



**IMPACT OF MATERNAL IMMUNIZATION DURING THE PERIOD OF COLLECTING EGGS FOR HATCHING ON MATERNAL IMMUNITY TRANSMITTED TO THEIR CHICKS.**

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**ABSTRACT:** A current study objective was to investigate the effect of maternal immunization during the period of collecting eggs for hatching on maternal immunity transmitted to their chicks at two weeks of age. The total number of chicks was 180 used from thirty-two hens. Hens were divided into three segments according to antibody titer of SRBCs (high, medium, and low) segments. The resulting fertilized eggs were divided into two groups (Group<sub>1</sub> and Group<sub>2</sub>) based on the period of egg collection after maternal SRBCs antigen injection. The obtained results were:

1.The transfer percentages for the three segments (high, medium, and low) were 2.88, 5.56, and 60.74%, respectively. Here we notice a clear inverse relationship between the level of maternal antibodies and the remaining percentage of immunity transmitted to the chicks at two weeks of age. Where, the transfer percentages for two separate groups (group<sub>1</sub> and group<sub>2</sub>) were 1.48, and 12.65%, respectively. This means that the second group is higher than the first group in the transfer percentage of remaining maternal antibodies at two weeks old chicks.

2.There were significant differences between dams' antibody titers to SRBCs antigen means, whether in the three segments (high, medium, and low) and, between the two groups.

3.There was a significant interaction between both segments (high, medium, and low) and groups (group<sub>1</sub> and group<sub>2</sub>). In antibody titers to SRBCs antigen means of dams and antibody titers to SRBCs antigen transformed means in two-week chicks old of dams for two-week-old chicks, but there were no significant differences between segments (high, medium, and low) in antibody titers to SRBCs antigen transformed means in two-week chicks.

**Keywords:** maternal immunity, chicks, Sheep Red Blood Cells, Antigen.

## INTRODUCTION

Maternal antibodies, passed from hens to their offspring, play a crucial role in avian disease prevention. Understanding this transmission is vital for improving chick health and overall flock resilience in the poultry industry (Hasselquist and Nilsson, 2009). Maternal antibodies serve as a critical first line of defense, safeguarding chicks from specific pathogens during their vulnerable early life stages (Heller et al., 1990; Mondal and Naqi, 2001; Ahmad and Akhter, 2003). Newly hatched chicks have an immature immune system (Bar-Shira et al., 2003). Passive immunity is important in early protection against pathogenic organisms (Kramer and Cho, 1970). Hen's transfer of antibodies against certain antigens plays a significant role in protecting its chicks against these antigens before the development of chicks' active immunity (Heller et al., 1990; Mondal and Naqi, 2001; Ahmad and Akhter, 2003). Gong et al., 2023 explained that maternal immune effects positively influence chicks' disease resistance beginning in the embryonic stage in birds.

The level of maternal-derived antibodies peaks at 72 to 96 hours post-hatch, and then decreases gradually to undetectable levels at 14 to 21 d. of age (Hamal et al., 2006). This makes birds vulnerable to infectious diseases, especially during the second-week post-hatch when the protection of passive immunity from hens has declined, and the immune system of chicks is not developed completely to respond properly to an early challenge. Antibodies transferred by the maternal against certain antigens play a significant role in protecting chicks against these pathogens before the maturation of their immune system (Ahmad and Akhter, 2003). Therefore, the present study objectives were to investigate the effect of maternal Immunization during the period of collecting eggs for hatching on maternal immunity transmitted to their chicks at two weeks of age.

## MATERIALS AND METHODS

### Experimental design:

The birds of this study were reviewed and approved by the Animal Welfare Committee of Menoufia University -Faculty of Agriculture- Shibin El-Kom. The study took place on a private farm in Menoufia Governorate for three months (from April and June 2019). The total number of chicks was 180 from 32 Norfa strain pullets. In that time pullets were 40 weeks old. Norfa is a synthetic local strain of chicken that is the product of a joint research and training project between the Department of Animal Science, Agricultural University of NORAD, and the Faculty of Agriculture, Menoufia University, Egypt (Kolstad and Abdou, 1999; Abdou et al., 2017; Abou-Elewa, 2024). Pullets were divided into three segments according to the ( $\bar{X} \pm S.D$ ) of antibody titer of SRBCs as follows:

- High: Includes hens with antibody titer greater than or equal ( $\bar{X} + 0.5 S. D$ ).
- Low: Includes hens with antibody titer less than or equal ( $\bar{X} - 0.5 S. D$ ).
- Medium: Includes hens with antibody titer equal ( $\bar{X}$ ) or at least it was not differed from the ( $\bar{X}$ ) significantly.

The resulting fertilized eggs were divided into two groups:

- Group<sub>1</sub>: Eggs were collected after dams were injected with SRBC antigen from the first day after injection to the fourth day.
- Group<sub>2</sub>: Eggs were collected after dams were injected from the fifth day after injection to the tenth day of injection.

### Immunization, collecting blood samples, and antibody titer determination:

Sheep blood was obtained in heparin solution from Ossimi sheep breed (15cm.) and were centrifuged and washed three times in Phosphate buffer saline (PBS., PH 7.2). After final wash, packed SRBCs were brought to

0.25 % vo/vo solution in PBS. and used for immunization (Kundu et al., 1999).

Every hen was injected intravenously with 0.1 ml of 0.25% SRBC suspension after three days from collecting eggs for hatching at seven days post-immunization hens blood samples were collected from the wing vein and immune sera were collected. Blood samples of pedigreed hatched chicks were collected from the wing vein and immune sera were collected at two weeks of age. Antibody titer was determined by the microtiter hemagglutination test assay described by (Siegel and Gross, 1980 ; Van der Zijpp and Leenstra 1980). Serum samples were incubated in incubator for half an hour at 56°C. Serum samples were individually titerated in 96-well plates. 25 ul of (0.9% Nacl saline) was added to all 96-well plates followed with 25 ul. of serum sample to first well where ,every row was allocated to an individual sample. Then serial dilutions were made from the first through the eleventh well. The twelfth well was used as control well. Next, 25ul of 0.25 % packed SRBC solution was added to each well. Then the 96-well plates were covered and shacked. After that, it was incubated at 37°C for one hour. Antibody titers were expressed as  $\log_2$  of the reciprocal of the last serum dilution ,in which there was positive heamagglination as shown in the folloing .

**Statistical analysis:**

Data were computerized and analyzed according to the following model by the SPSS Program (2004).

$$Y_{ijk} = \mu + S_i + G_j + (S \times G)_{ij} + e_{ijk}$$

Where:

$Y_{ijk}$  = The observation of the  $ij^{\text{th}}$

$\mu$  = The common (Overall) mean.

$S_i$  = Effect of the  $I^{\text{th}}$  segments.

$G_j$  = Effect of the  $J^{\text{th}}$  groups.

$(S \times G)_{ij}$  = Effect of interaction between segments and groups.

$e_{ijk}$  = Experimental error.

**RESULTS**

Results presented in Table (1) showed antibody titers to SRBCs antigen means in different three segments of dams (high, medium, and low) were 28.90, 11.33, and 1.35, respectively. Maternal antibody titers to SRBCs antigen transformed means at two weeks old for chicks of three different segments dams (high, medium, and low) were 0.83, 0.63, and 0.82, respectively.

Table (1) showed dam antibody titers to SRBCs antigen means and maternal antibody titers to SRBCs antigen transformed means at two weeks old for chicks of three in different two groups (group<sub>1</sub> and group<sub>2</sub>) were 15.59, 9.33, and 0.23, 1.18, respectively.

The remaining percentage of transferred maternal antibodies for two-week-old chicks was more important data presented in Table (1). It indicates the continuity of passive immunity from pullets to their chicks. Table (1) shows that the transfer percentages for three different segments (high, medium, and low) were 2.88, 5.56, and 60.74, respectively. Here we notice a clear inverse relationship between the level of maternal antibodies and the remaining percentage of immunity transmitted to the chicks at two weeks of age. The transfer percentages for two different groups (group<sub>1</sub> and group<sub>2</sub>) were 1.48, and 12.65, respectively. This means that the second group is higher than the first group in the transfer percentage of remaining maternal antibodies at two weeks old chicks.

Table (2) shows the interaction effect of segments and groups on antibody titers to SRBCs antigen of (dams and progeny) and their effects on the remaining percentage of transferred maternal antibodies for two-week-old chicks. Observed through the results displayed in Table (2) it was found that there were clear significant differences between dams' antibody titers to SRBCs antigen means, whether in the three segments (high, medium, and low) or the two groups (group<sub>1</sub> and group<sub>2</sub>). It is also noted that there is

significant interaction between both segments (high, medium, and low) and groups (group<sub>1</sub> and group<sub>2</sub>) in antibody titers to SRBCs antigen means of dams and antibody titers to SRBCs antigen transformed means in two-weeks chicks old of dams for two-week-old chicks. But no significant differences between segments (high, medium, and low) in antibody titers to SRBCs antigen transformed means in two-week chicks.

### **DISCUSSION**

The humoral immune response is a trait with a low heritability value as it is influenced by environmental factors therefore, the mean of humoral immune response in this study agrees with many studies and may differ from others, depending on the circumstances of each experiment. But means of the recent study are considered in the rolling range in previous studies (Pinard et al., 1992; Abou-Elewa, 2004; Abou-Elewa, 2010; Gebriel et al., 2021).

Heller et al. (1990) observed that the level of maternal antibodies transferred to chicks was influenced by the days after immunization of hens, genetic line, of SRBC antigen. This is consistent with the current study, where the significant differences between the two groups and the two lines of dams and chicks.

Yang et al. (1999) reported that maternal antibodies transferred to its chicks decrease over time after hatching. The antibody levels of chicks with a high antibody level decrease faster with time after hatching than chicks with a low antibody level. Also, Yang et al. (1999) found that the means of antibody titer transmitted to chicks of two lines of hens (high and low lines) at one day old were 5.5 and 3.2, respectively. While the means of antibody titer transmitted to chicks of two lines of hens (high and low lines) at 10 d. old were 3.2 and 2, respectively. Also, Ahmed (2011) reported there is positive correlated relationship between the level of antibodies in the chicks and the level of maternal antibodies, and the level of antibodies

transferred to the chicks' decreases over time after hatching. The antibody levels of chicks with a high antibody level decrease faster with time after hatching than chicks with a low antibody level. Ahmed (2011) found that means of antibody titer transmitted to chicks of three lines of hens (high, control, and low lines) at 6 d. old were 3, 1.64 and 1.5, respectively. While the means of antibody titer transmitted to chicks of three lines of hens (high, control and low lines) at 12 d. old were 1.21, 1, and 1.09, respectively. This supports what was previously concluded in the current study.

According to the results shown in Tables (1 and 2) and as stated in the previously mentioned studies (Yang et al., 1999; Ahmed, 2011). It is clear that the average of the low line is close to the high line in value of this may be explained by the fact that the immune system of chicks with high inherited immunity develops faster than those with low inherited immunity, which makes reliance on maternal immunity less than of chicks with low inherited immunity. Where the immune system develops more slowly, which makes reliance on maternal immunity greater.

As for the remaining percentages of antibody titers in chicks through research into previous studies, it was found that most of the calculated percentages of antibodies transmitted from dams to their chicks during the first week of age. For example, previous work by Hamal et al. (2006) showed that IgY, total or antigen-specific, in the hens' plasma or eggs was found to be a direct indicator of maternal antibody transfer to the chicks' circulation, with an expected approximate percentage transfer of 30%. They also reported discrepancies in meat lines of chickens concerning anti-Newcastle disease virus (NDV) and anti-infectious bronchitis virus (IBV) antibody levels in hens' plasma, egg yolks, and chicks' plasma. The transfer percentages were 79.2 and 100 % for infectious bursal disease (GD) and Newcastle

**maternal immunity, chicks, Sheep Red Blood Cells, Antigen.**

disease virus, respectively (Demissie et al., 2022).

In previous studies, (Fahey et al., 1987) the IBDV antibody ranged from 30 to 53% and its transfer rate was 45% in native Egyptian chickens. Abdel-Moneim and Abdel-Gawad (2006) reported that ratios of the inherited antibody level differences from hen to newly hatched chicks were found among different lines of chickens. Breed differences were found in regressions of levels of IBDV

antibody in yolk to that of hen or their chicks' sera were also found, so prediction of serum titer of chicks and/ or hens from yolk are varied among chicken lines. Also, Abdel-Moneim and Abdel-Gawad, 2006, showed that antibodies of the Newcastle disease virus had a transfer rate of about 79.2%. Whereas Hamal et al., 2006, reported that, the transfer rate of the Newcastle disease virus antibodies was 35.5 to 40.7% in two different lines of chickens.

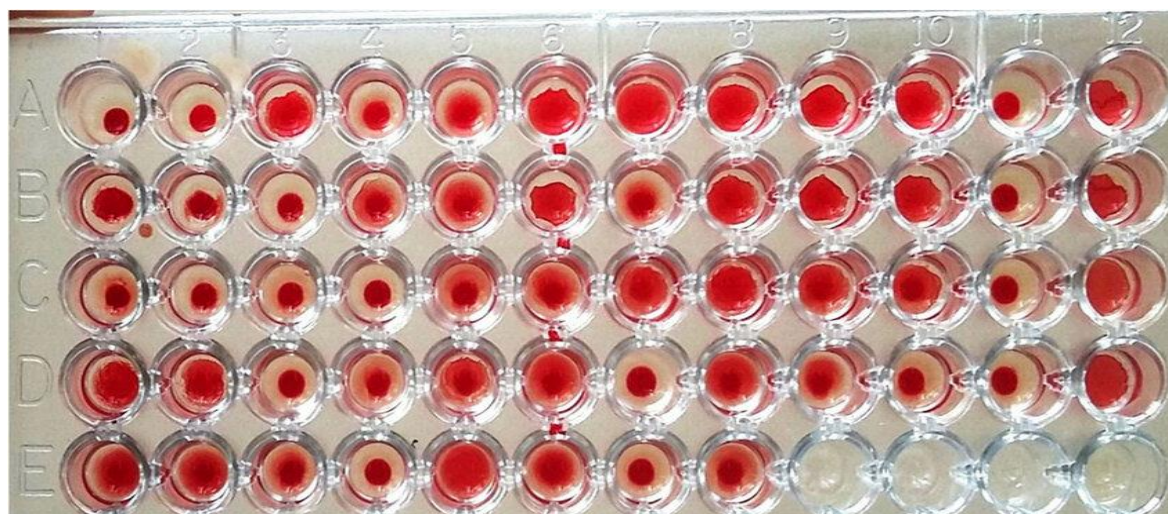
**Table (1):** Dams antibody titers to SRBCs antigen means ( $\bar{X} \pm S.E$ ) and maternal antibody titers to SRBCs antigen transformed means in different three segments of dams (high, medium, and low) and in two different groups of (group<sub>1</sub> and group<sub>2</sub>) and the remaining percentage of transferred maternal antibodies for two-week-old chicks.

Items		Dams ( $\bar{X} \pm S.E$ )	Progeny ( $\bar{X} \pm S.E$ )	The remaining percentage of transferred maternal antibodies for two-week-old chicks.
Segment	High	28.90 ± 2.21 <sup>a</sup>	0.83 ± 0.35	2.88
	Medium	11.33 ± 1.33 <sup>b</sup>	0.63 ± 0.11	5.56
	Low	1.35 ± 0.18 <sup>c</sup>	0.82 ± 0.17	60.74
Group	Group <sub>1</sub>	15.59 ± 1.69 <sup>a</sup>	0.23 ± 0.08 <sup>b</sup>	1.48
	Group <sub>2</sub>	9.33 ± 1.65 <sup>b</sup>	1.18 ± 0.16 <sup>a</sup>	12.65

**Table (2):** The interaction effect of segments and groups on antibody titers to SRBCs antigen of (dams and progeny) and the remaining percentage of transferred maternal antibodies for two-week-old chicks.

segments	Dams' antibody titer ( $\bar{X} \pm S.E$ )	Progeny antibody titer ( $\bar{X} \pm S.E$ )	The remaining percentage of transferred maternal antibodies for two-week-old chicks.
<b>Group<sub>1</sub></b>			
High	27.52 ± 2.64 <sup>c</sup>	0.22 ± 0.17 <sup>c</sup>	0.80
medium	13.66 ± 1.86 <sup>b</sup>	0.26 ± 0.10 <sup>c</sup>	1.90
Low	1.00 ± 0.30 <sup>c</sup>	0.05 ± 0.05 <sup>c</sup>	5.00
<b>Group<sub>2</sub></b>			
High	32.11 ± 4.06 <sup>a</sup>	2.26 ± 0.86 <sup>a</sup>	7.04
medium	8.82 ± 1.83 <sup>bc</sup>	1.03 ± 0.18 <sup>b</sup>	11.70
Low	1.48 ± 0.21 <sup>c</sup>	1.12 ± 0.19 <sup>b</sup>	75.67
<b>Means of squares</b>			
Segment	4048.24**	0.49 <sup>n.s</sup>	-
Group	1177.89**	26.87**	-
Interaction	1729.22**	7.00**	-

\*\* significant differences at  $P \leq 0.01$ , N.S. non-significant



**Picture (1):** An example of 96-wells plates used titration of antibody titer for SRBC antigen.

- The remaining percentage of transferred maternal antibodies for two-week-old chicks = (mean titer in chicks/ mean titer in hens) x 100.  
to describe the percentages of transferred antibodies.

**REFERENCES**

- Abdel-Moneim, M.A.S. and M.M.A. Abdel-Gawad 2006.** Genetic variations in maternal transfer and immune responsiveness to infectious bursal disease virus. *Vet. Microbiol.*, 114:16 – 24.
- Abdou, F. H.; A. A. Enab; A. A. El-Fiky and N. Kolstad, 2017.** Improving indigenous chickens in developing countries outlet of the Norwegian-Egyptian project "NORFA" in Egypt (1980 - 2017). Proceeding of the Poultry Science Association Annual Meeting, 17- 20 July 2017, Orlando, Florida, USA.
- Abou-Elewa, E. M. 2004.** Selection for general immune response and its relation to some economic traits in chickens. M. Sc. Thesis, Facu. Agric., Minufiya Univ., Egypt. Ph. D. Thesis, Minufiya Univ., Egypt.
- Abou-Elewa, E. M. 2010.** Some genetic parameters of the immune response trait and its utilization in different selection methods in chickens. Ph. D. Thesis, Facu. Agric., Minufiya Univ., Egypt.
- Abou-Elewa, E. M. 2004.** Evaluation of Norfa productive performance as an EGYPTIAN synthetic strain. *Egyptian Poult. Sc. J.*, 44 (1): 41 - 64.
- Ahmad, Z., and S. Akhter 2003.** Role of maternal antibodies in protection against infectious bursal disease in commercial broilers. *Int. J. Poult. Sci.*, 2:251–255.
- Ahmed, A. S. 2011.** Antibody response in early-hatched chicks is influenced by vaccination and different maternal antibody levels. *Egy. J. Anim. Prod.*, 48: 77-85.
- Bar-Shira, E.; D. Sklan and A. Friedman 2003.** Establishment of immune competence in the avian GALT during the immediate post-hatch period. *Developmental & Comparative Immunology*. Vol. 27, Issue 2, 147-157.
- Demissie, E.; K. Melese; M. Shimellis; B. Urge, and T. Seyoum 2022.** Maternal Antibody Transfer to Pathogen in Dual-Purpose Koekkoek Breed Chickens. *Livestock Research Results*, 2.
- Fahey, K.J., J.K. Crooks and R.A. Fraser 1987.** Assessment by ELISA of passively acquired protection against infectious bursal disease virus in chickens. *Aust. Vet. J.* 64, 203–207.
- Gebriel G.M.; A.A. El-Fikey; N. M. Sebea and E.M. Abou-Elewa 2021.** Application of immunoglobulin g(IGg) as an early genetic marker to improve some egg production traits in chickens. *Menoufia J. Animal, Poultry & Fish Prod.*, 5: 109-123. (24).
- Gong, H.; T. Wang; M. Wu; Q. Chu; H. Lan; W. Lang; L. Zhu; Y. Song; Y. Zhou; Q. Wen; J. Yu, B. Wang, and X. Zheng. 2023.** Maternal effects drive intestinal development beginning in the embryonic period based on maternal immune and microbial transfer in chickens. *Microbiome* 11, 41 (2023). <https://doi.org/10.1186/s40168-023-01490-5> Volume 11, article number 41.
- Hamal K.R., S.C. Burgess, I.Y. Pevzner, and G.F. Erf 2006.** Maternal antibodies transfer from dams to their egg yolks, egg whites, and chicks in the meat lines of chickens. *Poult. Sci.* 85:1364–1372.
- Hasselquist D., Nilsson J.Å. 2009.** Maternal transfer of antibodies in vertebrates: trans-generational effects on offspring immunity. *Philos Trans R Soc Lond B Biol Sci.* 364:51–60. doi: 10.1098/rstb.2008.0137
- Heller E.D.; G. Leitner; N. Drabkin and D. Melamed 1990.** Passive immunization of chicks against *Escherichia coli*. *Avian Pathol.* 19:345–354.
- Kramer, T.T. and H.C., Cho 1970.** Transfer of immunoglobulins and antibodies in the hen's egg. *Immunology* 19:157– 167.

- Kolstad, N. and F.H. Abdou 1999.** indigenous chickens in developing countries outlet of the Norwegian-Egyptian project "NORFA" in Egypt (1980-2017). Proceeding of the Poultry Science Association Annual Meeting, 17-20 July 2017, Orlando, Florida, USA.
- Kundu,A.; D.P. Singh; S.C. Mohapatra; B.B. Dash; R.P. Moudgal and G.S. Bisht 1999.**Antibody response to sheep erythrocytes in Indian native vis-à-vis imported breeds of chickens. British Poultry Sci., 40(I): 40-43.
- Mondal S.P. and S.A. Naqi. 2001.** Maternal antibody to infectious bronchitis virus: its role in protection against infection and development of active immunity to vaccine. Vet. Immunol. Immunopathol. 79:31-40.
- Pinard, M.H.; J.A.M. Arendonk, M.G.B. Neuwland and A.J. Van der Zijp 1992.** Divergent selection for immune responsiveness in chickens: estimation of realized heritability with an animal model. J. Anim. Sci., 70: 2986-2993.
- Siegel, P.B. and W.B., Gross 1980.** Production and persistence of antibodies in chicken to sheep erythrocytes.1. Directional selection. Poultry Sci.,59:1-6.
- SPSS Program 2004.**User's guide statistic. Release 10.01, Copyright SPSS Inc., USA.
- Van der Zijpp and Leenstra 1980.** Genetic analysis of humoral immune response of White Leghorn chicks. Poultry Sci., 59:1363-1369.
- Yang N.C.; E.A. Dunnington and P.B. Siegel 1999.** Kinetics of antibody responses in hens from chicken lines selected for response to sheep red blood cells. Poultry Sci. ,78:1081- 1084.

### الملخص العربي

## تأثير التحصين للأمهات خلال فترة جمع البيض للفقس على المناعة المنقولة إلى الكتاكيت إيمان متولى ابو عليوه

قسم إنتاج الدواجن والأسماك. الزراعة - جامعة المنوفية - شبين الكوم

هدفت الدراسة الحالية إلى معرفة تأثير تحصين الأمهات خلال فترة جمع البيض للفقس على المناعة الأمية التي تنتقل إلى النسل الناتج من الكتاكيت عند عمر أسبوعين. بلغ إجمالي عدد الكتاكيت 180 كتكوت ناتجة 32 دجاجة. تم تقسيم الدجاج إلى ثلاثة شرائح وفقاً لمستوى الأجسام المضادة لخلايا دم الغنم الحمراء SRBCs (عالية، متوسطة ومنخفضة)، وتم تقسيم البيض المخصب الناتج إلى مجموعتين (المجموعة 1 والمجموعة 2) على أساس فترة جمع البيض بعد حقن الأنتيجين SRBC للأمهات. وكانت أهم النتائج:

1. بلغت نسب الأجسام المضادة المتبقية للثلاث خطوط (عالي، وسط ومنخفض) 2.88%، 5.56%، 60.74%. علي التوالي. وهنا نلاحظ وجود علاقة عكسية واضحة بين مستوى الأجسام المضادة للأمهات ونسبة المناعة المتبقية التي تنتقل إلى الكتاكيت عند عمر الأسبوعين. حيث بلغت النسب المتبقية المنقولة للمجموعتين (المجموعة 1 والمجموعة 2) 1.48% و 12.65% على التوالي. وهذا يعني أن المجموعة الثانية أعلى من المجموعة الأولى في نسبة نقل الأجسام المضادة الأموية المتبقية للكتاكيت عند عمر أسبوعين.

2. توجد فروق معنوية بين مستوي الأجسام المضادة ضد SRBCs للأمهات سواء في الخطوط الثلاثة وبين المجموعتين. مكررة هي هي رقم 3 (تحذف)

3. كان هناك فارق معنوي بين الثلاث خطوط (العالي، الوسط والمنخفض) والمجموعتين (المجموعة 1 والمجموعة 2). في مستوي الأجسام المضادة ضد SRBCs في الأمهات وكذلك مستوي الأجسام المضادة ضد SRBCs المنقول الي الكتاكيت عند عمر أسبوعين، ولكن لم تكن هناك فروق معنوية بين الخطوط (العالية، الوسط والمنخفضة) في مستوي الأجسام المضادة SRBCs المنقول الي الكتاكيت بعمر أسبوعين.