Effect of Adding Guanidinoacetic Acid to Semen Diluents, Duration of *in Vitro* Storage on Semen Quality, Hatchability of Iraqi Chickens

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ABSTRACT

This study was conducted to examine the effect of adding different levels of Guanidinoacetic Acid (GAA) to the semen Diluents of the Iraqi rooster in the semen quality characteristics and the hatchability of two Iraqi chicken breeds during different storage periods *in vitro*. It was used 80 Cockerels (40 white + 40 naked red), and 256 chickens (128 white + 128 naked red) at 29 age weeks. Birds of each strain were divided in to four groups, these groups including concentration (Conc), 0, 1, 2, 3% of GAA were added to semen. Then, stored for four *in vitro* storage (SP) 0, 24, 48, 72 hours at a temperature of 4 Cº. The results indicated a significant increase in mass motility (MM) and individual motility (IM) hatchability (Ha) with significant reduction of Dead sperm (DS), abnormal spermatozoa (AS) and Acrosomal abnormalities (AA) when adding GAA with concentration of 1, 3, 5 % compared with 0% concentration, and there was no significant effect of breed (Br), and interaction Br and Conc in previous qualities. While a significant effect of interaction was found between Br and SP with significant effect of interaction and Br and Conc and SP. It is therefore possible to conclude that adding GAA to sperm diluents improves sperm quality and hatchability characteristics. Therefore, GAA can be used to improve the productive qualities of Iraqi chickens.

Key Words: Guanidinoacetic Acid, Semen diluents, *in vitro* storage, Semen quality, Iraqi chickens.

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INTRODUCTION

The local Iraqi breeds of chicken were characterized by a decrease in their reproductive efficiency, reflected in a decrease in local production of egg and meat [1]. Therefore, the studies recommended artificial insemination of birds in Iraq to overcome the problems of production and decrease in the local breeds, as the adoption of artificial insemination provides the possibility of election cocks and chicken with good production and rejection of the weak [2]. Then, obtaining sperm with good specifications and desirable quality and hatching rates [3].

In spite of the advantages of artificial insemination in birds, it focuses on a number of problem such as the short life of sperm *in vitro,* and the short time of

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artificial insemination, which does not exceed 10 – 15 min due to the rapid rates of high metabolism of birds [4], which opened the doors to using of Diluents to storage semen, to prolong the life of sperm and prevent the causes of death, the most important are free radicals [5]. Therefore, it is important to search for every metabolite, add to the sperm diluents that are presented by energy, reduce the activity of free radicals and then extended he length of storage [6, 7]. The hypothesis of sperm diluents is based on providing an environment that allows prolongation of life intravenous insomnia provides energy substrate to sustain metabolism or antioxidants that inhibit free radicals during storage [8]. In this sense, it was necessary to search for materials that are equipped with energy – efficient and one of these materials Guadinoacetic Acid [9].

Guadinoacetic (GAA), or Guanidinoacetate or Glycocyamineo is derived from the reaction of arginine and glycine in the liver and kidney [10]. Contributes to the synthesis of creation (Ct) in the reactions of the transfer of the group of the instance of S – adenosy methionine to GAA [11], Ct is a high energy phosphate that is important in the metabolism of the body's energy, especially in the male reproductive system [12], as Vigue et al. [13] report the Ct is responsible for the transfer of ATP energy to the sperm tail (sperm), to provide adequate energy from sperm movement.

The disturbance in energy metabolism reduces the mobility of the sperm and the movement of the sperm is a key factor of fertilization [14]. Tapeh et al. [15] reported that the use of GAA in roosters' diets improved the IM and MM and the reduction of DS, AA, AS. So that, this study examines the possibility of adding GAA to sperm diluents and indicates its role in semen, fertility and hatchability at four SP for two Iraqi Br.

MATERIALS

The study was carried out in the Poultry Research center, Agricultural Research office, Ministry of Agriculture, Iraq. In the period of time from 1/2/2016 to 1/3/2016 to study the effect of adding different levels of the GAA to Semen diluents of Iraqi rooster in the semen quality and hatchability for two Br from Iraqi Chicken's during different *in vitro* storage.

Experimental design and treatments

This study involved 80 Cockerels (40 white + 40 naked red), and 256 hens (128 white + 128 naked red) at the age of 29 weeks, these birds were housed in halls their environmental conditions perfect, and given diet contained 2850 Kcal and 16.71% crud protein. Birds were divided into four groups, included adding the levels 0, 1, 2, 3% of the GAA to sperm Diluents.

Sample collections and preparations

Then collecting sperm by massage the abdominal area [16] by using semen suction device [17], reduced sperm using Lake diluent [18], after the preparation of GAA solution by dissolving 1gm of GAA in 10 ml distilled water, and soluble fully complete in size to 100 ml, then added 1, 3, 5 ml of aqueous solution of GAA to semen diluted by Lake diluents [18]. And then stored semen samples *in vitro* under the temperature of 4 Cº for the SP 0, 24, 48, 72 h, the following tests were carried out at the previous periods. The microscope used to study the qualitative characteristics to sperm during the four SP (0, 24, 48, 72 h) to calculate the collective MM on the strength of the 40x and IM on 100x [19] and the percentage of DS by uses of Eosin – nigrosin stain [16], and the percentage of AS and AA by using Fast Green – Eosin stain [20, 21]. Then, artificial insemination Hens by using 0.05 of diluents / hen [2, 17], the lap of the eggs of the product for the following days, the second day of artificial insemination and reached 50 eggs/group, after 21 days Ha calculated by the following equation:

$$\text{Hatchability } \% = \frac{\text{Chicks hatched}}{\text{Total eggs}} \times 100$$

Statistical analysis

Analysis data according to factorial experiment in Completely Randomized Design –CRD to effect of different factors (GAA, Br, SP) in qualities under study. Statistical analyses were performed using the All Statistical Package for Social Science [22], version 21.0 for windows (SPSS Inc., Chicago, IL, USA) according to a Complete Randomized Design. Means were compared by Duncan's Multiple Range Test [23] with a significance level of 5%.

RESULTS

Mass motility

Table 1 show that there is no significant effect for Br in MM, but the addition of the GAA in $Conc_{1, 3, 5}$ to the semen diluent had significant increase of MM (P<0.01) compared to $Conc._0$, while noting the significant decrease (P<0.05) in MM when increasing storage periods (SP), as the best MM reached at SP_0 and then SP_{24} and SP_{48} and SP_{72} h respectively.

It also indicated that of interaction between Br and Conc. in MM. However, the interaction between Br and SP reached the level of significant (P<0.05), noting that the interaction between Br and SP_0 achieved the best MM, then Br With SP_{24} trailed by interaction Br with SP_{48}, the interaction between Br and SP_{72} has recorded less than with regard to the interaction between the Conc and SP notable deterioration of the MM with the increase of the SP significantly (P<0.05), noting that it absorbs the interaction of the four (0, 1, 3, 5%) achieved best MM when interaction with SP_0. While the interaction between $Conc_0$ and SP_{72} less than MM. The interaction between it $Conc_{1, 3, 5}$ with durations of 24, 48, 72 h achieved MM best interaction of $Conc_0$ with the same duration. At the same time notes that interaction each of Br and Conc and SP_0 achieved the best MM interaction with other SP, then the interactions of Br and Conc with the SP_{24}, and thus interaction SP_{48}, and that the interaction with the SP_0 indicated lower levels of significant ($P<0.05$). Also, the interaction of Br and $Conc_0$ with the SP_{72} recorded the lowest significant ($P<0.05$).

Individual motility

As can be seen from table 2 that there are no significant differences between Br_1 and Br_2 in IM, as indicated above $Conc_{1,3,5}$ significantly ($P<0.01$) on the $Conc_0$ in IM, with the significant decrease ($P<0.01$) in IM when $SP_{24,48,72}$ when compared with SP_0. no significant difference of the interaction between the Br and Conc, with the interaction significant ($P<0.05$) between Conc and SP, noting that the $Br_{1,2}SP_0$ indicated significant increase ($P<0.05$) IM interactions with the other, followed by $Br_{1,2}SP_{24}$, then $Br_{1,2}SP_{48}$ and then $Br_{1,2}SP_{72}$ with regard to the interaction between the Conc and SP notes that the $Conc_{0,1,3,5}SP_0$ indicated significant increase ($P<0.01$) in IM compared to other groups, while noting that the $Conc_{1,3,5}SP_{48,72}$ indicated significant increase ($P<0.01$) compared with $Conc_0SP_{48,72}$. When the interaction of the three factors notes from the same table (table 2): $Br_1Conc_1SP_0$ and $Br_2Conc_0SP_0$ a record significant increase ($P<0.05$) in IM compared to other groups. The $Br_2Conc_5SP_{24}$ did not differ from the significant of $Br_{1,2}Conc._{0,1,3,5}SP_0$. While $Br_{1,2}Conc_0SP_{48}$ indicated significant decrease ($P<0.05$) in IM compared to $Br_2Conc_5SP_{48}$ and significant decrease ($P<0.05$) $Br_{1,2}Conc_0SP_{72}$ comparing to $Br_{1,2}Conc_{1,3,5}SP_{48,72}$.

Dead sperm

As can be seen from table 3 that there is no significant differences in DS between Br_1 and Br_2, as shown in the low significant ($P<0.01$) in the DS in favor of $Conc_{1,3,5}$ compared with $Conc_0$, also notes the significant decline ($P<0.01$) in the SD in favor of the SP_0 compared to $SP_{0,24,48}$ and the SP_{24} recorded less SD than $SP_{48,72}$ and SP_{72} posted a significant increase ($P<0.01$) compared to SP_{48} in SD. While not observed significant differences in interaction between Br and Conc. It also notes that the $Br_{1,2}SP_0$ recorded significant decline ($P<0.05$) in SD compared to groups remaining, and $Br_{1,2}SP_{24}$ Best of $Br_{1,2}SP_{48}$ and $Br_{1,2}SP_{72}$ recorder the highest rate($P<0.05$) of SD compared with other groups. It also noted from table 3 that the $Conc_0SP_0$ recorded a significant increase ($P<0.05$) in SD compared to all interactions, including $Conc_{1,3,5}SP_{72}$, as well as notes that $Conc_{0,1,3,5}SP_0$ the lowest SD compared to other interactions. As can be seen from table 3 that the $Br_{1,2}Conc_0SP_{72}$ significant decrease ($P<0.05$) in SD compared to all interactions especially $Br_{1,2}Conc_{1,3,5}SP_{72}$.

Abnormal spermatozoa

It is clear from table 4 that there is no significant difference between Br_1 and Br_2 in AS, with the existence of a significant decrease ($P<0.01$) in AS for $Conc_{1,3,5}$ compared $Conc_0$. At the same time noted the increase in AS significant ($P<0.01$) progress of the SP. No significant differences interaction between Br and Conc in AS. While noting that the $Br_{1,2}SP_0$ recorded a lower average ($P<0.05$) AS compared to all interactions followed by $Br_{1,2}SP_{24}$ and then $Br_{1,2}SP_{48}$ and then $Br_{1,2}SP_{72}$. It also notes that the $Conc_5SP_0$ significant decrease ($P<0.05$) in AS compared to the groups remaining, while the $Conc_0SP_{72}$ the highest values ($P<0.05$) AS compared to the rest of the intersections. As can be seen from the same table that $Br_1Conc_5SP_0$ significant decrease ($P<0.05$) compared to $Br_{1,2}Conc_{0,1,3,5}SP_{48,72}$ and $Br_1Conc_{0,1,3}SP_{24}$ and $Br_2Conc_{0,3}SP_{24}$. In the same time in which the $Br_{1,2}Conc_0SP_{72}$ higher significant ($P<0.05$) values of AS compared to other interactions.

Acrosomal abnormalities

Table 5 indicates the absence of significant differences between Br_1 and Br_2 in AA, with the existence of a significant decrease ($P<0.05$) in AS for $Conc_{1,3,5}$ compared $Conc_0$, and the significant increases of the AS registered progress of SP. At the time did not observe the significant difference of the interaction between the Br and Conc in the AA as indicated the existence of a significant decrease ($P<0.05$) in favor of $Br_{1,2}SP_0$ compared to $Br_{1,2}SP_{24,48,72}$ in AA, and significant decrease ($P<0.05$) in favor of $Br_{1,2}SP_{24}$ compared to $Br_{1,2}SP_{48,72}$ and $Br_{1,2}SP_{48}$ record the less significant ($P<0.05$) in AA of $Br_{1,2}SP_{72}$. The table also indicates $Br_{1,2}Conc_{0,1,3,5}SP_0$ had achieved a significant decrease ($P<0.05$) in the AA compared to $Br_{1,2}Conc_{0,1,3,5}SP_{24,48,72}$. and $Br_{1,2}Conc_0SP_{72}$ posted a significant increase ($P<0.05$) in AA compared to groups remaining. The $Br_{1,2}Conc_{1,3,5}SP_{48}$ recorded significant decrease ($P<0.05$) in AA compared to $Br_{1,2}Conc_0SP_{48}$ and the $Br_{1,2}Conc_{1,3,5}SP_{72}$ record low significant ($P<0.05$) in the AA compared to $Br_{1,2}Conc_0SP_{72}$.

Hatchability

Table 6 indicated that there is no significant effect Br in Ha, but that the use of GAA $Conc_{1,3,5}$ had led to a significant increase ($P<0.01$) in Ha compared to $Conc_0$. Increasing the duration of storage of 0 to 72 h led to significant decrease ($P<0.01$) in Ha, the SP_0 first and then the SP_{24} and SP_{48} and SP_{72}. It notes the no significant effects of the interaction between the Br and Conc. The significant effects were observed interaction between Br and SP, as $Br_{1,2}SP_{0,24}$ excelled Significantly ($P<0.05$) on $Br_{1,2}SP_{48,72}$, and $Br_{1,2}SP_{48}$ than $Br_{1,2}SP_{72}$. Shows that $Conc_{0,1,3,5}SP_{0,24}$ excelled significantly ($P<0.05$) on $Conc._{0,1,3,5}SP_{48,72}$. The $Conc_0SP_{72}$ record less significant ($P<0.05$) than the value of Ha compared to other groups, no significant difference between $Conc_5SP_{72}$ and $Conc_0SP_{48}$, the significant decrease($P<0.01$) in comparison with $Conc_{1,3,5}SP_{48}$. It is the same table clear $Br_2Conc_1SP_0$ significant increase ($P<0.05$) on $Br_2Conc_0SP_{24}$ and $Br_{1,2}Conc_{0,1,3,5}SP_{48,72}$, without significant effects differ from other interactions. In the record of $Br_{1,2}Conc_0SP_{72}$ less than the values per Ha compared to the rest of the intersections. The $Br_{1,2}Conc_{1,3,5}SP_{48}$, recorded a significant increase ($P<0.05$) in Ha when compared to $Br_{1,2}Conc_0SP_{48}$, and did not differ from the interactions of Br and Conc with the SP_{72}.

DISCUSSION

It also noted that the results showed no significant effect of Br on the specific characteristics of the specific characteristics of the semen and the hatchability of the previously mentioned results. This may be due to the convergence of the productive efficiency of the two breeds. The Iraqi breeds characterized by their yield [1].

The significant influence of Conc significant improvment is evident in MM and IM, which may be due to the fact that adding GAA to semen diluents contributes to the Create of Ct [11], which provides energy substrates for the sperm [13]. Lee et al. [24] has indicates that the seminal vesicles in the reproductive system of mammals produce Ct to be a major source of energy and an important responsible for the movement of the sperm [25]. Due to the absence of the attached gonads in the roosters, GAA may be a direct source of Ct extract after supplementation with semen driers. Ct has an anti – oxidant effect [26], which may be due to low SD, AS, AA, as well as simple signs of GAA's antioxidant roles [27, 28].

The GAA's anti – properties reduce concentrations of polyunsaturated fatty acids (PUFA) in the semen diluted, as these acids increase the likelihood of peroxides and thus reduce the fertilization capacity of the sperm. Peroxides reduce the life of span of sperm within the *in vivo* during inoculation or *in vitro* [29]. Peroxide lead to significant changes in sperm composition, especially in the acrosome region, and cause a sharp decline in the rate and vitality of the sperm, resulting in the prevention of the reaction of the acrosome of the sperm with the egg membrane [30, 31, 32] reinforcing the role of Ct in maintaining the movement of the sperm [25].

That the degradation of the studied traits of the SP effect may be due to the penetration of the energy substrates important for the movement of the sperm, as well as the free radicals by the oxidative processes that affect the plasma membrane of the sperm, which contains a part of the phospholipid [3, 6, 7, 8], noting differences between each and every period in the specific characteristics of semen.

Interaction was not significantly because the effect of Br is greater than the Br Conc the effect of Conc this is illustrated by the interaction of BrSP, in which the effect of SP is equal for Br_1 and Br_2.

The interaction between Conc. SP, which has a significant effect on semen quality and hatchability, shows an inverse relationship between the studied SP properties of the peroxides that attack the plasma membrane of the sperm, accompanied by a positive relationship between the studied traits and Conc on reducing the negative effects of SP on the effect and resistance of peroxides caused by oxidation processes as well as protection of the plasma membrane of the sperm for GAA anti-oxidation and its importance in the production of Ct, one of the most important sources of energy for the movement of sperm [11, 27, 28].

When you notice BrConcSP interactions, the role of Conc in reducing the effect of SP for each of $Br_{1,2}$, in semen characteristics, Conc's role is confirmed when the SP is rendered as observed in the MM for example, the Conc effect is equal. When SP_0, 24 is only Conc effects, increases at $SP_{48,\ 72}$, especially at $Conc_5$. The $Conc_5$ effect is becoming clearer. When SP_{72} is in IM that was close to its effects at SP_{48}, the same is true in DS. As shows the effect of Conc when SP_{48}. This reinforces the hypothesis of the role of GAA in improving the storage conditions of the semen of the Iraqi roosters, and the antioxidant antagonist [28].

The improvement in Ha was due to the characteristics (tables 1, 2, 3, 4 and 5), with the effect of GAA, which provided protection against oxidizing factors, or worked on processing the tail of sperm with energy needed for the movement and fertilization [2, 17]. That decrease in the movement of the fetus reduces the sperm stored in the uterovaginal sperm – host glands, because the movement is necessary to cross the vagina and access to the glands, and that the reduction of sperm in those glands reflected negativity in the fertility of birds [33], as well as the importance of movement of the embryos to move from the uterovaginal sperm – host glands to the funnel during ovulation to fertilize the ovarian egg from the ovary [3]. In addition, protection against oxidative damage to the ovaries increases in IM, which is expressed in the rapid transfer of the embryos to the repression. The movement of the sperm is a major condition for the fertilization process, and the reduction of SD, AS and AA increases the fertility rates of fertilization [10], and the fertility ratio is positively correlated with Ha [2, 17].

CONCLUSION

According to previous results, it can be concluded that adding GAA to semen diluents improves the quality characteristics of semen and hatching rate. It limits the negative effects of the in vitro storage. It can therefore be used in the Iraqi rooster's semen diluents to improve its productive qualities and looking for better ways to prolong semen storage.

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Table 1. Effect of Adding Guanidinoacetic Acid to the Semen Diluents, and Duration of *in vitro* Storage on mass motility (%) of two Iraqi chickens' breeds.

Breed	Concentration (%)	Storage Periods (h) 0	24	48	72	Breed * Concentration	Mean: Breed
White	0	75.06± 1.59^{A}	57.81±4.96^{B}	33.95±5.11^{DE}	5.15±0.87^{G}	42.99±8.08	48.24 ±3.42
	1	75.64± 1.45^{A}	58.80±5.53^{B}	35.47±3.21^{DE}	6.45±0.67^{G}	49.94±6.77	
	3	75.87±1.81^{A}	63.48±1.72^{B}	43.60±2.20^{CD}	16.81±1.39^{F}	49.77±6.40	
	5	76.58±3.81^{A}	61.83±1.97^{B}	41.40±4.07^{CDE}	17.48±1.22^{F}	50.28±6.64	
Naked brown	0	76.80±0.93^{A}	59.14±4.37^{B}	43.03±2.78^{CD}	20.10±1.32^{F}	44.09±7.96	49.00 ±3.39
	1	77.81±0.85^{A}	62.97±1.53^{B}	41.20±1.83^{CDE}	20.97±2.10^{F}	49.32±6.82	
	3	74.56±3.47^{A}	64.78±2.67^{B}	43.89±1.96^{CD}	17.89±1.70^{F}	50.74±6.54	
	5	76.45±1.02^{A}	64.93±3.23^{B}	44.88±1.49^{C}	21.07±0.27^{F}	51.83±6.40	
White		75.57± 0.96^{A}	61.30±1.79^{B}	41.12±1.87^{C}	14.99±1.84^{D}		
Naked brown		76.62± 0.94^{A}	62.14±1.61^{B}	40.74±1.58^{C}	16.49±1.88^{D}		
Concentration (%)						Mean: Concentration (%)	
0.00		75.35±0.97^{A}	58.31±3.33^{C}	34.71±2.72^{E}	5.80 ± 0.57^{G}	43.54±5.55^{B}	
1.00		76.22±1.89^{A}	62.66±1.23^{BC}	42.50±2.13^{D}	17.14 ± 0.84^{F}	49.63±4.70^{A}	
3.00		77.30±0.61^{A}	61.06±2.24^{BC}	42.12±1.54^{D}	20.53 ± 1.13^{F}	50.25±4.48^{A}	
5.00		75.50±1.67^{A}	64.86±1.87^{B}	44.38±1.12^{D}	19.48 ± 1.05^{F}	51.06±4.51^{A}	
Mean Storage Periods (h)		76.10±0.67^{A}	61.72±1.18^{B}	40.93±1.20^{C}	15.74 ± 1.30^{D}		

S. O. V	P – Value	R-Square (%)	CV (%)	Over All Mean	Over All Stander Error
Breed	N.S	97.3	9.49	48.62	2.40
Concentration	0.01				
Storage Periods	0.05				
Breed* Concentration	N.S				
Breed* Storage Periods	0.05				
Concentration* Storage Periods	0.05				
Breed* Concentration* Storage Periods	0.05				

Table 2. Effect of Adding Guanidinoacetic Acid to the Semen Diluents, and Duration of *in vitro* Storage on individual motility (%) of two Iraqi chickens' breeds

Breed	Concentration (%)	Storage Periods (h) 0	24	48	72	Breed * Concentration	Mean: Breed
White	0	81.73±1.76^{AB}	66.09±1.68^{D}	21.26±2.77^{F}	6.01±1.20^{G}	43.77±9.42	49.84±3.74
	1	83.74±3.40^{A}	66.98±3.83^{D}	31.99±2.33^{E}	30.83±2.11^{EF}	53.38±6.98	
	3	77.33±4.40^{ABC}	65.25±3.36^{D}	27.77±2.58^{EF}	29.19±0.21^{EF}	49.88±6.71	
	5	81.70±1.27^{AB}	69.23±2.39^{CD}	29.52±1.76^{EF}	28.91±1.99^{EF}	52.34±7.14	
Naked brown	0	83.50±3.17^{A}	68.61±1.44^{CD}	21.16±2.51^{F}	5.23±1.45^{G}	44.63±9.80	50.71±3.84
	1	79.88±4.04^{AB}	67.16±6.26^{D}	30.40±4.24^{EF}	32.35±0.85^{E}	52.45±6.75	
	3	81.12±4.06^{AB}	67.70±2.61^{D}	29.53±3.89^{EF}	28.75±3.38^{EF}	51.78±7.13	
	5	82.08±4.26^{AB}	73.25±0.84^{BCD}	33.86±3.69^{E}	26.77±1.86^{EF}	53.99±7.35	
White		81.13±1.46^{A}	66.89±1.33^{B}	27.63±1.57^{C}	23.73±3.17^{C}		
Naked brown		81.65±1.71^{A}	69.18±1.66^{B}	28.74±2.09^{C}	23.28±3.32^{C}		
Concentration (%)						Mean: Concentration (%)	
0.00		82.62±1.67^{A}	67.35±1.14^{B}	21.21±1.67^{D}	5.62±0.86^{E}	44.20±6.65^{B}	
1.00		81.81±2.51^{A}	67.07±3.28^{B}	31.19±2.19^{C}	31.59±1.07^{C}	52.92±4.75^{A}	
3.00		79.23±2.81^{A}	66.47±1.98^{B}	28.65±2.12^{C}	28.97±1.52^{C}	50.83±4.79^{A}	
5.00		81.89±1.99^{A}	71.24±1.45^{B}	31.69±2.07^{C}	27.84±1.31^{C}	53.17±5.02^{A}	
Mean Storage Periods (h)		81.39±1.10^{A}	68.03±1.07^{B}	28.18±1.29^{C}	23.50±2.24^{D}		

S. O. V	P – Value	R-Square (%)	CV (%)	Over All Mean	Over All Stander Error
Breed	N. S				
Concentration	0.01				
Storage Periods	0.01				
Breed* Concentration	N. S				
Breed* Storage Periods	0.05	97.4	10.26	50.28	2.66
Concentration* Storage Periods	0.01				
Breed* Concentration* Storage Periods	0.05				

Table 3. Effect of Adding Guanidinoacetic Acid to the Semen Diluents, and Duration of *in vitro* Storage on Dead sperm (%) of two Iraqi chickens' breeds.

Breed	Concentration (%)	Storage Periods (h) 0	24	48	72	Breed * Concentration	Mean: Breed
White	0	14.88±1.43^{H}	24.81±3.98^{G}	69.46±3.56^{CDE}	93.87±1.43^{A}	50.76 ± 9.81	44.45±4.12
	1	15.92±1.18^{H}	22.02±2.81^{GH}	66.62±2.79^{DE}	77.19±2.11^{BC}	45.44 ± 8.15	
	3	14.83±1.77^{H}	20.61±1.98^{GH}	68.05±2.11^{DE}	74.65±3.05^{BCD}	44.54 ± 8.20	
	5	13.87±2.28^{H}	14.03±1.96^{H}	53.73±2.72^{F}	66.71±3.53^{DE}	37.08 ± 7.20	
Naked brown	0	14.30±0.90^{H}	25.74±2.19^{G}	71.60±1.03^{CDE}	97.77±1.23^{A}	52.35 ± 10.23	45.60±4.22
	1	15.50±1.76^{H}	19.41±3.25^{GH}	65.08±4.15^{E}	70.33±4.14^{CDE}	42.58 ± 7.75	
	3	13.73±1.74^{H}	18.68±4.00^{GH}	56.81±2.32^{F}	71.19±4.53^{CDE}	40.10 ± 7.52	
	5	14.46±1.52^{H}	24.26±3.16^{G}	69.82±0.02^{CDE}	80.95±0.17^{B}	47.37 ± 8.63	
White		14.88±0.76^{D}	20.36±1.69^{CD}	64.46±2.25^{B}	78.11±3.19^{A}		
Naked brown		14.50±0.68^{D}	22.02±1.65^{C}	65.83±2.01^{B}	80.06±3.59^{A}		
Concentration (%)						Mean: Concentration (%)	
0.00		14.59±0.77^{F}	25.27±2.04^{E}	70.53±1.72^{BC}	95.82±1.21^{A}	51.55 ± 6.93 ^{A}	
1.00		15.71±0.95^{F}	20.71±2.01^{EF}	65.85±2.26^{CD}	73.76±2.58^{B}	44.01 ± 5.51 ^{B}	
3.00		14.28±1.14^{F}	19.64±2.04^{EF}	62.43±2.88^{D}	72.92±2.56^{B}	42.32 ± 5.46 ^{B}	
5.00		14.16±1.23^{F}	19.14±2.83^{EF}	61.78±3.80^{D}	73.83±3.55^{B}	42.23 ± 5.60 ^{B}	
Mean Storage Periods (h)		14.69±0.50^{D}	21.19±1.17^{C}	65.15±1.48^{B}	79.08±2.36^{A}		

S. O. V	P – Value	R-Square (%)	CV (%)	Over All Mean	Over All Stander Error
Breed	N. S				
Concentration	0.01				
Storage Periods	0.01				
Breed* Concentration	N. S				
Breed* Storage Periods	0.05	98.3	10.02	45.03	2.93
Concentration* Storage Periods	0.05				
Breed* Concentration* Storage Periods	0.05				

Table 4. Effect of Adding Guanidinoacetic Acid to the Semen Diluents, and Duration of *in vitro* Storage on abnormal spermatozoa (%) of two Iraqi chickens' breeds

Breed	Concentration (%)	Storage Periods (h) 0	24	48	72	Breed * Concentration	Mean: Breed
White	0	16.69±2.00^{DEFG}	24.63±2.14^{DE}	60.11±1.49^{C}	95.22±1.74^{A}	49.16±9.45	45.98±4.23
	1	16.93±2.04^{DEFG}	23.70±2.90^{DEF}	53.22±2.86^{C}	85.41±2.08^{B}	44.81±8.25	
	3	16.19±2.03^{FG}	23.52±3.23^{DEF}	56.45±1.70^{C}	87.71±1.33^{B}	45.97±8.64	
	5	15.15±2.04^{G}	22.41±3.95^{DEFG}	52.23±3.85^{C}	86.11±1.80^{B}	43.97±8.55	
Naked brown	0	16.67±2.51^{DEFG}	24.86±1.71^{D}	58.92±1.71^{C}	95.39±1.31^{A}	48.96±9.42	45.45±4.20
	1	17.00±3.03^{DEFG}	22.58±2.53^{DEFG}	52.96±3.39^{C}	86.03±1.64^{B}	44.64±8.38	
	3	16.44±2.08^{EFG}	24.44±3.38^{DE}	53.63±3.74^{C}	85.09±2.14^{B}	44.90±8.24	
	5	15.99±2.47^{FG}	19.52±0.25^{DEFG}	52.17±2.60^{C}	85.54±1.93^{B}	43.30±8.54	
White		16.24±0.89^{D}	23.57±1.35^{C}	55.50±1.46^{B}	88.61±1.40^{A}		
Naked brown		16.52±1.09^{D}	22.85±1.16^{C}	54.42±1.50^{B}	88.01±1.49^{A}		
Concentration (%)						Mean: Concentration (%)	
0.00		16.68±1.43^{FG}	24.74±1.23^{E}	59.52±1.05^{C}	95.31±0.97^{A}	49.06±6.52 ^{A}	
1.00		16.97±1.63^{FG}	23.14±1.74^{E}	53.09±1.98^{D}	85.72±1.19^{B}	44.73±5.75 ^{B}	
3.00		16.31±1.30^{FG}	23.98±2.10^{E}	55.04±1.94^{D}	86.40±1.27^{B}	45.43±5.84 ^{B}	
5.00		15.57±1.44^{G}	20.96±1.88^{EF}	52.20±2.08^{D}	85.82±1.19^{B}	43.64±5.91 ^{B}	
Mean Storage Periods (h)		16.38±0.69^{D}	23.21±0.88^{C}	54.96±1.03^{B}	88.31±1.00^{A}		

S. O. V	P – Value	R-Square (%)	CV (%)	Over All Mean	Over All Stander Error
Breed	N. S	98.6	9.24	45.72	2.96
Concentration	0.01				
Storage Periods	0.01				
Breed* Concentration	N. S				
Breed* Storage Periods	0.05				
Concentration* Storage Periods	0.05				
Breed* Concentration* Storage Periods	0.05				

Table 5. Effect of Adding Guanidinoacetic Acid to the Semen Diluents, and Duration of *in vitro* Storage on Acrosomal abnormalities (%) of two Iraqi chickens' breeds

Breed	Concentration (%)	Storage Periods (h) 0	24	48	72	Breed * Concentration	Mean: Breed
White	0	7.39±0.42 ^{I}	24.40±2.06^{GH}	56.11±6.46^{E}	99.14±0.39^{A}	46.76 ± 10.63	42.90±4.80
	1	7.29±0.89 ^{I}	19.23±0.80^{H}	49.26±0.84^{F}	92.53±2.36^{B}	42.08 ± 9.94	
	3	7.95±0.55 ^{I}	21.21±1.21^{GH}	49.13±1.42^{F}	91.42±3.16^{B}	42.43 ± 9.67	
	5	7.22±0.59 ^{I}	18.40±0.30^{H}	49.03±1.07^{F}	86.69±3.33^{BCD}	40.34 ± 9.33	
Naked brown	0	7.24±0.57 ^{I}	26.36±2.14^{G}	56.22±4.66^{E}	99.19±0.54^{A}	47.25 ± 10.52	41.95±4.59
	1	7.60±0.81 ^{I}	19.86±1.09^{GH}	49.61±1.33^{F}	83.69±2.42^{CD}	40.19 ± 8.89	
	3	7.15±0.61 ^{I}	18.70±0.34^{H}	49.42±0.91^{F}	88.87±4.67^{BC}	41.03 ± 9.60	
	5	7.61±1.18 ^{I}	19.71±1.19^{GH}	48.06±0.01^{F}	81.88±2.23^{D}	39.31 ± 8.65	
White		7.46±0.29 ^{D}	20.81±0.88^{C}	50.88±1.70^{B}	92.45±1.74^{A}		
Naked brown		7.40±0.36 ^{D}	21.16±1.08^{C}	50.83±1.42^{B}	88.41±2.37^{A}		
Concentration (%)						Mean: Concentration (%)	

0.00	7.31±0.32 H	25.38±1.40 F	56.17±3.56 D	99.16±0.30 A	47.01 ± 7.31 A
1.00	7.45±0.54 H	19.55±0.62 G	49.44±0.71 E	88.11±2.49 BC	41.14 ± 6.52 B
3.00	7.55±0.41 H	19.95±0.80 G	49.28±0.76 E	90.14±2.59 B	41.73 ± 6.66 B
5.00	7.42±0.59 H	19.06±0.62 G	48.54±0.53 E	84.29±2.09 C	39.83 ± 6.22 B
Mean Storage Periods (h)	7.43±0.22 D	20.99±0.68 C	50.86±1.08 B	90.43±1.50 A	
S. O. V	P – Value	R-Square (%)	CV (%)	Over All Mean	Over All Stander Error
Breed	N. S	99.11	8.77	42.43	3.30
Concentration	0.05				
Storage Periods	0.01				
Breed* Concentration	N.S				
Breed* Storage Periods	0.05				
Concentration* Storage Periods	0.05				
Breed* Concentration* Storage Periods	0.05				

Table 6. Effect of Adding Guanidinoacetic Acid to the Semen Diluents, and Duration of *in vitro* Storage on hatchability (%) of two Iraqi chickens' breeds

Breed	Concentration (%)	Storage Periods (h)				Breed * Concentration	Mean: Breed
		0	24	48	72		
White	0	88.00±3.46 ABC	81.33±1.76 ABCD	43.33±4.06 GH	7.33±2.67 I	55.00 ± 9.85	66.17±3.88
	1	92.67±1.76 AB	88.67±5.46 ABC	60.00±1.15 F	33.33±1.33 H	68.67 ± 7.34	
	3	90.67±4.37 AB	87.33±4.37 ABC	64.67±6.77 EF	34.67±5.81 H	69.33 ± 7.13	
	5	90.00±3.06 AB	84.67±5.46 ABCD	73.33±4.81 DE	38.67±2.40 GH	71.67 ± 6.28	
Naked brown	0	84.00±2.31 ABCD	79.33±4.67 BCD	48.00±5.03 G	10.00±5.03 I	55.33 ± 9.12	67.58±3.61
	1	93.33±1.76 A	86.67±3.53 ABC	66.67±4.37 EF	36.67±2.91 GH	70.83 ± 6.79	
	3	92.00±4.16 AB	84.67±3.71 ABCD	66.00±3.46 EF	40.67±.67 GH	70.83 ± 6.14	
	5	90.67±5.33 AB	85.33±2.91 ABCD	76.67±3.33 CDE	40.67±4.37 GH	73.33 ± 6.14	
White		90.33±1.49 A	85.50±2.11 A	60.33±3.84 B	28.50±4.02 C		
Naked brown		90.00±1.91 A	84.00±1.81 A	64.33±3.57 B	32.00±4.16 C		
Concentration (%)						Mean: Concentration (%)	
0.00		86.00±2.07 AB	80.33±2.28 BC	45.67±3.07 E	8.67±2.62 G	55.17 ± 6.57 B	
1.00		93.00±1.13 A	87.67±2.94 AB	63.33±2.51 D	35.00±1.61 F	69.75 ± 4.90 A	
3.00		91.33±2.72 A	86.00±2.63 AB	65.33±3.41 D	37.67±2.94 F	70.08 ± 4.61 A	
5.00		90.33±2.75 A	85.00±2.77 AB	75.00±2.72 C	39.67±2.28 EF	72.50 ± 4.30 A	
Mean Storage Periods (h)		90.17±1.19 A	84.75±1.37 B	62.33±2.60 C	30.25±2.85 D		
S. O. V	P – Value	R-Square (%)	CV (%)	Over All Mean	Over All Stander Error		
Breed	N.S	95.34	10.16	66.88	2.64		
Concentration	0.01						
Storage Periods	0.01						
Breed* Concentration	N.S						
Breed* Storage Periods	0.05						
Concentration* Storage Periods	0.01						
Breed* Concentration* Storage Periods	0.05						

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