

# The Impact of Re-Mating Interval and Genotype on Physiological Response of Rabbits after First Kindling

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## Abstract

This is an experiment aimed to study the effect of re-mating interval on rabbit does after first kindling on hormonal (insulin, leptin, and T3) and metabolites (triglycerides, urea, and glucose) levels. DNA damage in ovary cells of rabbit does during the 2nd parity was also studied. Two varieties were used: APRI (synthetic line) and Baladi Black (BB, Egyptian breed). A total number of 120 mature rabbit does (60 does for each breed) were 6 months of age and were used at the beginning of the breeding season. Does of each breed were divided into three equal groups according to reproductive rhythm. The 1<sup>st</sup> group was postpartum (PP). The 2<sup>nd</sup> group was 11 days after parturition (P11). The 3<sup>rd</sup> group was post-weaning (PW). There were significant ( $P \geq 0.05$ ) differences in plasma leptin concentration during 1<sup>st</sup> parity. The highest value of plasma leptin concentration was recorded by the PW group at mating. Also, there were significant differences in plasma insulin and T3 hormones concentrations of doe rabbits. The highest value of plasma insulin concentration was recorded by the PW group at mating in 1<sup>st</sup> parity and the highest value of plasma T3 hormone concentration was recorded for the PS group at mating. While there were insignificant differences during 2<sup>nd</sup> parity in T3 hormone concentration in rabbits, the differences of plasma glucose and triglyceride concentrations of doe rabbits during 1<sup>st</sup> parity and 2<sup>nd</sup> parity were significant. However, the highest significant value of plasma glucose concentration was recorded by the PW group at mating. On the other hand, there were insignificant differences in plasma urea concentration of doe rabbits during 1<sup>st</sup> parity and 2<sup>nd</sup> parity. Finally, no significant effects were observed on comet length, head diameter, tail length, or DNA % tail.

**Keywords:** DNA, hormone, metabolism, parity, nutrition

## 1. Introduction

Food shortage is a global problem facing the world. In 2030, man may suffer hunger due to a higher population with more economic complications caused by the COVID-19 pandemic, especially in food production (FAO, 2021). The necessity of food varies from one foodstuff to another, but protein records the most needed one; so many searches were done to increase the source of animal protein production. Rabbits (*Oryctolagus cuniculus*) may participate to solve this problem as they provide man with protein, and many foodstuffs, beside their ability to fast reproduce with many offspring (Para et al., 2015).

The most important factor that determines the rabbit does' production is their body condition (Pascual, 2010), and that includes nutritional system (De Blas, 2013); energy balance (Andrea et al., 2019); lactation (Bolet and Fortun-Lamothe, 2002); and the reproduction plan (Saha et al., 2013).

To trace the nutritional system, many hormones can be investigated such as leptin (KloK et al., 2007); T3 and T4 (Nishanth et al., 2016); and insulin (Slater et al., 2019).

On the other hand, in order to monitor the nutritional system, many metabolites can be monitored, such as glucose (Jaiswal et al., 2019) and urea and triglycerides which are two important metabolites indicating body food metabolism and energy conservation (Niels and Jens, 2009).

## 2. Literature Review

The most controlling factors that regulate the rabbit does' production is their body condition (Pascual, 2010). Several works indicate that modern primiparous rabbit does show a clear energy deficit during lactation (Bolet and Fortun-Lamothe, 2002; Pascual et al., 2002). During the final phase of gestation that coincides with the lactation peak, the does show energy deficit (Xiccato, 1996); thus, feed intake cannot provide them with their energy demand. The primiparous lactating does lose an exceptionally large part of their fat depots and that can be explained with the growth of their own body as they are inseminated after reaching 75-80% of their adult weight. The does' condition is the most critical after the first kindling (Matics et al., 2011).

The reproductive plan of rabbit does may have a significant impact on the optimization of the rabbit doe fertility and growth rate based on the alternative mating interval. The optimum rabbit re-mating interval may play a principle role in increasing reproductive activity of does and the growth of their kits (Saha et al., 2013). Several hormones such as leptin, insulin, and T3 link growth, metabolism, energy homeostasis, and reproduction functions (Hornick et al., 2000).

Leptin is a mediator of long-term regulation of energy balance, inhibiting food intake and thus inducing weight loss (KloK et al., 2007). It is synthesized by adipocytes (Zhang et al., 1994), and it regulates the food consumption, energy outflow, cell metabolism (Barb, 1999), and control of reproductive harmony (Zerani et al., 2004). Leptin is capable of effectively reducing food intake and body weight (Andrea et al., 2019). Cardinali et al. (2008) described that about 1/3 of does which are inseminated at 11 days postpartum have a good body condition. So, it is not only hormonal parameters that influence reproductive performances, but also body status. Cardinali et al. (2009) differentiated between the hormones and metabolites concentrations in blood of nulliparous does (just gestation effect) and primiparous does (interference between gestation and lactation). They concluded that it is possible to approve that leptin level decreases during gestation, while the parity order has a little effect. Non-esterified fatty acids level replicates the energy shortage in primiparous does.

Also, T3 is an active form of thyroid gland hormone. It is an important indicator to analyze the metabolic activity, glucose level, and the energy balance. T3 is synthesized by deiodination of T4 (Nishanth et al., 2016).

Insulin is an anabolic hormone that stimulates metabolic effects throughout the body. It is secreted by beta cells inside the pancreas. By following glucose levels, amino acids, keto acids, and fatty acids circulating within the plasma, beta cells regulate the production of insulin accordingly. The role of insulin is to control energy conservation and utilization during feeding and fasting states (Zhao et al., 2017; Najjar and Perdomo, 2019; Slater et al., 2019).

Glucose is the main and central molecule of energy production. The metabolism of carbohydrate, protein, and lipid synthesizes glucose, which acts as the chief metabolic fuel for both mature and fetus mammals. Glucose has anabolic reactions that create hormones, cell membranes, essential molecules, nerve impulse conduction, cell division and growth, and other physiological functions (Jaiswal et al., 2019). Glucose metabolism takes place through several ways like glycolysis, gluconeogenesis, and glycogenolysis. Glycolysis occurs in the liver and needs definite enzymes that participate in glucose catabolism in cells. Particularly, glucokinase is the enzyme which permits the liver to adjust serum glucose levels and to utilize glucose when serum glucose levels rise after meals (Han et al., 2016). Castillo et al. (2005) concluded that low maternal serum glucose at parturition may be a result of the higher needs of glucose required for final foetal growth. The liver exhibits a decrease in gluconeogenesis and an increase in glycogenesis in response to the presence of insulin. Insulin effect stretches to lipid and protein metabolism as well. It stimulates lipogenesis and protein synthesis and conversely inhibits lipolysis and protein degradation (Zhao et al., 2017; Najjar and Perdomo, 2019; Slater et al., 2019).

Beside glucose monitoring, urea and triglycerides are two important metabolites indicating body food metabolism and energy conservation (Niels and Jens, 2009). Urea is the chief nitrogenous end product of the metabolic breakdown of proteins in all mammals and some fishes. In the course of the breakdown of proteins, amino groups ( $\text{NH}_2$ ) are removed from the amino acids that partly comprise proteins. These amino groups are converted to ammonia ( $\text{NH}_3$ ), which is toxic to the body and thus must be converted to urea by the liver. The urea then passes to the kidneys and is eventually excreted in the urine (Britannica, 2021). Since urea is used as an indicator for nitrogen metabolism, more plasma urea means more protein catabolism (Lise et al., 1996).

Triglyceride is an important indicator of fat metabolism. It is re-esterified free fatty acids in the liver, kept and deposited as an adipose tissue (Tuvdendorj et al., 2015). The liver plays the master controlling role of fat metabolism. This important role is to collect free fatty acids (FFAs) from the plasma, re-esterify them into triglycerides (TGs), and then push them again into the plasma in the form of very low density lipoprotein triglycerides (VLDL-TGs). Under normal conditions, the rate of FA release from adipose tissues is more than the

rate of fat oxidation; nevertheless, the extra FAs deliver a readily available substrate to allow a rapid increment in fat oxidation when required, e.g., at the onset of exercise (Lavoie and Gauthier, 2006; Yki-Järvinen, 2010; Reddy and Rao, 2006). The FAs in VLDL-TGs can be utilized as an energy reservoir by different tissues or taken up and stored in adipose tissue (Jackson, 1983; Wolfe, 1989).

The aim of the present study is to examine the influence of re-mating intervals after the first parturition on hormonal assay and metabolites assays of rabbit does controlling the glycaemia.

### 3. Material and Methods

#### 3.1 Experimental Design and Animals

In this experiment, we studied the effect of re-mating interval after first kindling (parity) only on hormonal assays [insulin, leptin, and triiodothyronine (T<sub>3</sub>)] and the metabolites assays of plasma triglycerides (TG); urea and plasma levels of glucose were determined in both APRI (synthetic line) and Baladi Black (BB, Egyptian breed) rabbits. We also studied DNA damage in ovary cells of rabbit does during the 2<sup>nd</sup> parity. A total number of 120 adult rabbit does (60 does for each breed) were 6 months of age and were used at the beginning of the breeding season. After the first parturition, all does from each strain (primiparous) were allocated to three equal groups, each of 20 does. The grouping after the first kindling was based on three mating intervals and where represented as follow:

- Group 1 (PP): mating interval 1 day post kindling.
- Group 2 (PS): mating interval 11 days post kindling.
- Group 3 (PW): mating interval 35 days post kindling (Weaning age).

During next parities in all groups, the does mated at 11 days from the second kindling to the sixth kindling or parity.

#### 3.2 Housing and Feeding

All rabbits were raised under the same environmental and managerial conditions, in a semi-closed rabbit system in which does were kept in individually wire cages (60x50x35 cm) and provided with feeders and automatic watering nipple drinkers at all times. The nest boxes for suckling rabbits measurement was 30x30x25 cm. Does were fed ad libitum, a commercial rabbit pelleted diet containing 16% crude protein, 13% crude fiber, and 2.4% fat. The diet was done according to the National Research Council (NRC) (1977) and formulated to cover all the essential nutrients requirements for rabbits. The free suckling system was applied. Young rabbits were weaned at 5 weeks, ear tagged, sexed, and transferred to progeny wire butches equipped with feeding hoppers and drinking nipples.

Cages of all animals were cleaned and disinfected regularly before each kindling. Manure that dropped from cages on the floor was collected and removed outside; the process was repeated daily, in the morning.

#### 3.3 Management

Animals were kept under natural light. Sex ratio was ranged from 3-5 females to 1 male, depending upon the available numbers throughout the experiment where the natural mating was used during the whole experimental period. Every doe was transported to a buck's cage randomly, where it was left for about 5-10 minutes after success of copulation and then re-backed to its cage. Twelve days after mating, the does were tested for pregnancy by abdominal palpation.

#### 3.4 Blood Parameters

Blood samples were taken individually from does of each group in the two genotypes, after 3 hours of fasting. They were taken two times at kindling and mating during 1<sup>st</sup> and 2<sup>nd</sup> parity only for hormonal and metabolites assay. The total number of collected samples was 96 samples (48 for the 1<sup>st</sup> and 48 for the 2<sup>nd</sup> parity) and divided as follows:

In 1<sup>st</sup> parity: 4 samples representing each subgroup of both breeds (APRI line and the same form Baladi Black) were collected randomly at kindling and the same at mating. 2<sup>nd</sup> parity: the same as followed in the 1<sup>st</sup> parity.

The blood was drawn into sterilized tubes containing EDTA, from the ear vein drawn. Blood samples were immediately centrifuged at 3000 rpm for 15 minutes for obtaining plasma and then stored frozen at -20°C until assayed for hormones and metabolites.

Hormonal assays included leptin, insulin, and T<sub>3</sub>, and plasma metabolites included urea, triglycerides, and glucose.

### 3.5 Hormonal Assays

Analyses were determined using kits according to the manufacturer's protocol. Plasma leptin concentrations were determined by ELISA method (DRG Diagnostics, Germany) according to Anubhuti and Arora (2008), while insulin and total triiodothyronine (T3) were measured by ELISA kits, Accu Bind, Monobind Inc., USA, according to Eastham (1986) and Comeau et al. (1991), respectively.

### 3.6 Metabolites Assays

Plasma triglycerides (TG) and urea were analyzed using kits, BioMed- Diagnostic, EGY-CHEM, according to Fossati and Prencipe (1982) and Chaney and Marbach (1962), respectively. Plasma levels of glucose were determined using blood glucose monitoring system, a specific device for the measurement of sugar. The device works by electrochemical method to measure blood sugar and comes with tapes dedicated to the device to help read the results of the test (Kahn and Weir, 1994).

### 3.7 Ovary Activity

Effects of experimental groups on ovarian structure were studied by assessment of follicular apoptosis in ovarian sections and were measured by comet assay that detects the disruptions of DNA happening during the follicular progression. In each group (after first kindling), two rabbit does from each group and strain at time of mating were slaughtered and their ovaries were dissected. They were used in comet tests. The whole ovaries were removed and then each ovary was stored frozen until used in the comet test as described by Singh et al. (1988).

### 3.8 Protocol Comet Test

Alkaline comet assay was done by applying the protocol of Singh et al. (1988) and Tice et al. (2000) with minor changes.

Microscopic slides were painted with 1% normal melting point agarose and left for at least 48h to dry at room temperature. After 2 h incubation, centrifugation (5 min at 2000 rpm) was done, and then 100  $\mu$ L of cell suspension was mixed with 100  $\mu$ L of 1% low melting point agarose (LMPA). The suspension was rapidly pipetted onto the first agarose layer and spread using a cover-slip and placed in the fridge to solidify. After removal of the cover-slip, the slides were immersed in a cold lysing solution at pH 10 (2.5 M NaCl, 100 mM EDTA, 10 mM Tris pH 10, 1% Triton X-100, 10% DMSO) for 60 min at 4°C in the dark. After lysis, the slides were subjected to a horizontal gel electrophoresis tank to allow DNA unwinding in cold alkaline electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH>13) for 30 min. Electrophoresis was done at 4°C with electric current of 35 V and 300 mA for 30 min. All these steps were performed in the dark (the tank was covered with a black cloth) to prevent additional DNA damage. The slides were then cleaned with DI H<sub>2</sub>O for 2 min and repeated twice. The slides were fixed with cold 70% Ethanol for 5 min then removed and allowed to air dry. Before drying completely, the slides were stained with 50  $\mu$ L of 20  $\mu$ g/mL ethidium bromide for 15 min at room temperature until a yellowish-brown color developed. The stained slides were examined by fluorescence microscopy using a FITC filter.

### 3.9 Scoring of Comets

Slides were examined at 400 $\times$  magnification on a fluorescent microscope (Leica, UK). One hundred comets (50 nuclei from each replicate slide) were scored according to Collins et al. (1995). Scoring of the comet visually as belonging to one of five classes according to tail intensity and given values 0, 1, 2, 3, or 4 as shown in Figure 13. Thus, the total score for 100 comets could range from 0 (all undamaged) to 4 (all maximally damaged). These visual scorings were analyzed in website [www.cometassay.com](http://www.cometassay.com) to obtain comet length, head diameter, tail length, and % of DNA in tail as parameters for DNA damage.

### 3.10 Statistical Analysis

Obtained data were subjected to SAS programmers (2001) to compare the two genotypes in the corresponding subgroup in addition to between each subgroup of each breed as well as different parities.

## 4. Results

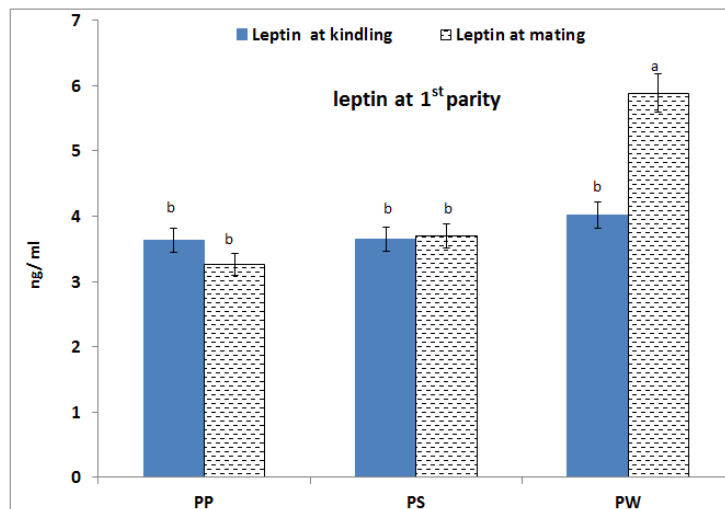
### 4.1 Influence of Re-Mating Interval Groups On

#### 4.1.1 Hormonal Assay

Results in Figures 1 and 2 showed that there were significant ( $P \leq 0.05$ ) differences in plasma leptin concentration during 1<sup>st</sup> parity. The highest ( $P \leq 0.05$ ) value of plasma leptin concentration was recorded by the PW group at mating compared with other different groups, whether at mating or kindling during 1<sup>st</sup> parity and 2<sup>nd</sup> parity. On the other hand, it is noticed that there are significant differences ( $P \leq 0.05$ ) in plasma insulin and T3 hormone (Figures 3-6) concentrations of doe rabbits due to the effect between stage and re-mating interval during 1<sup>st</sup>

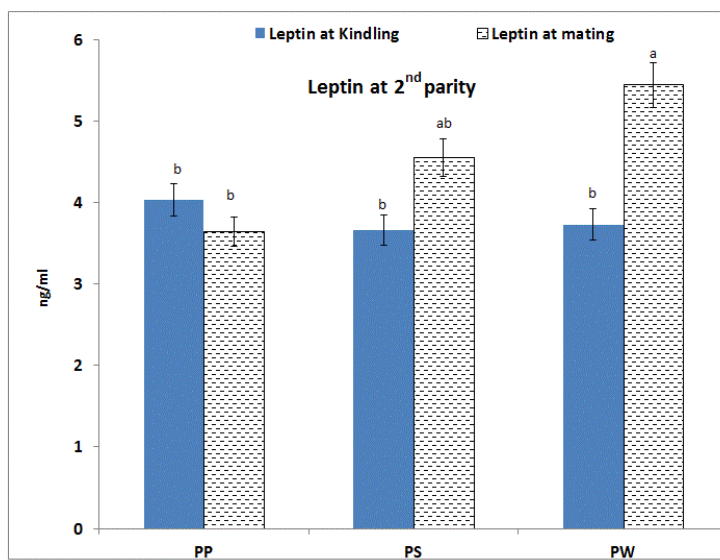
parity. The highest ( $P \leq 0.05$ ) value of plasma insulin concentration was recorded by the PW group at mating in 1<sup>st</sup> parity compared with other groups at mating and at kindling.

However, the highest ( $P \leq 0.05$ ) value of plasma T3 hormone concentration was recorded for the PS group at mating compared to both the PW group and the PP group, while there were insignificant ( $P \leq 0.05$ ) differences during 2<sup>nd</sup> parity in T3 hormone (Figure 6) concentration in rabbits.



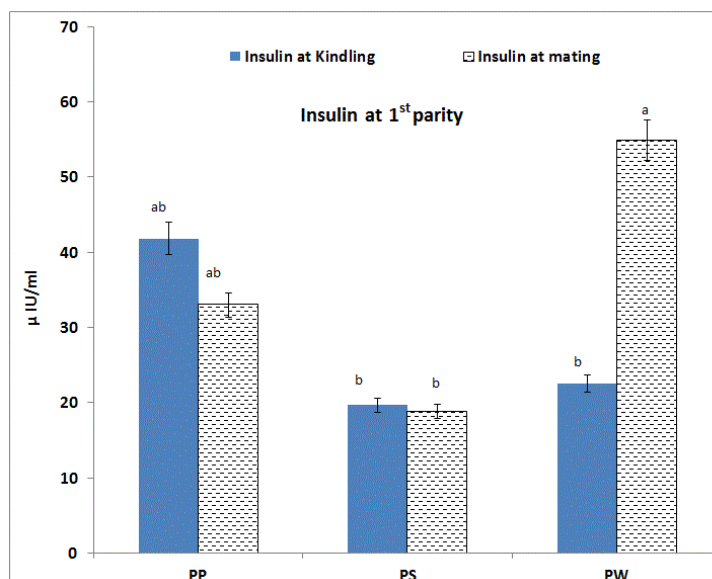
(PP): mating interval 1 day post kindling, (PS): mating interval 11 days post kindling, (PW): mating interval 35 days post kindling (weaning age).

Figure 1. Effects of mating interval groups on plasma leptin concentrations in rabbit does during 1st parity (Means  $\pm$  SE)



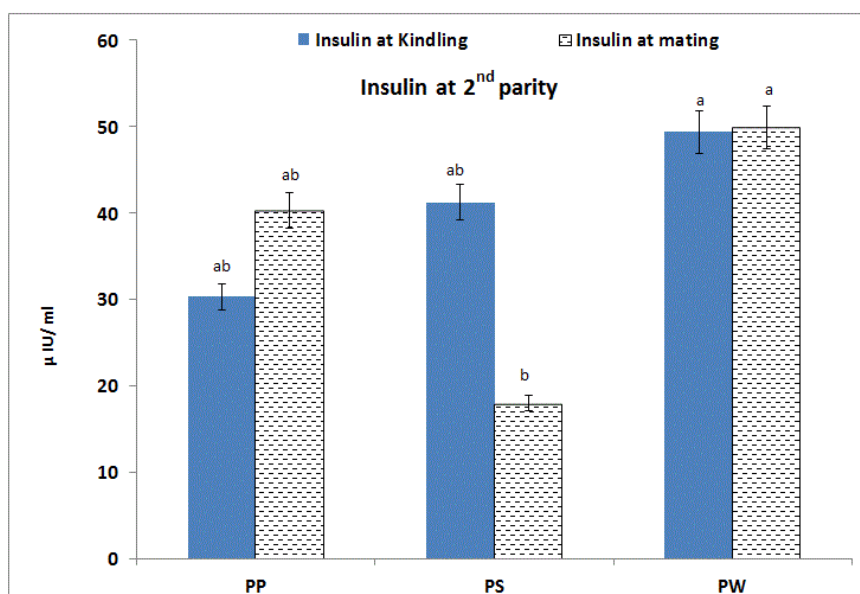
(PP): mating interval 1 day post kindling, (PS): mating interval 11 days post kindling, (PW): mating interval 35 days post kindling (weaning age).

Figure 2. Effects of mating interval groups on plasma leptin concentrations in rabbit does during 2nd parity (Means  $\pm$  SE)



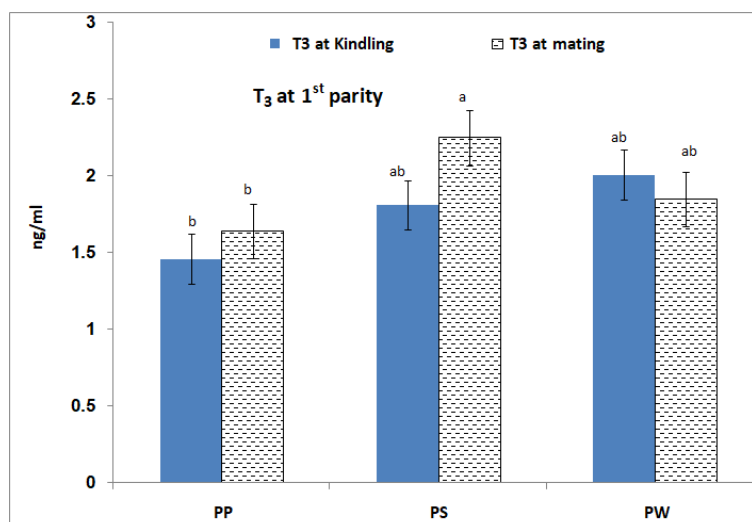
(PP): mating interval 1 day post kindling, (PS): mating interval 11 days post kindling, (PW): mating interval 35 days post kindling (weaning age).

Figure 3. Effects of mating interval groups on plasma insulin concentrations in rabbit does during 1st parity (Means  $\pm$  SE)



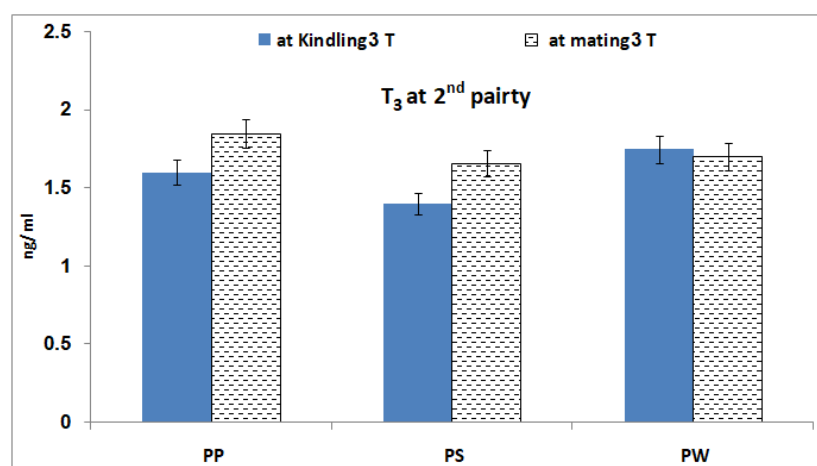
(PP): mating interval 1 day post kindling, (PS): mating interval 11 days post kindling, (PW): mating interval 35 days post kindling (weaning age).

Figure 4. Effects of mating interval groups on plasma insulin concentrations in rabbit does during 2nd parity (Means  $\pm$  SE)



(PP): mating interval 1 day post kindling, (PS): mating interval 11 days post kindling, (PW): mating interval 35 days post kindling (weaning age).

Figure 5. Effects of mating interval groups on plasma T<sub>3</sub> concentrations in rabbit does during 1<sup>st</sup> parity (Means ± SE)

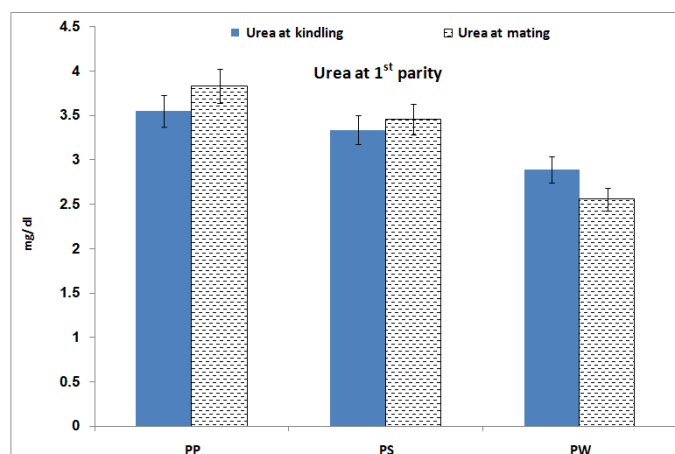


(PP): mating interval 1 day post kindling, (PS): mating interval 11 days post kindling, (PW): mating interval 35 days post kindling (weaning age).

Figure 6. Effects of mating interval groups on plasma T<sub>3</sub> concentrations in rabbit does during 2<sup>nd</sup> parity (Means ± SE)

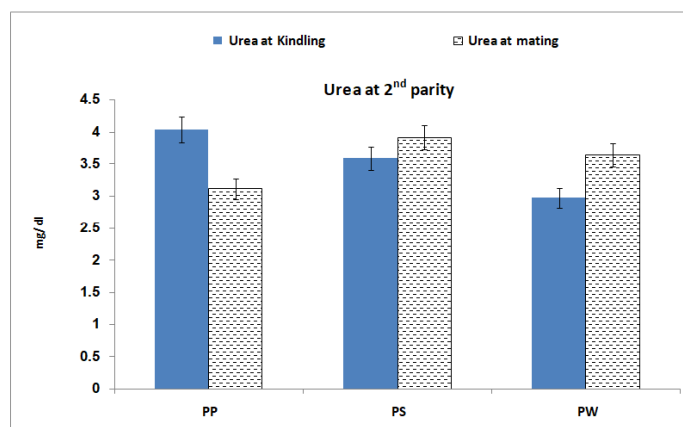
#### 4.1.2 Blood Biochemical Parameters

Results in Figures 9-12 showed significant differences ( $P \leq 0.05$ ) in plasma glucose and triglyceride concentrations of doe rabbits during 1<sup>st</sup> parity and 2<sup>nd</sup> parity. However, the highest significant ( $P \leq 0.05$ ) value of plasma glucose concentration was recorded by the PW group at mating compared with both PP group and PS group in 1<sup>st</sup> parity and 2<sup>nd</sup> parity. While the PS group at kindling in 1<sup>st</sup> parity recorded significantly ( $P \leq 0.05$ ) lower plasma triglyceride concentration than other groups at mating and kindling during 1<sup>st</sup> parity and 2<sup>nd</sup> parity. On the other hand, there were insignificant ( $P \leq 0.05$ ) differences in plasma urea concentration of doe rabbits during 1<sup>st</sup> parity and 2<sup>nd</sup> parity (Figures 7 and 8). Moreover, the lowest insignificant ( $P \leq 0.05$ ) value of plasma urea concentration was recorded by the PW group at mating during the 1<sup>st</sup> parity compared to other groups at mating and at kindling in 1<sup>st</sup> parity and 2<sup>nd</sup> parity. While the highest insignificant ( $P \leq 0.05$ ) value of plasma urea concentration was recorded by the PP group at kindling compared to other groups during 2<sup>nd</sup> parity at mating and at kindling in 1<sup>st</sup> parity and 2<sup>nd</sup> parity. The glucose levels did not show any significant variation in both stages within each group (Figures 9 and 10).



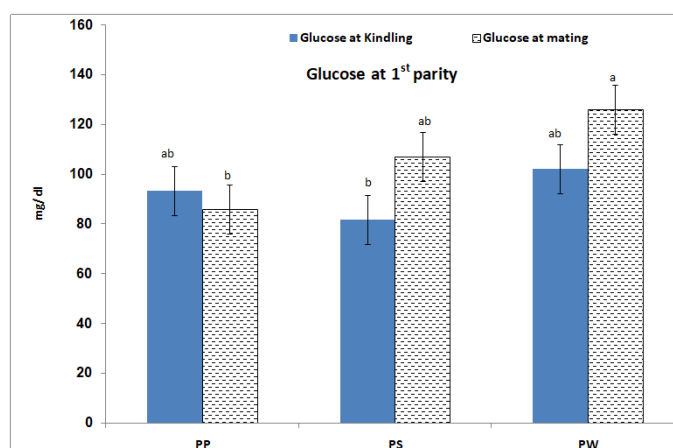
(PP): mating interval 1 day post kindling, (PS): mating interval 11 days post kindling, (PW): mating interval 35 days post kindling (weaning age).

Figure 7. Effect of mating interval groups on plasma urea concentration in rabbit does during 1st parity (Means  $\pm$  SE)



(PP): mating interval 1 day post kindling, (PS): mating interval 11 days post kindling, (PW): mating interval 35 days post kindling (weaning age).

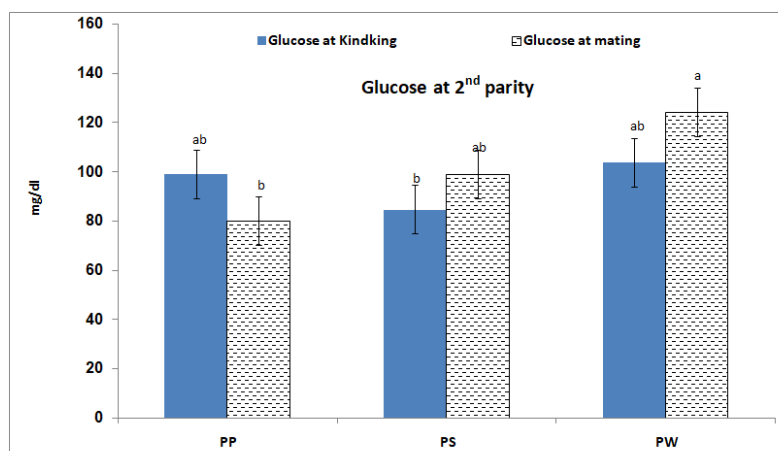
Figure 8. Effect of mating interval groups on plasma urea concentration in rabbit does during 2nd parity (Means  $\pm$  SE)



(PP): mating interval 1 day post kindling, (PS): mating interval 11 days post kindling, (PW): mating interval 35 days post kindling (weaning age).

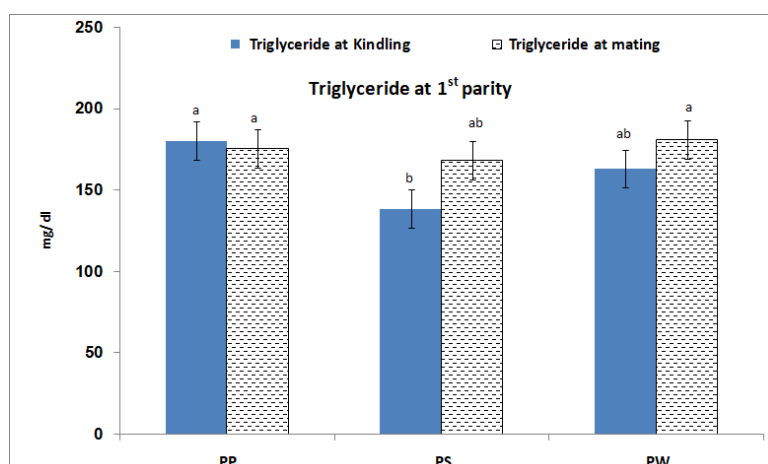
Figure 9. Effects of mating interval groups on plasma glucose concentration in rabbit does during 1st parity (Means  $\pm$  SE)





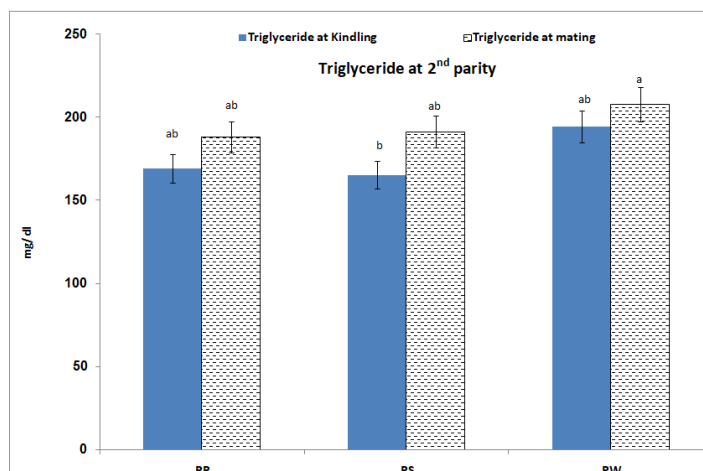
(PP): mating interval 1 day post kindling, (PS): mating interval 11 days post kindling, (PW): mating interval 35 days post kindling (weaning age).

Figure 10. Effect of mating interval groups on glucose in rabbit does during 2<sup>nd</sup> parity (Means  $\pm$  SE)



(PP): mating interval 1 day post kindling, (PS): mating interval 11 days post kindling, (PW): mating interval 35 days post kindling (weaning age).

Figure 11. Effect of mating interval groups on triglyceride in rabbit does during 1<sup>st</sup> parity (Means  $\pm$  SE)



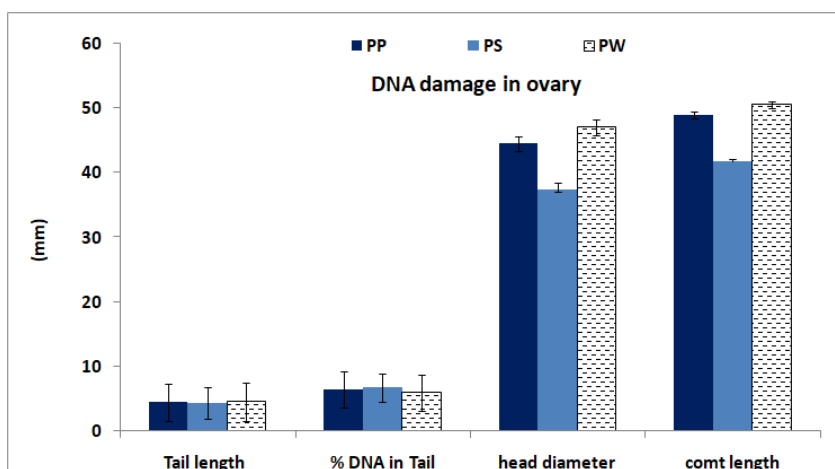
(PP): mating interval 1 day post kindling, (PS): mating interval 11 days post kindling, (PW): mating interval 35 days post kindling (weaning age).

Figure 12. Effects of mating interval groups on triglyceride in rabbit does during 2<sup>nd</sup> parity (Means  $\pm$  SE)

### 4.1.3 Ovarian Status

#### 4.1.3.1 Results of Comet Test

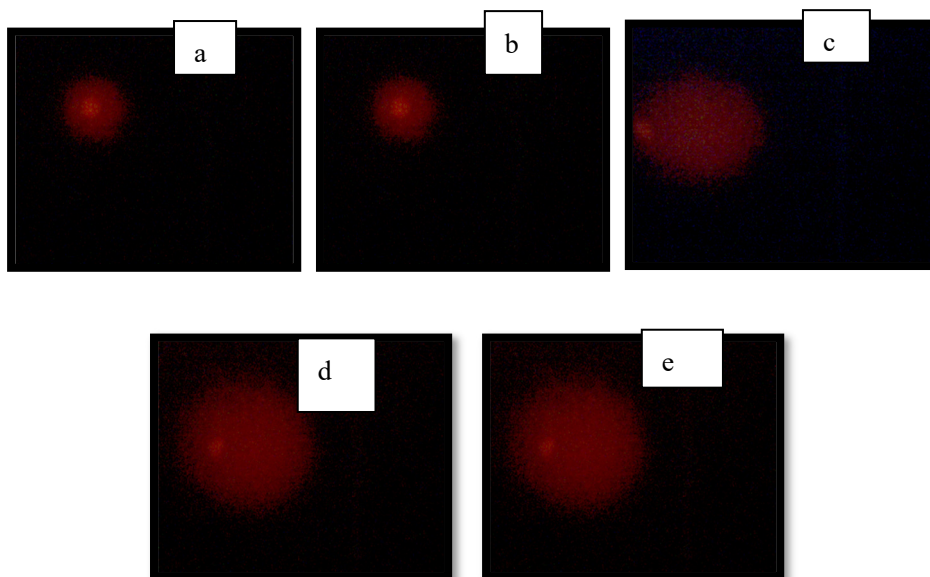
Data presented in Figure 13 illustrate the effect of re-mating interval groups after 1st parity on DNA damage in ovary cells of doe rabbits. No significant effects were observed on comet length, head diameter, tail length, or DNA % tail. However, PW group had the highest values of both comet length and head diameter, followed by PP group then PS group, which had the lowest value in the same previous parameters. On the other hand, the PW group recorded the lowest values of tail length and % DNA in tail compared with PP and PS doe rabbits. Besides, PP doe rabbits had the highest value of tail length compared to PS and PW doe rabbits. While the highest value of % DNA in the tail was recorded by the PS group compared to PP and PW groups.



(PP): mating interval 1 day post kindling, (PS): mating interval 11 days post kindling, (PW): mating interval 35 days post kindling (weaning age).

Figure 13. Effect of mating interval groups on DNA damage in ovary cells of rabbit does during the 2<sup>nd</sup> parity (Means  $\pm$  SE)

#### 4.1.3.2 Plate



\* Relationship between blood parameters

Plate 1. Various levels of DNA damage in ovary of rabbit in the comet assay: (a) none = 0, (b) low = 1, (c) medium = 2, (d) high = 3 and (e) total = 4

#### 4.2 Interaction Analysis Between Different Parameters

Results in Table (1) demonstrated the degrees of the correlation coefficient between the investigated parameters. There were seven strong correlation coefficients between leptin and triglycerides, glucose and triglycerides, leptin and urea, insulin and triglycerides, insulin and urea, T<sub>3</sub> and triglycerides, and finally insulin and urea in descending order. The moderated correlation coefficients were five relations. They were for leptin and insulin, leptin and glucose, leptin and T<sub>3</sub>, insulin and glucose and, at the end, insulin and T<sub>3</sub>, in descending order. The last three mild correlation coefficients were those of triglycerides and glucose, urea and glucose, while the last one for urea and T<sub>3</sub>, in descending order.

Table 1. Correlation between the different blood parameter interaction analysis between different parameters

Parameter	Urea	Glucose	Triglycerides	T <sub>3</sub>	Insulin	Leptin
Urea		0.1769	0.5318	0.1448	0.5652	0.9034
Glucose	0.1769		0.2788	0.9164	0.3275	0.3923
Triglycerides	0.5318	0.2788		0.5613	0.6061	0.9660
T <sub>3</sub>	0.1448	0.9164	0.5613		0.3261	0.3443
Insulin	0.5652	0.3275	0.6061	0.3261		0.4269
Leptin	0.9034	0.3923	0.9660	0.3443	0.4269	

#### 5. Discussion

In this study, there were significant ( $P \leq 0.05$ ) differences in plasma leptin concentration, plasma insulin, and T<sub>3</sub> hormones during 1<sup>st</sup> parity, and there were insignificant ( $P \leq 0.05$ ) differences during 2<sup>nd</sup> parity in T<sub>3</sub> hormone. These results may be related to the body growth or body composition status which is in agreement with other studies in rabbits (Rommers et al., 2004), heifers (Yambayamba et al., 1996), bulls (Hornick et al., 1998), sows (Quesnel et al., 1998), and chickens (Bruggeman et al., 1997). It is well-known that leptin hormone is produced and secreted by adipose cells, and it has an important role in the long-term regulation of body weight and body fat mass content, since leptin regulates food intake and metabolism via the central nervous system, where its leptin receptors are expressed in high quantities in the hypothalamus (Fei et al., 1997).

On the other hand, insulin and T<sub>3</sub> increased in mating as a physiological reply to energetic requirements during the suckling (Martinez-Paredes et al., 2012). In the present study, leptin increased at mating in the PW group which may reflect the body condition status (BCS). Plasma T<sub>3</sub> hormone concentrations were higher in the PS group (primiparous does) which may be due to the energy shortage as a result of the production of milk, which contributed to the forcefulness of the energy mobilization (Martinez-Paredes et al., 2012). In this study, the overlapping between gestation and lactation was observed in the PP and PS groups and had reduced the blood concentration of leptin, and this may be reflecting the reduction of fat reserves. So, it is not just hormonal participation regulating reproductive functions, but it is also a body status (Cardinali et al., 2008).

Also, we observed significant differences ( $P \leq 0.05$ ) in plasma glucose and triglyceride concentrations of doe rabbits during 1<sup>st</sup> parity and 2<sup>nd</sup> parity, while there were insignificant ( $P \leq 0.05$ ) differences in plasma urea concentration of doe rabbits during 1<sup>st</sup> parity and 2<sup>nd</sup> parity. These results of plasma urea concentration were similar to those of Habeeb et al. (1997) who concluded that the decrease in urea nitrogen may be as a result of the decrease in feed intake in summer than in winter; meanwhile, the decrease in blood metabolites may be correlated with the reduction of metabolism, whereas the increase of its concentrations may be dependant on the increase in cell protein catabolism.

In this study, we observed that in 2<sup>nd</sup> parity there were significant differences ( $P \leq 0.05$ ) in plasma glucose and triglyceride concentrations of doe rabbits, while glucose concentrations did not give any significant difference in both stages within each group, and this can be discussed through the homeostatic mechanisms controlling the glycaemia (Cardinali et al., 2009). Secretion of fatty acids as triglycerides leads to more potential energy source as circulating lipids (Tuvdendorj et al., 2015). The concentration of triglyceride was significantly lower ( $P \leq 0.05$ ) in the later pregnancy that is associated with Chodová et al. (2017) in their experiment that examined restricted feeding and age in rabbits.

Also, in this study, no significant effects were observed on comet length, head diameter, tail length, or DNA % tail; so, this indicated that the PS group was more stressed than both the PP and PW groups. In the present study, we applied the alkaline comet assay which is reported as a sensitive, rapid, and simple method that has been widely used for detection of DNA damage (Chen et al., 2008; Azqueta et al., 2016), since alkaline comet assay is a very

important method for studying cells exposed to genotoxic or another oxidative stress which can cause increased DNA damage in vitro or in vivo. Also, it provides DNA damage quantitatively in single cells and is established as a valuable tool in fundamental DNA damage and repair studies (AL-Ahmed et al., 2016). The follicles are in turn specialized layers of theca cells (derived from stroma) and granulosa cells that surround and nourish the oocyte. The stroma and theca cells are the only ovarian cells in direct contact with systemic circulation (Johnston and Wallace, 2009). The follicles with granulosa cell-enriched populations were more responding with levels of DNA damage (Roti Roti et al., 2012).

The interaction result obtained in Table 1 can help us advise applying similar mating interval times, i.e., 35 days, 11 days, and 1 day post kindling, in descending order, to improve the health and physiological activity of both does and their kits.

## 6. Conclusion

Based on the results, it can be concluded that applying a lengthened period after the first kindling (by more than 10 days) or after weaning had a favorable effect on the does.

- 1) Hormones and metabolites: The highest ( $P \leq 0.05$ ) value of plasma leptin concentration was recorded by the PW group at mating compared with that recorded by different groups both of mating and kindling during 1<sup>st</sup> parity and 2<sup>nd</sup> parity.
- 2) The highest significant ( $P \leq 0.05$ ) value of plasma glucose concentration was recorded by the PW group at mating compared with both the PP group and the PS group.
- 3) Mating interval groups had insignificant ( $P \leq 0.05$ ) differences during 1<sup>st</sup> parity and 2<sup>nd</sup> parity of doe rabbits on urea concentration.
- 4) Data of the comet assay test presented the effect of mating interval groups during 2<sup>nd</sup> parity on DNA damage in ovary cells of doe rabbits. No significant effects were observed on comet length, head diameter, tail length, or DNA % in tail.

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