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Research Article

Estrous Cycle Length in the Algerian Arbia Goat: Exfoliative Vaginal Cytology and Serum Progesterone Levels

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Abstract

This study aimed to estimate the estrous cycle length of the Algerian Arbia goat in Northern Algeria. For this, eighteen (18) Arbia goats, aged between 2 and 6 years, were used in our work that took place in the experimental farm of the Saad Dahlab University (Blida, Algeria). Blood samples were taken from each goat twice a week (at a 2 or 3-day interval) for 3 months. The serum progesterone concentration was determined by Radio-Immuno-Assay. Smears of the vaginal mucosa were taken at the same time as the blood samples. The predominance of superficial cells on the smear of the vaginal mucosa as well as a serum progesterone level less than 1 ng/mL expressed the return to estrous which was considered the beginning of a new cycle. A negative correlation was observed between the percentage of superficial cells (SC) and serum progesterone (P4) levels in all goats. Our results showed a significant difference ($P<0.05$) between the means of different cycle lengths obtained among the females. In addition, normal cycles had an average of 20.11±1.85 days (17-25 days) representing 59.6% of cycles. Besides, a large number of short cycles (<17 days) with an average of 14.41±1.51 days were found representing 25.5% of recorded cycles. The number of long cycles (>25 days; with an average of 32.14±5.58 days), represented 14.9% of recorded cycles. Following these results, it can be concluded that the local goat in Northern Algeria had different types of cycles (normal, short, and long) with a large percentage of normal cycles.

Keywords: *Arbia goat*, *Cycle length, Serum progesterone, Vaginal cytology smears*

Introduction

Small ruminant production is one of the most important sources of meat in Algeria and plays a vital role in the country's food security [1]. Furthermore, the reproductive performance of ruminants is among the major concerns of breeders and their technical supervisors [2]. Knowledge of the reproductive physiology of the estrous cycle is important for animal management and to determine the reproductive and productive potential of animals [3]. The latter plays a key role in farm economics, not only in determining animal performance but also in decisions concerning selection and culling [2].

Perfect knowledge of the characteristics of the sexual cycle and its different stages is of decisive value in the success of breeding. In females with normal cycles, morphological, endocrine and secretory changes that occur in the ovaries and tubules usually represent the stage of the cycle. These changes have been associated with sexual steroid hormone levels. In the absence of infection, circulating levels of progesterone and estradiol 17β are the main determinants of the model of vaginal cytology [4,5].

It is well known that the female reproductive tract is a target for sex steroid hormones. The endometrium and the vaginal epithelium are especially influenced by sex hormones which determine their development and function [6]. The serum levels of estradiol 17β (E2) and progesterone (P4) directly influence the cytology pattern of the vagina. Examination of vaginal cells is a useful indicator of the estrous cycle in different species [7,8].

Vaginal cytology changes during the estrous cycle have been studied in sheep [9], goats [6], bovine [10] and rodents [11]. The morphology of exfoliated cells has been found to be very useful to determine the physiological and pathological status of the female as well as a tool for hormonal bioassay in several animal species [5,12]. Many researchers have studied the variations which occur in the vaginal mucosa at different phases of estrus cycle by using vaginal smears [13,14]. These variations occur under the influence of steroid hormones [15]. Exfoliated cells are a normal occurrence during the estrous cycle due to the increase of estrogen. As the stages of the cycle advance to estrus, mostly cornified epithelial cells are present [14].The relative proportion of different types of vaginal epithelial cells can be used as a marker of the endocrine environment [15,16].

In Algeria, as far as the authors know, there is little or no work done on the nature of reproductive cyclicity in local goats and the characterization of sexual cycle parameters. These data are imperfectly known and are still unclear. Thus, the purpose of the present work was to study the reproductive cyclicity by determining the estrous cycle length of the Algerian Arbia goat.

Material and Methods

Ethical Statement

All the animal studies were conducted with the utmost regard for animal welfare, and all animal rights issues were appropriately observed. No animal suffered during the course of the work. All the experiments were carried out according to the guidelines of the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research (Agreement Number 45/DGLPAG/DVA.SDA.14).

Study Area

This current study took place in the experimental farm of the Saad Dahlab University of Blida 1, in Blida region, located between latitude 36°28' North and longitude 2°49' East. It is bordered in the North by Tipaza and Algiers, East by Boumerdes and Bouira, South by Medea and West by Ain Defla *(Fig. 1)*. It has a Mediterranean climate characterized by cold and rainywinters and hot and dry summers.

Animals

Eighteen Arbia goats, aged between 2 to 6 years, with an average live weight of 31.28 kg were used in our experiment.

To ensure that the females were not pregnant, an ultrasound examination was performed for each goat. Throughout the study period, the goats were separated from the males (buck) to avoid any unwanted protrusions.

Before the start of the study, the entire herd used for the experiment underwent internal and external antiparasitic treatment using Ivermectin (Ivomec1%®, Merial). The study took place during September, October, and November.

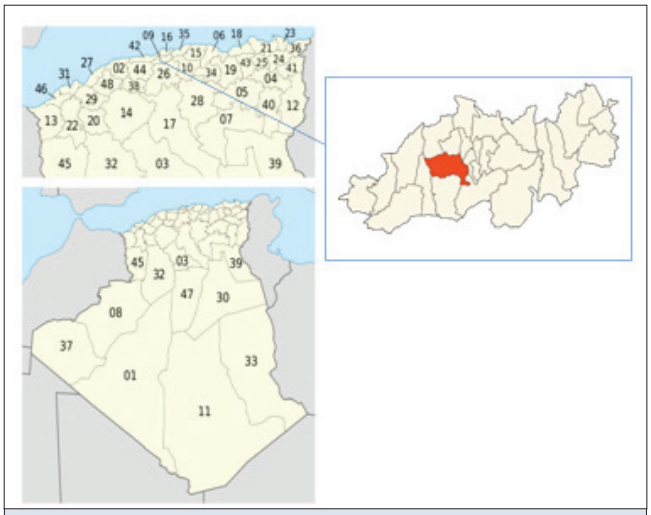

Fig 1. Study area localization

Diet

The entire herd received an identical diet throughout the experiment period. The animals received a daily feed ration consisting of oat hay, and a concentrate based on crushed barley, corn and bran distributed at a rate of 500 g/day/animal (In addition to the contribution acquired by free grazing in the meadow of the experimental station). Water was supplied *ad-libitum*.

Vaginal Smear and Cytology

Vaginal smears were collected from each female twice a week (at 2 or 3-day interval) for 3 months. We studied 312 smears in total. The smear collection procedure involved using a vaginoscope for parting the vulva lips and inserting a 10 cm long cotton-tipped sterile swab into the vagina to a depth of about 5-7 cm. The swab inside the vagina was rotated through 2-3 revolutions against the vaginal wall. It was withdrawn and rolled on a clean glass slide to form two parallel tracks of smear material on the glass surface. The smear was immediately fixed with absolute methanol, air-dried and stained with the Giemsa stain. The vaginal cells were classified under a light microscope into three basic cell types with different diameters; superficial squamous cells with light cytoplasm, intermediate squamous cells and parabasal cells [14]. Each cell type was counted and then expressed as a percentage of the total [15]. The predominance of superficial cells on the smear of the vaginal mucosa means the return to heat which was considered the beginning of a new cycle [17].

Hormonal Assay

In order to measure serum progesterone, blood samples (5 mL) were taken from the jugular vein of all females

twice a week (at a 2 or 3-day interval) for 3 months (in the same time as vaginal cytology samples). Following centrifugation at 3000 rpm for 20 min, serum was harvested and stored at -20°C until assayed. Progesterone concentration was determined by the RIA technique using an Immunotech kit (RIA Progesterone from IMMUNOTECH SAS France). The analysis was carried out at the Nuclear Research Center in Draria, Algiers.

Estrous cycle length was defined as the number of days between the onsets of two consecutive estrous periods [18]. Serum progesterone levels below 1 ng/mL associated with the predominance of superficial epithelial cells on vaginal smears indicated the beginning of a new cycle [19]. We were therefore able to determine the different lengths of estrous cycles by the detection of estrous, the perception at the same time of a large percentage of superficial cells on vaginal smears and the decrease of serum progesterone (<1 ng/mL).

Twenty-four samples were taken from each goat during the 3 months of the study (vaginal smears and blood samples), corresponding to 864 samples analyzed (432 smears and 432 serums).

Statistical Analysis

The differences between the percentage of epithelial cell types as well as the cycle length recorded after analysis of vaginal cytology and the determination of serum progesterone levels were calculated by the ANOVA test (statistical software Past3).

Results

According to our results *(Table 1)*, in 75.7% of all vaginal smears, intermediate epithelial cells predominated, followed by the superficial epithelial cells with 20% of smears, and a rate of 4.3% for the parabasal epithelial cells that were rarely encountered (P<0.05) *(Table 2)*.

Table 1. *Percentage of recorded epithelial cells*

Sample	Percentage of Superficiel Cells (%)																	
	goat 1	**goat 2**	**goat 3**	**goat 4**	**goat 5**	**goat 6**	**goat 7**	**goat 8**	**goat 9**	**goat 10**	**goat 11**	**goat 12**	**goat 13**	**goat 14**	**goat 15**	**goat 16**	**goat 17**	**goat 18**
1	2	9	30	30	9	9	11	0	0	20	2	22	12	10	1	29	76	2
2	7	12	50	40	3	52	4	1	0	80	4	0	16	64	5	87	23	13
3	12	65	7	10	50	76	10	3	80	5	7	3	75	30	23	38	2	73
4	21	43	0	1	64	33	32	2	33	3	0	9	26	12	68	18	7	33
5	26	5	2	0	13	15	99	0	11	2	80	20	23	0	12	2	0	5
6	73	8	90	0	38	5	65	5	67	9	76	39	0	2	4	6	28	2
7	25	12	20	12	17	0	8	10	32	18	11	18	20	5	0	12	64	12
8	2	15	10	34	14	0	2	55	11	74	2	8	65	88	0	0	23	69
9	5	43	1	80	71	0	1	92	39	20	4	30	25	20	15	24	10	21
10	11	16	3	0	77	10	5	42	34	2	34	11	4	15	56	77	75	11
11	12	6	45	10	22	8	7	22	56	12	42	22	5	9	40	12	9	5
12	28	7	70	5	17	22	11	30	25	65	76	11	10	12	11	5	2	3
13	29	8	0	0	78	32	15	27	19	7	10	50	22	25	10	2	16	6
14	56	52	0	0	49	49	55	0	4	5	8	22	78	90	82	17	22	56
15	0	62	2	22	5	38	78	4	25	12	2	5	33	33	24	22	11	11
16	15	5	1	0	10	15	5	2	65	0	56	10	12	20	2	92	73	20
17	32	18	0	8	85	21	13	0	45	6	31	65	5	12	1	53	20	8
18	33	19	78	23	20	9	5	4	32	66	78	21	8	6	21	25	8	4
19	46	12	23	0	15	78	0	0	19	1	23	54	0	23	90	19	11	5
20	3	86	3	11	12	14	0	0	20	9	3	32	0	62	28	3	4	3
21	33	82	4	42	10	8	4	2	5	32	15	77	10	27	17	1	9	0
22	4	5	12	90	79	6	60	0	10	30	3	81	15	5	6	67	94	1
23	63	3	30	67	9	0	88	1	72	28	5	17	24	10	11	18	6	9
24	10	11	88	30	71	10	76	2	12	90	2	32	17	2	65	2	0	2

Table 2. *Percentage of epithelial cell type encountered on analyzed smears*

Cell Type	Parabasal	Superficial	Intermediate
Smear rate where cell type predominates	4.3%	20%	75.7%
P-value	<0.05		

Table 3. *Progesterone levels*

Sample	Progesterone Levels (ng/mL)																	
	goat 1	goat 2	goat 3	goat 4	goat 5	goat 6	goat 7	goat 8	goat 9	goat 10	goat 11	goat 12	goat 13	goat 14	goat 15	goat 16	goat 17	goat 18
1	1.8	1.6	1.1	0.8	1.5	1.6	0.9	1.8	1.1	1.3	0.9	1.2	1.8	1.9	1.7	2	0.2	1.8
2	1.9	1.6	0.5	0.5	1.3	1.1	1	1.6	1.4	0.4	0.6	1.7	2	0.2	0.9	0	0.9	1.1
3	1.3	0.6	1.6	1.2	0.7	0.6	1.1	1.5	0.6	1.2	0.8	1.3	0.3	1.6	1.5	1.3	1.9	0.4
4	1.2	1	1.9	1.4	0.8	1.5	1	1.5	1.5	1.4	0.9	1.5	1.1	1.3	0.2	1.8	1.3	1.9
5	0.9	0.8	1.5	1.3	1.3	1.2	0.6	1.7	1.5	1.3	0.7	0.6	1.3	1	1.5	1.2	2.4	2.1
6	0.3	0.8	0.6	1.2	1.1	1.7	1	1.4	0.3	1.1	0.8	1.5	1.9	1.9	1.3	1.4	1.5	1.9
7	0.8	0.9	1.3	1.1	1.2	1.3	1.9	1.6	1.6	1.3	1.4	1.3	1.1	1.7	1.9	1.1	0.6	0.9
8	2	1.2	1.4	1.3	0.9	1.6	1.2	1.8	1	0.5	1.3	1.9	0.6	0.7	1.2	2.3	0.8	0.3
9	1.2	0.5	1.2	0.7	0.6	1.7	1	0.1	0.9	1.7	1.2	1.2	1.1	0.9	1.7	1.8	1.1	1.8
10	0.8	0.7	1.6	1.6	1	1.7	1.3	0.9	1	1.8	0.6	1.4	1.5	1	0.6	0.4	0.3	1.9
11	1.1	1	1.4	1.2	1	3	1.1	1	0.5	1.1	0.7	0.8	1.9	1.4	0.8	1.9	2	1.5
12	1.7	1.2	0.8	0.8	0.6	1.6	1.5	1.2	1.3	0.7	0.6	1.5	1.7	2.1	1.1	1	1.5	1.5
13	2.3	1.1	6.2	1.2	0.5	1.3	1.2	1.1	1.7	1.3	1.5	0.4	1.2	1.1	1.2	1.2	1.3	1.2
14	0.1	0.8	1.3	1.5	0.7	1.2	1.7	1.8	5.9	1.4	1.2	1.2	0.7	0.5	0.3	1.3	1.1	0.7
15	1.6	0.6	1.5	1.2	0.9	0.8	0.5	4.8	3.3	1.5	1.1	1.3	1.3	1.5	1	0.9	1.6	1.1
16	0.9	1.2	1.2	1.1	1.5	3	1.5	1.4	0.2	1.2	0.5	1.2	1.8	3	1.8	0.2	0.4	1.5
17	1.8	1	1.4	0.8	0.1	1.5	1.2	1.4	0.9	0.9	0.5	0.5	2.1	1.9	2.2	1.4	1.7	1.2
18	1.8	0.9	0.1	1.5	1.2	1.2	1	1.5	1.6	0.5	0.9	1.1	1.5	1.6	1.3	1.6	1.1	1.7
19	1.6	1.3	1.2	1.3	1.1	0.4	2	1.2	1.6	1.8	1.3	1.4	2	1.1	0.2	1.2	1.9	1.8
20	0.4	0.1	1.2	1.2	1	1.6	1	1.2	1.3	1.5	1.2	1.2	1.8	0.3	1.1	1	1.3	1.2
21	1.4	0.5	1.4	0.8	1.2	1.2	1.3	1.5	0.8	0.7	1	0.8	0.9	1.8	1.4	1.1	1.5	1.3
22	1.3	1.4	1.2	0.8	0.2	1.4	1	1.5	1	1.1	1.2	1.2	1.3	2	1.5	0.3	0.1	2.3
23	0.3	1.2	1.1	0.9	1.3	1.3	0.7	2.2	0.4	1.3	1.4	1.1	1.5	1.8	0.3	2.4	1.8	2.2
24	1.2	1.3	0.7	1.1	0.3	1	0.8	1.1	1.4	0.8	1.4	1.2	0.7	0.9	0.9	1.2	2.1	1.3

In this study, variations in progesterone levels ranging from 0.1 ng/mL to 6.2 ng/mL were found *(Table 3)*. A negative correlation was observed between the percentage of superficial cells (SC) and serum progesterone (P4) levels in all goats (when the percentage of the superficial cells increased, the serum progesterone level decreased) *(Fig. 2)*.

Throughout the study, we recorded for all the goats, 47 cycles of different types (lengths) which are presented in *Table 4*.

The average cycle length observed was; 20.11±1.85 days for normal cycles, 14.41±1.51 days for short cycles, and 32.14±5.58 days for long cycles *(Table 5)*. There was significant difference in the estrous cycle lengths among the different goats (P<0.05).

Fig. 3 shows the frequency of the different cycle lengths revealed throughout the study. It was noted that the majority of cycles were between 14 and 24 days with some cycles less than 14 days and some cycles more than 25 days.

Data in *Table 3* indicate that the frequency of normal cycles (from 17 to 25 days) was the most important which reached a rate of 59.6% (28 cycles of 47cycles recorded) followed by short cycles (<17 days) with a rate of 25.5%

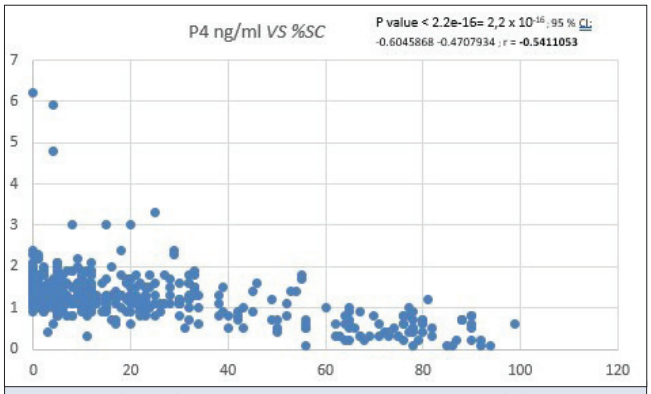

Fig 2. Correlation between serum progesterone rates and the percentage of superficial cells

Table 4. *Number and type of cycle recorded in all females throughout the study*

Goat Number	Number and Duration of Cycle		
	Short (< to 17d)	Normal (17 to 25d)	Long (> to 25d)
1	-	-	(31d), (35d)
2	-	(21d), (18d), (21d)	-
3	(14d), (14d)	(17d), (21d)	-
4	(16d)	-	(42d)
5	(15d)	(21d)	-
6	-	(18d)	(26d)
7	-	-	(28d), (35d)
8	-	-	-
9	(11d)	(17d), (19d), (24d)	-
10	(15d)	(21d), (21d), (21d)	-
11	(15d)	(24d)	-
12	(15d), (15d)	-	-
13	(16d)	(21d)	-
14	-	(20d), (19d), (20d)	-
15	(15d)	(18d), (21d), (19d)	-
16	-	(20d), (22d)	(28d)
17	(12d)	(21d), (19d), (22d)	-
18	-	(17d), (20d)	-
Average +/- SD	14.41±1.51d	20.11±1.85d	32.14±5.58d
P value	0.02623		

d: day

(12 cycles of 47 cycles recorded) and finally the lowest rate was that of long cycles (>25 days) with a rate of 14.9% (7 cycles of 47 cycles recorded).

Table 5. *Frequency and percentage of different cycle lengths*

Parameter	Cycle Length (days)			
	Short (< 17d)	Normal (17d - 25d)	Long (> 25d)	Total
Number of cycle	12	28	7	47
Percentage of cycles	25.5	59.6	14.9	100
P-value	0.005			

d: day

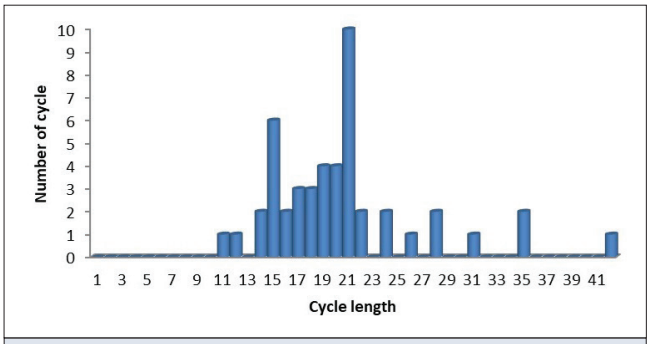

Fig 3. Frequency of recorded cycle lengths

A significant difference (P<0.05) was recorded between the percentages of cycle lengths found.

Discussion

The predominance of intermediate cells in the majority of analyzed smears seems to be related to the luteal phase with a high rate of serum P4 (which is the longest phase of the estrous cycle). On the other hand, smears dominated by superficial epithelial cells corresponded to samples with a decrease in P4 levels, which indicates the estrous phase as reported previously [7]. Sitaresmi [17]found that intermediate cells dominated the majority of the smears, especially on the metestrus and diestrus. Ola et al.[6] noted that intermediate and parabasal cells were more commonly encountered in the smears from days corresponding to the luteal phase under progesterone control. These results are in agreement with those reported by Zarkaoui and soukouti [20] in Damascus does, which have shown that the follicular phase had an average length of 3.1±0.6 days (range: 2-5 days), with a mean progesterone level of 0.68±0.79 nmol L^{-1} (range: 0.00-2.81 nmol L^{-1}).

These cyclical relationships between the exfoliated cells and the ovarian steroid hormones have been strongly established for small ruminants and for other species [5,6,8,21].

In the current study, data showed that the average normal estrous cycle length in the Algerian Arbia goat was 20.1±1.85 days (17-25 days). In addition to the normal cycles, the females presented a great percentage of short

cycles (25.5%) with an average of 14.41±1.51 days. Long cycles were found with a percentage of 14.9% and an average of 32.14±5.58 days. These results are consistent with the study conducted by Yahia et al.[22]. These latter concluded that the average length of normal cycles for Algerian local goats was 19.23 days. In the same context, Charallah [23] confirmed that the normal estrous cycle length was 20 days in the Bedouin goat, with the existence of other types (short and long cycles). Derquaoui and El Khaldi [24] noticed that the average duration of the estrous cycle in D'man goats was20.96±2.84 days for normal cycles and 10.5±3.45 days for short cycles. In local Moor goats in Tunisia, the average duration of normal cycles was 21.1±1.5 days [25]. Corteel [25] found that the goat showed only 6-8estrous cycles during each year and the frequent duration was 21 days.

On the contrary, longer or shorter cycles (less or more than 21 days) were observed by several authors. Lahirigoyen [27] mentioned that the average duration of short cycles was 6 days and those of long cycles ranged between 30 and 44 days. The west African dwarf goats exhibited medium (regarded as normal) cycle lengths of between 19-22 days [28,29]. In fact, 86%, 32%, of cycles were short, respectively, in the Nubian goat [30], and in Creole [31]. A study with Alpine goats during the breeding season recorded 77% of normal cycles (17-25 days) with an average duration of 20.7 days, 14% were short cycles (8 days on average) and 9% were long cycles (39 days on average) [32], which is in agreement with our results. It would seem that the relatively high incidence of short cycles was a characteristic of the goat species [24,32]. The origin and etiology of short cycles in small ruminants are not fully elucidated, but they can be explained by the fact that the corpus luteum of short cycles is of bad quality and that its secretory function is limited as a consequence [31]. This fact is strongly influenced by food level [33].

In addition, the periods found in this work are similar to those reported for a certain tropical alpine breed in different countries. Greyling [34] showed that the normal estrous cycle length was 20.7±0.7 days.The normal cycle duration of 19.7±1.5 was revealed in the Matou goat in China by Moaeen-ud-Din et al.[35]. The normal estrous cycle length recordedin our study (20.11±1.85 days), is very close to those reported in Red Sokoto goats in Nigeria [36] and in Criollo goats in Chile [37] (21.3 and 20.7, respectively).

The present study showed a negative correlation between the percentage of superficial cells (SC) and serum progesterone (P4) levels in all goats, and the average normal estrus cycle length in Algerian Arbia goats was 20.11±1.85 days with a percentage of 59.6%. To this type of cycles, were added short and long cycles with different rates. In addition, it has been suggested that a vaginal cytology exam is highly indicative of the effect of progesterone in the goat reproductive tract. It was possible through this work, for the first time, to characterize the estrous cycle length of the Algerian Arbia goat and to determine the estrus phase by researching the high rate of superficial epithelial cells and the low level of serum progesterone. These findings are important and useful in assessing the physiology of the estrous cycle of the Algerian Arbia goat as a primary parameter of reproduction.

Declarations

Availability of Data and Materials: The datasets during and/or analyzed during the current study available from the corresponding author (N. Mimoune) on reasonable request.

Funding Support: There is no funding source.

Ethical Statement: All the experiments were carried out according to the guidelines of the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research (Agreement Number 45/DGLPAG/DVA.SDA.14).

Competing Interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions: YA, NH, KS, KH, NM: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Writing - Original Draft, Writing - Review & Editing.

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