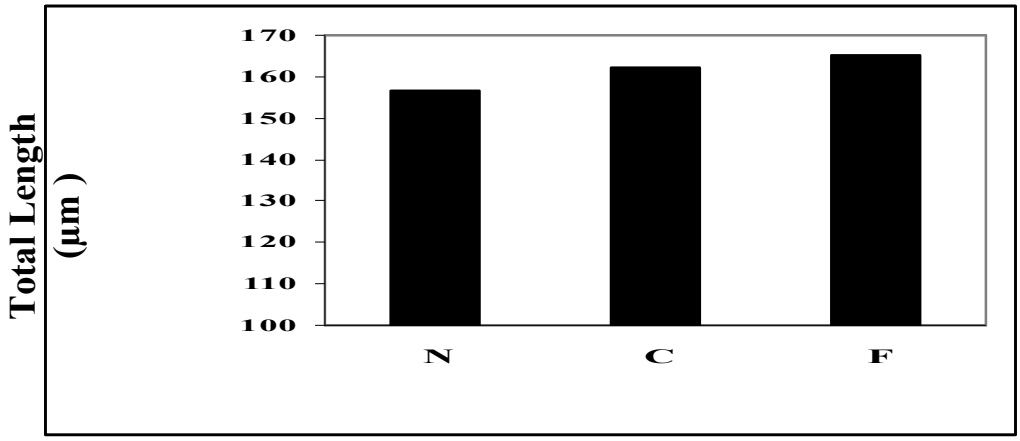

Figure (10): Comparison of the Total Haploid Set of the Chromosomes of the Rabbit Breeds. N= New Zealand White, C= Californian breed, and F = Flander.

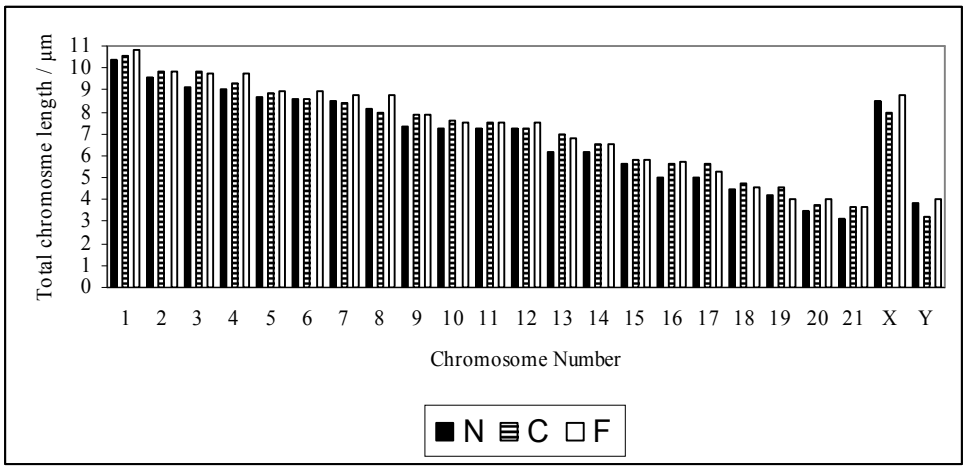

Figure (11): Comparative Histogram for Total Length (µm) for The Studied Rabbit Breeds. N=New Zealand White, C=Californian, and F= Flander rabbit breeds.

II. Statistical Analysis of the Total Chromosomal Length in the Rabbit Breeds:

In general, there were significant differences ($P \leq 0.05$) between chromosomes number 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 17, 19, X and Y. while no significant differences ($P \geq 0.05$) among chromosomes number 2, 13, 14, 15, 18, 20, and 21, Table (2).

The correlation between the changes in both chromosomal number and morphology has been used to support the idea that the karyotype evolution and the creation of new species are mostly due to chromosomal rearrangements (**Denton, 1973**). The change in chromosome number and / or morphology for the same species may be also due to the geographical locality and the prevailing environmental conditions (***Kirpichnikov*, 1981**).

***Arai*, (1982); *Ihssen et al.*, (1981) and *Kornfield*, (1984)** have cautioned about comparing the karyotyping morphologies driver from a number of different sources. This is due to the differences in chromosomal fixation and preparation techniques which can cause differential contraction of chromosomes also can change the relative number of chromosome types.

Table (2): The Total Chromosome Lengths in Californian, New Zealand White and Flander Breeds.

Breeds	N	Mean± S.E							
		Ch.1	Ch.2	Ch.3	Ch.4	Ch.5	Ch.6	Ch.7	Ch.8
C	10	10.56 ± 0.09^{a}	9.64 ± 0.08^{a}	9.30 ± 0.11^{b}	9.13 ± 0.06^{b}	8.79 ± 0.08^{ab}	8.59 ± 0.10^{ab}	8.38 ± 0.08^{b}	8.07 ± 0.08^{b}
N	10	10.19 ± 0.10^{b}	9.60 ± 0.05^{a}	9.23 ± 0.08^{b}	9.09 ± 0.08^{b}	8.58 ± 0.14^{b}	8.52 ± 0.11^{b}	8.44 ± 0.05^{b}	8.03 ± 0.07^{b}
F	10	10.54 ± 0.07^{a}	9.76 ± 0.08^{a}	9.58 ± 0.07^{a}	9.60 ± 0.11^{a}	9.09 ± 0.13^{a}	8.88 ± 0.13^{a}	8.67 ± 0.07^{a}	8.38 ± 0.10^{a}

Breeds	N	Mean± S.E							
		Ch.9	Ch.10	Ch.11	Ch.12	Ch.13	Ch.14	Ch.15	Ch.16
C	10	7.82 ± 0.06^{a}	7.63 ± 0.04^{a}	7.42 ± 0.05^{a}	7.19 ± 0.05^{b}	6.73 ± 0.08^{a}	6.23 ± 0.08^{a}	5.71 ± 0.04^{a}	5.46 ± 0.10^{a}
N	10	7.32 ± 0.03^{b}	7.26 ± 0.06^{b}	7.23 ± 0.04^{b}	7.15 ± 0.05^{b}	6.51 ± 0.10^{a}	6.20 ± 0.04^{a}	5.62 ± 0.05^{a}	5.13 ± 0.06^{b}
F	10	7.86 ± 0.05^{a}	7.64 ± 0.11^{a}	7.46 ± 0.03^{a}	7.34 ± 0.05^{a}	6.62 ± 0.05^{a}	6.28 ± 0.10^{a}	5.76 ± 0.06^{a}	5.40 ± 0.10^{a}

Breeds	N	Mean± S.E						
		Ch.17	Ch.18	Ch.19	Ch.20	Ch.21	X	Y
C	10	5.32 ± 0.09^{a}	4.71 ± 0.06^{a}	4.27 ± 0.09^{a}	3.84 ± 0.04^{a}	3.42 ± 0.09^{a}	8.49 ± 0.15^{ab}	3.16 ± 0.05^{b}
N	10	5.02 ± 0.05^{b}	4.54 ± 0.06^{a}	4.22± 0.05^{a}	3.67 ± 0.07^{a}	3.35 ± 0.07^{a}	8.35 ± 0.07^{b}	3.89 ± 0.04^{a}
F	10	5.20 ± 0.06^{ab}	4.26 ± 0.31^{a}	3.92 ± 0.05^{b}	3.56 ± 0.15^{a}	3.39 ± 0.16^{a}	8.75 ± 0.04^{a}	3.95 ± 0

Means carrying different superscripts are significant at ($P \leq 0.05$) otherwise they do not. C= Californian breed, N= New Zealand White, and F = Flander. Ch = chromosome.

REFERENCES

Aria, R. (1982): a chromosome study of two cyprinid fishes, Acrossochelus Labitus and Pseudorasbora. Pumila pumila, with notes on Eurasian cyprinids and their karyotypes. Bull. Nat. Sc. Mus. (Tokyo) 8:131-152.

Battaglia, F.R. (1952): *Appaiamento crmosomico primario, appaiamento crmosomico secondario nella. Attidella Soc. Toscana 59:166.*

Denton T. E. (1973): Evolution of the fish karyotype**.** *In* Fish chromosome methodology. Illinois: springfield; 129-148.

Finzi, A. and Amici, A. (1991): Traditional and alternative rabbit breeding systems for developing countries. Rivista di Agricultura Subtropicale Tropicale, 6 (1): 103-125.

Gaillard, R. C.; Ferrand, N; and Hayes, H. (2009): Genome Mapping and Genomics in Animals. 3:175-176.

Ihssen, P. E.; Booke, H. E.; Casselman, J. M.; McCilade, J. M.; Payne, N. I.; and Utter, R. M. (1981): Stock identification material and methods. Can. J. Fish. Aquatic Sci., 38:1838-1855.

Kirpichnikov, R. S. (1981): Genetic basis of fish selection. Springer, Berlin – Hedilberg – New York. pp. 410.

Kornfield, I. L. (1984). Descriptive Genetics of Cichlid fishes. In: Evolutionary Genetics of Fishes, Turner, B.J. (Ed.), Plenum Press, New York. p. 591-616.

Lebas, F.; coudert, P.; Thebault, R. G.; and De Rochambeau, H. (1997): The rabbit. Third edition .chapter 1, pages 13-14.

Lukefahr, S. W. D.; Hohenbaken, P. R.; and potton, N. M. (1989): Genetic effects on maternal performance and litter pre-weaning and pos-weaning traits in rabbit. Anim.Prod.38:293-300.

Melander, Y. (1956): The chromosome complement of rabbit. Hereditas 42:432-435.

Mona, H. A.; Elrabey, H.; Khalaf, R. M.; El- Hadary, M. H. and El-halafawy , K. (2009) : Cytological and Molecular Charactrization of some wheat (Triticum aestivum L.) Cultivars. Egypt. J. Genet. Cytol., 38: 235-250.

Nichols W. W.; Leven, A.; Melander, E.; and Mic, Y. (1964): The Idiogram of the Rabbit. Hereditas 53- 6.

Ortaya, C. and Agulogyu, S. (1990): Karyotype of rabbit (Oryctolagus cuniculus) chromosomes from leucocyte cultures. Doga, Türk Biyoloji Dergisi 14(3):173-179.

Paul, M. P.; Nehete, S. B.; Pawar, V. D.; Bangar, M. G.; Pingle, M. S.; **and** ***Umrikar, U. D. (2004):*** Chromosome analysis of domestic rabbit. Journal of Bombay Veterinary College, 12(1/2):28-29.

Poulsen, B. S.; Shibasaki, Y.; and Ronne, M. (1988): The high-resolution R-banded karyotype of *Oryctolagus cuniculus L*. Hereditas, 109:57–60.

Ray, M. and Williams, T. W. (1966): Karyotype of rabbit chromosomes from leukocyte cultures. Canadian Journal of Genetics and Cytology, 8: (3) 393-397.

Schlolaut, W. (1992): Management in rabbit production-graduator for transfer of knowledge into production level. Fifth Congress of the World Rabbit Science Association. July 25-30, 1992. Oregon, U.S.A. Vol. A, pp 594-614.

Serebryakova, E. V. (1972): Some data on the chromosome complexes in Acipenseridae. In Genetic selection and hybridization (ed. B. I. Cherfas). Israel program for Scientific Translation, Jarusalem (translated from Russian), 98-106.

SPSS (1995): **SPSS user's guide. SPSS Inc., USA.**

Yerle, M.; Echard, G.; and Gillois, M. (1987): The high-resolution GTG- banding pattern of rabbit chromosomes. Cytogenet Cell Genet 45:5–9.

الملخص العربي

دراسات مقارنة للهيئة الكروموسوميه على بعض سلالات الارانب

أجريت دراسات سيتوجينيه على ثلاث سلالات من الأرانب، وهي نيوزيلندا الأبيض، فلاندرز(الفلمنكي العملاق)، وكاليفورنيا لمقارنة الخريطة الكروموسومية وبعض الدراسات الكروموسومية .كشفت نتيجة هذه الدراسة أن العدد الزوجي للكروموسومات في سلالات الأرانب الثلاثة (نيوزيلندا الأبيض، كاليفورنيا وفلاندر) كان2N = 44 ، و تقريبا نفس الخريطة الكروموسومية لهذه السلالات .كانت هناك فروق ذات دلالة إحصائية ($P \leq 0.05$)بين الكروموسومات رقم1 ،3 ،4 ،5 ،6 ،7 ،8 ،9 ،10 ،11 ،12 ،16 ،17 ،19 ، X و Y. بينما لا توجد فروق معنوية ($P \geq 0.05$) بين الكروموسومات2 ،13 ،14 ،15 ،18 ،20 ، و ٢١. خلصت هذه النتائج بأن الدراسات الوراثية الخلوية يمكن أن تستخدم كأداة للمقارنة بين سلالات الأرانب.