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Productivity and Progesterone Profile of Nulliparous and Second-Parous Rabbits Characterized by Low and High Litter Size

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Abstract: This study aimed to evaluate the effect of the litter size (LS) category on the reproductive performance of nulliparous (NP) and second-parous (SP) NZW rabbits and the performance of their offspring. A total of 39 NP and 33 SP doe rabbits were divided into low (LLS, <7) and high (HLS, ≥7) after parturition. Reproductive measurements of each LS category and doe type were determined. The results show LS of 3-8 and 4-10 NP and SP, respectively. Doe rabbits with 6 LS show the highest distribution. Doe body weight at mating and from mating to parturition was higher (P<0.0001) for HLS than LLS, and SP than NP. Kit weight at birth and weaning, LS and viability were higher in HLS than in LLS does. At weaning, LS was higher (P<0.0001) in SP than NP. Levels of P4 at mid-pregnancy (P<0.001) were higher in HLS than in LLS, and in SP than in NP. In conclusion, a high litter size category (≥7/doe) was obtained from heavier NP or SP doe rabbits (LBW around 3 kg) and from second-parous compared with nulliparous does, with near similarity in conception rate of does. Weight and viability rate at birth and weaning as well as plasma progesterone level at mid-pregnancy were higher for does with high than low litter size category. In conclusion, body weight and parity may have a significant role in improving the litter size of doe rabbits, which may be useful in breeding and managerial programs to increase the economic value of rabbit production.

Keywords: Litter Size, Progesterone, Pregnancy, Rabbit, Viability

1. Introduction

With increasing human population in developing countries, especially in Egypt, rabbit production requires to gain momentum to solve the shortage in white meat production. Rabbit production reliance, as a cheap resource of animal proteins in solving malnutrition problems, is not overemphasized, which is attributed to the high rate of reproduction to produce high nutritional value meat from rabbits [1].

Genetic and environmental factors affect rabbit reproductive efficiency [2]. The productive performance of commercial rabbits mainly depends on the litter size at birth and the kit survival rate at weaning [3]. One of the most important economic components in intensive rabbit meat production is litter size [4] and most of the maternal lines are selected by litter size at weaning because this trait reflects the prolificacy of the rabbit doe. The increase observed in litter size is usually due to an increase in ovulation rate [5], which depends on the genetic parameters of its components [6]. Litter size affects growth traits of young rabbits from birth to weaning [7, 8] as well as the productive performance of the future reproductive females [9]. Thus, larger litters show a lower average birth weight, lower birth-to-weaning growth rate, and higher mortality than smaller litters [5]. It seems advisable to perform a limited standardization level (9 kits/doe) after kindling to improve reproductive performance [9].

The economic efficiency of a rabbitry depends mainly on the reproductive performance of the doe, which in turn is affected by their fertility and prolificacy [10]. Female reproduction is a more interesting and attractive subject to study and is preferred by many researchers and this is essential and more important and beneficial for successful rabbit breeding. Litter size, birth weight and conception rate are so important economic traits in any productive animal [11]. For great profit, special attention must be focused on these traits, so studying characterization of the reproductive performance and profile of progesterone in rabbit does that are characterized by low or high litter size is important and must be taken into consideration.

Therefore, the present work aimed to study the reflection of the interaction between litter size of doe and parity order on reproductive traits, profile of progesterone in NZW rabbit does and the performance of their litters.

2. Materials and Methods

This study was carried out within the framework of scientific cooperation between Animal Production Department, Faculty of Agriculture, Mansoura University and Animal Production Research Institute, Agricultural Research Center, Egypt, during the period from September 2020 to April 2021.

2.1. Animals, Feeding, and Management

Sexually mature NZW rabbit does (nulliparous, NP, n=39) and (second-parous, SP, n=33) with an age of 6-7 mo and body weight of 2.75-2.90 kg, as well as 10 fertile NZW rabbit bucks aging 10-13 mo and weighing 3.5-4.1 kg body weight, were used in this study. Does and bucks were housed in a naturally ventilated building. They were kept individually in flat deck cages (50×60×40 cm) equipped with automatic water dispensers and supplied with internal nest boxes only for does. Animals were fed *ad libitum* on a commercial pelleted diet (10.5 MJ ME/kg, 18.5% CP and 12.5% CF) according to their physiological condition. All animals were healthy and clinically free of external parasites. Does and bucks were kept under the same management system and similar normal conditions. Cages and nest boxes were cleaned and disinfected regularly before each kindling. Urine and feces dropped from the cages on the floor and cleaned daily in the morning.

All animals were managed and cared humanely with an approved protocol following the Animal Care Committee of the Faculty of Veterinary Medicines. At the beginning of the experimental period, all does were naturally mated with fertile bucks. Doe rabbit was i.m. injected with 0.2 ml Receptal premating and transferred to the cages of buck to natural mating and returned to its cage after mating. The mated does were palpated 10-11 d post-mating to diagnose pregnancy. On day 27 of pregnancy, the nest boxes were supplied with wooden straw to help the doe in preparing a worm comfortable nest for the bunnies.

2.2. Experimental Design

After the parturition, doe rabbits (NP or SP) were divided according to their total litter size at birth into does with low (<7 kits/litter) or high (≥7 kits/litter) litter size. The number of NP and SP does was 42 does with low and 30 with high litter size. Data concerning the reproductive parameters, profile of progesterone (P4), and litter performance from birth to weaning were recorded for all does that delivered low or high litter size.

2.3. Experimental Procedures

Live body weight of does was recorded at mating, on day 9, 14 and 28 of pregnancy, and at parturition. After parturition, kit weight, and litter size (total and live) per doe were determined at birth. Also, weight, litter size, viability rate, and sex ratio of kits were recorded at weaning (28 d of age). Conception rate (number of pregnant does/number of mated rabbit's x 100) and performance index (live litter size x conception rate/100) were calculated.

2.4. Blood Samples and Hormonal Assay

Blood samples were collected at mid-pregnancy (15 d), and late-pregnancy (28 d) from the ear vein into heparinized clean test tubes and immediately centrifuged (4000 rpm) for 15 min. Then, plasma was separated and stored at -20°C until hormonal assay. Plasma concentration of P4 was determined at mid- and late-pregnancy by using a P4 specified kit (Oxis International, Inc. 323 Vintage Park Dr Foster City, CA 94404) according to Ross *et al.* [12]. The intra- and inter-assay coefficients of variation were 6 and 13% for P4 assay. The assay sensitivity, expressed as the value of 2 standard deviations below maximum binding (zero standard), was 0-3 ng/ml.

2.5. Statistical Analysis

Data were statistically analyzed using SAS [13] software, version 8.1 (SAS Institute, Cary, North Carolina, USA). The data of the experiment were analyzed employing the two-way analysis of variance (ANOVA) to study the effect of litter size category (low and high), type of doe rabbits (NP and SP), and their interactions on different parameters studied using the GLM procedure of SAS (SAS Institute Inc., Cary, Nc.).

3. Results

3.1. Frequency Distribution of Litter Size

Data obtained revealed that the minimum-maximum litter size (LS) was 3-8 and 4-10 kits/litter for NP and SP, respectively. Among the different types of LS, the highest frequency distribution was for NP and SP doe rabbits with 6 LS (Table 1).

Table 1. *Frequency distribution of does with different types of litter size of NP and SP doe rabbits.*

Doe type	Frequency distribution (%) of litter size								LS type	
	3	4	5	6	7	8	9	10	<7	≥7
NP	7.69	12.82	17.95	25.64	23.08	12.82	0.00	0.00	64.10	35.90
SP	0.00	2.56	17.95	28.21	15.38	23.08	10.26	2.56	48.72	51.28
Total	3.85	7.69	17.95	26.92	19.23	17.95	5.13	1.28	56.41	41.59

3.2. Mating and Conception Parameters

Results in Table 2 showed that the live body weight of rabbit does at mating was higher (P<0.0001) for high (HLS) than low (LLS) litter size does, and SP than NP does. The effect of interaction between LS and doe type was significant (P<0.0001). Conception rate was not affected by LS, doe type and their interaction. Reproductive index (RI) was higher in HLS than in LLS (P<0.001) and in SP than in NP does (P<0.05). The effect of interaction on RI was not significant.

Table 2. *Live body weight, conception rate, and reproductive index of does with different litter sizes of NP and SP doe rabbits.*

Variable	Doe LBW at mating (g)	Conception rate	Reproductive index
Litter size			
Low (<7)	2883.4±21.33	81.5±5.80	3.60±1.87
High (≥7)	3101.8±49.60	87.9±5.61	6.23±2.48
P-value (Sig)	0.0001***	0.4560	0.000***
Doe type			
Nulliparous (NP)	2789.4±7.62	84.9±5.82	4.43±0.246
Second-parous (SP)	3167.8±33.43	84.5±5.82	5.40±0.237
P-value (Sig.)	0.0001***	0.9642	0.017*
Interaction (litter size x doe type)			
Low*NP	2786.4±9.010	84.0±7.40	3.29±0211
Low*SP	3011.1±27.96	78.9±9.63	3.91±230
High*NP	2794.6±14.22	85.7±9.70	5.57±0.264
High*SP	3316.8±35.48	90.0±0.068	6.89±2571
P-value (Sig.)	0.0001***	0.5852	0.5741

* Significant at P<0.05. *** Significant at P<0.001.

3.3. Change in Live Body Weight of Does

Results based on the overall mean of LS type or parity order (PO) are illustrated in Figure 1. Results revealed that NP and SP doe rabbit show a gradual increase from mating to late pregnancy (28 d), and then showed a marked reduction in their weight at parturition. From mating up to parturition, LBW was higher in HLS than LLS does and SP than NP does (P<0.0001).

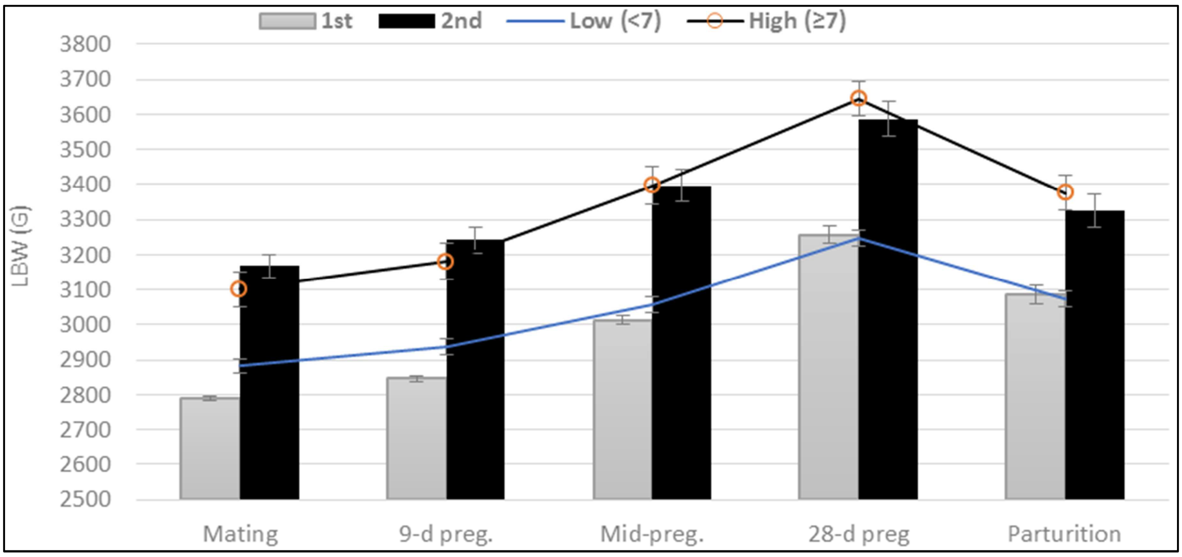

Figure 1. *Change in live body weight of doe rabbits during the 1st parity (NP) and second parity (SP), and doe rabbits with low and high litter size at different reproductive stages.*

The effect of interaction between LS and doe type on doe LBW at all times was significant (P<0.0001). In NP does, LBW of HLS was similar to that of LLS at mating, mid-pregnancy, and early pregnancy. At late pregnancy, LBW of HLS was higher than LLS for NP and SP does. Generally, the heaviest LBW at all reproductive times was for HLS does in the 2^{nd} parity (Figure 2).

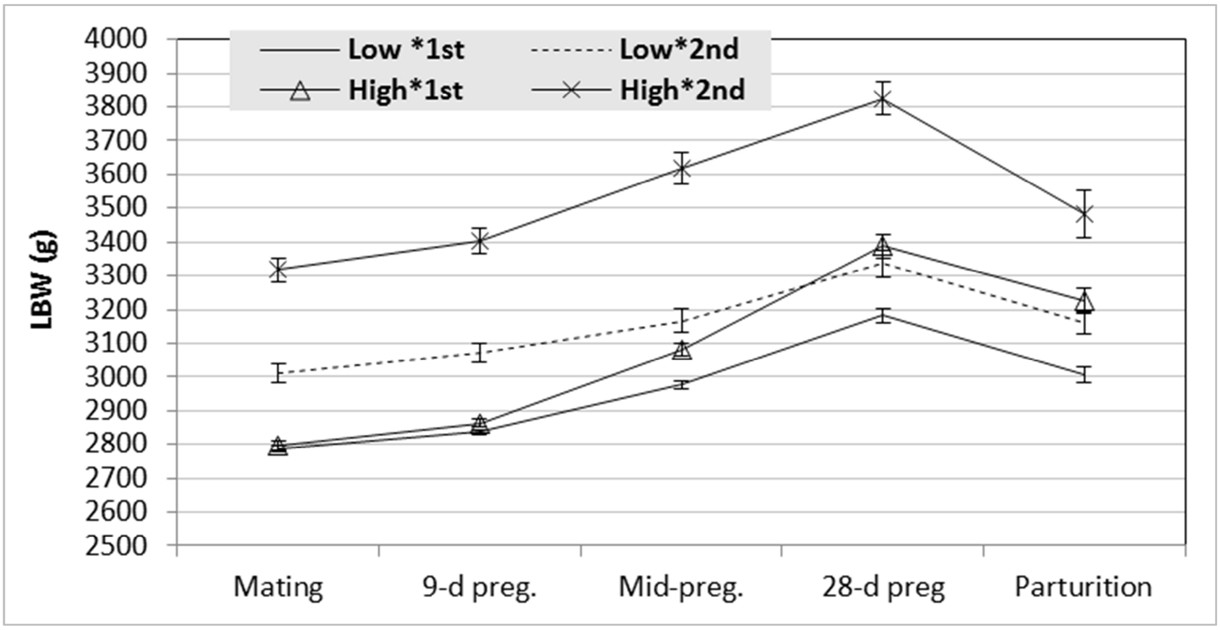

Figure 2. *Change in live body weight of NP (1^{st}) and SP (2^{nd}) doe rabbits with low or high litter size at different reproductive stages.*

3.4. Average Weight, Litter Size and Viability Rate of Kits at Birth

Average kit weight at birth was higher (P<0.001) in HLS than in LLS does. At birth, LS (total and live, P<0.001) and viability rate (P<0.05) were higher in HLS than in LLS does and for SP than NP does (P<0.01). The insignificant interaction effect (LS x doe type) on average kit weight, litter size and viability rate reflected the highest values in SP with HLS, and the lowest values in NP does with LLS (Tale 3).

Table 3. *Average kit weight, litter size and kit viability rate at the birth of does with different litter sizes of NP and SP doe rabbits.*

Variable	**Average kit weight (g) at birth**	**Kit litter size at birth**		**Kit viability rate at birth**
		Total	**Live**	
Litter size				
Low (<7)	56.64±0.51	5.20±0.140	4.36±0.166	84.05±2.32
High (≥7)	62.53±0.66	7.74±0.135	7.18±0.210	92.53±1.93
P-value $^{(Sig)}$	0.0001***	0.0001***	0.0001***	0.0214*
Doe type				
Nulliparous (NP)	58.48±0.707	5.82±0.234	4.85±0.255	82.97±2.381
Second-parous (SP)	59.92±0.770	6.80±0.232	6.33±0.278	92.53±1.938
P-value $^{(Sig)}$	0.3937	0.0023**	0.01**	0.0085**
Interaction (litter size x doe type)				
Low*NP	56.88±0.705	4.96±0.211	3.92±0.199	79.93±3.026
Low*SP	56.31±0.749	5.53±0.140	4.95±0.222	89.47±3.296
High*NP	61.35±1.202	7.36±0.132	6.50±0.272	88.39±3.530
High*SP	63.35±0.733	8.00±0.191	7.65±0.264	95.43±1.991
P-value $^{(Sig)}$	0.1289	0.8425	0.8005	0.6838

Ns: not significant. * Significant at P<0.05. ** Significant at P<0.01. *** Significant at P<0.001.

3.5. Average Weight, Litter Size and Sex Ratio of Kits at Weaning

At weaning, average kit weight (P<0.01), litter size (P<0.0001), and kit viability rate (P<0.05) were higher for HLS than LLS does. Only litter size at weaning increased (P<0.0001) for SP in comparing with NP does. Results of the interaction (LS x PO) reveal that SP does with HLS showed the highest average kit weight, litter size, and viability rate of kits, while NP does with LLS showed the lowest values. On the other hand, the sex ratio of kits at weaning was not affected by LS, doe type or their interaction (Table 4).

Table 4. *Average kit weight, litter size and sex ratio of kits at weaning of does with different litter sizes of NP and SP doe rabbits.*

Variable	Average kit weight (g) at weaning	Kit litter size at weaning	Viability rate at weaning	Kit sex ratio at weaning
Litter size				
Low (<7)	622.54±7.47	4.11±0.163	93.17±2.123	51.52±2.927
High (≥7)	685.83±10.37	6.53±0.239	100.0±0.00	54.26±2.614
P-value (Sig)	0.0021**	0.0001***	0.0425*	0.5766
Doe type				
Nulliparous (NP)	650.27±8.535	4.38±0.216	93.96±1.451	50.45±2.729
Second-parous (SP)	658.13±11.218	5.95±0.272	99.21±0.738	54.97±2.914
P-value (Sig)	0.2833	0.0001***	0.075	0.3892
Interaction (litter size x doe type)				
Low*NP	625.68±9.450	3.76±0.210	87.92±2.026	47.87±3.706
Low*SP	619.41±9.630	4.58±0.220	98.42±1.296	56.32±4.587
High*NP	674.85±11.182	5.50±0.291	100.0±0.00	55.07±3.579
High*SP	696.85±10.544	7.25±0.250	100.0±0.00	53.70±3.743
P-value (Sig)	0.1561	0.4052	0.1614	0.2335

Ns: not significant. * Significant at P<0.05. *** Significant at P<0.001.

3.6. Progesterone Profile

Results in Table 5 show that plasma P4 concentration on days 15 of pregnancy was affected significantly by LS. Plasma P4 concentration was higher at mid-pregnancy (P<0.001) in HLS than LLS does. Concentrations of P4 at mid-pregnancy was higher (P<0.001) in SP than NP does. The significant interaction cleared more effect of LS than doe type on increasing P4 at mid-pregnancy. However, P4 concentration at late pregnancy was affected significantly (P<0.01) only by LS, being lower for HLS than LLS does.

Table 5. *Plasma profile of progesterone in blood plasma of does with different litter sizes of NP and SP doe rabbits.*

Variable	Plasma progesterone (ng/ml)	
	Pregnancy (Day 15)	Pregnancy (Day 28)
Litter size		
Low (<7)	10.265±0.137	3.911±0.115
High (≥7)	12.100±0.207	3.511±0.074
P-value (Sig)	0.0001***	0.0092**
Doe type		
Nulliparous (NP)	10.422±0.155	3.764±0.109
Second-parous (SP)	11.708±0.233	3.710±0.106
P-value (Sig)	0.0001***	0.9367
Interaction (litter size x doe type)		
Low*NP	10.042±0.180	3.918±0.155
Low*SP	10.559±0.198	3.903±0.175
High*NP	11.101±0.184	3.488±0.089
High*SP	12.799±0.219	3.527±0.111
P-value (Sig)	0.005**	0.8610

Ns: not significant. ** Significant at P<0.01. *** Significant at P<0.001.

4. Discussion

Several factors are affecting the reproductive performance of rabbit does. The profitability of rabbit farms has increased mainly due to improvements in management and genetic selection. The effect of kindling order, litter size, weaning age and reproductive rhythm on the reproductive performance and welfare of females and mechanisms implicated in these effects are discussed by Castellini *et al.* [14]. Does with LS of six kits have high sexual receptivity, fertility rate, and milk suckled per kit. This greater milk availability determines high body weight and lower mortality rate of kits [10]. Relationship between body weight at first mating and subsequent body weight development, feed intake, and reproductive performance of rabbit does were studied by Rommers et al. [15]. They reported that young rabbit does are vulnerable to body energy deficit in the first lactation, causing a reduction in the reproductive efficiency and high replacement rate. However, heavy does at first mating might be able to benefit from the extra amount of body weight to cope with the energy deficit during the first lactation.

The present results indicated a pronounced increase in the frequency distribution of NZW does with HLS (>7) from 35.9% for NP to 51.28% for SP does. Litter size was 3-8 in NP and 4-10 in SP does; the most frequent LS was 6 kits for all does. A similar trend was observed in NZW rabbits, LS was 1-12; the most frequent LS was 6 kits, ranging from 4-8 and frequency of does with LS of ≤6 was about 60% [11].

These findings represent a proper number of kits to the teat number in the female rabbit (8-10) and may ensure good management of the kits after parturition.

The obtained data reveal significant effects of LS, doe type and their interaction on body weight of does at mating, but these effects were not significant on conception rate and the number of services per conception. At mating, overall mean of body weight of does was heavier (P<0.05) in HLS than LLS does (3101.8±49.60 vs. 2883.4±21.33 kg), and for SP than NP does (3167.8±33.43 vs. 2789.4±7.62). However, the significant (P<0.001) interaction between LS and doe type reflected higher LBW at the mating of SP does with LLS than NP does with HLS. These results indicate improved LS of doses by increasing LBW at the mating for both NP and SP does. The observed higher LBW at the mating in HLS than LLS for SP does, is associated with improving body weight of the same rabbit does, besides the improving in LS in SP as compared to NP does. In agreement with the present study, Rommers *et al*. [15] found that a higher body weight improved LS of NP does, being 8.9, 7.7, and 6.4 for heavy, medium, and small does, respectively (P<0.05). Extra body weight at the start of reproduction in NP does improves LS. On the other hand, an opposite trend was reported by Ayo-Ajasa *et al*. [16] on local rabbits in Nigeria. They observed that does with moderate weights (1901-2200 g) recorded the highest LS at birth and weaning as compared to low (1600-1900 kg) and high weights (2201-2500 kg). Also, Mahmoud [11] recorded higher LS for NZW does of 2.5-3 kg than in those with 3-3.5 or 3.5-4 kg.

It is of interest to note that body weight of does at mating in our study showed a positive relationship with total LS at birth and weaning for SP and NP does. Studying the change in doe body weight due to pregnancy as affected by LS and doe type, the present study reveals a similar trend of changes in the overall mean of LBW of all does with HLS and LLS at different stages of pregnancy and parturition. Increasing overall mean of body weight in SP does with HLS than NP with LLS at different reproductive times is in association with LBW of does at mating. Increasing body weight of NP does with HLS in comparing with SP does with LLS, particularly at late pregnancy was mainly related to increasing their LS. Rommers *et al*. [15] found that heavy does were heavier at first mating and remained so throughout the reproductive stages, but they followed a similar body weight curve as medium and small does. However, Oguike and Okocha [17] found a non-significant change in the doe body weight at mating and parturition.

Although the conception rate was nearly similar in HLS and LLS and for NP and SP, reproductive index increased in HLS versus LLS, and for SP versus NP does. These findings are in association with higher LS at birth and weaning for HLS than LLS for NP and SP does. These findings are often attributed to higher body weight of HLS than LLS does, which may be in association with poor body condition [18] or a deficit in body energy [21] of LLS as compared to HLS does. In agreement with the present results, LS of NZW does was higher in SP than NP does [11] and a higher fertility rate was recorded in multiparous than primiparous does [19]. Moreover, the smallest LS was recorded at the first kindling [19, 20]. In contrast, Xiccato *et al*. [21] found no change in fertility rate at the second kindling in comparing with the 1st one.

Average kit weight at birth and weaning was higher for HLS than LLS does for NP and SP does. This means that litter weight at birth and weaning was higher for HLS than LLS does in parallel with LS at birth and weaning. Contrary, Mahmoud [11] found that kid birth weight was inversely affected by its litter size. Also, Xiccato *et al*. [21] found that litter weight increased by the third kindling and then declined. The litter size at birth was increased as parity increased reaching the peak at the 3rd parity then decreased gradually thereafter [11]. Generally, the growth of kits until weaning is limited by the milk production of the doe [20], and milk yield was mainly affected by body weight of does during the suckling period. Although the number of kits per litter affects the quantity of produced milk, and kits reared in larger litters have access to a smaller amount of milk [22], higher average kit weight of HLS than LLS does may be probably due to sufficient milk yield of HLS than LLS, in reference with body weight. Mahmoud [11] revealed a significant effect of the dam weight on the LS and the kit birth weight. However, Jimenez et al. [23] reported no consistent relation between the mother's weight and the total weight of the kits at birth. Rommers *et al*. [15] found that LS was in a direct relationship with the body weight of the dam being higher in the heavy dams and low in the lighter ones only in the 1st parity. The present study clear that neither LS nor doe type has a pronounced effect on the sex ratio of kits, although Mahmoud [11] reported that the sex ratio of kits increased by increasing LS. Our results reveal increased P4 level during mid-pregnancy for HLS does is in association with improving LS and viability rate at weaning due to increasing milk production during the suckling period. The recorded increase in P4 at mid-pregnancy was in a strong relationship with LS.

5. Conclusion

The obtained results indicated that, high litter size category (≥7/doe) was obtained from heavier doe rabbits (LBW around 3 kg) and during the second parity, with near similarity in conception rate of does. Weight and viability rate at birth and weaning as well as plasma P4 level at mid-pregnancy were higher for high than low litter size does. In conclusion, body weight and parity may have a significant role in improving the litter size of doe rabbits, which may be useful in breeding and managerial programs to increase the economic value of rabbit production.

Data Availability Statement

The data sets generated and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Compliance with Ethical Standards

The experimental procedures were conducted according to the Directive 2010/63/EU of the European Parliament and of the Council of 22 September, 2010 on the Protection of Animals Used for Scientific Purposes.

Authorship Contribution Statement

Substantial contributions to conception and design (Abo-Farw M. A.; Nagy W. M.; Zayed Sh. M.; Rehab F. Ismail); acquisition of data (Abo-Farw M. A.; Zayed Sh. M.; Rehab F. Ismail, Younan G. E.); analysis and interpretation of data (Nagy W. M.; Zayed Sh. M.; Rehab F. Ismail, Younan G. E.); statistical analyses (Nagy W. M.; Zayed Sh. M., Younan G. E.), drafting the manuscript; critically revising the manuscript for important intellectual content and final approval of the manuscript for publication (All authors).

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