Effects of prostaglandin F 2 alpha injection and rabbit does age on reproductive, hematological parameters and level of progesterone

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ABSTRACT: *Data of a total number of 96 V-line rabbit does (two parities of 48 does) were analyzed to evaluate the effects of prostaglandin F 2 alpha ($PGF_{2\alpha}$) injection and age of rabbit does on some reproductive, hematological parameters, and progesterone level. The $PGF_{2\alpha}$ injection group had significant increase in vulva color, sexual receptivity, conception rate, litter size (at birth, 21 days of age and weaning), litter weight (at birth, 21 days of age and weaning), red and white blood cells count. However, it had significant decrease in gestation period, mean corpuscular volume and mean corpuscular hemoglobin compared to the negative and positive control. Significant increase were found in litter size (at birth, 21 days of age and weaning) of rabbit does at 18 months compared to at 30 months. However, does at 30 months have higher levels of progesterone than does at 18 months It is concluded that in rabbit does having low reproductive performance which may be output due to increase plasma progesterone concentration or/and advance age, the $PGF_{2\alpha}$ could be effective in removing the progesterone block and improving the most of reproductive parameters studied.*

INTRODUCTION

The improvement of reproductive performance on farms is conditioned by the use of methods enabling the induction and synchronization of oestrus. This concerns hormonal treatments or non-hormonal alternative methods. Hormonal treatments have been widely used in recent years. Several authors have suggested using Prostaglandin F 2 alpha ($PGF_{2\alpha}$) or its analogues to improve doe receptivity and improving reproductive parameters (**Boiti *et al.*, 2006; Theau-Clement, 2008 and Gogol 2009)**. In domestic animals, the most important and the practical utility of prostaglandin (PG) appears to be $PGF_{2\alpha}$ (**Lauderdale, 2002**). PG are found in most tissues and organs. They are produced by all nucleated cells except lymphocytes. They are autocrine and paracrine lipid mediators that act upon platelets, endothelium, uterine and mast cells. They are synthesized in the cell from the essential fatty acids (**Laneuville, 2003).** Synthetic PG analogues are molecules which are manufactured to bind to a PG receptor. Most PGs act at or near the site of production and can affect a variety of reproductive functions including ovulation, fertilization, implanatation, embryonic development, luteolysis, parturition and lactogenesis **(McNitt,1992**).

Fertility of doe rabbits in terms of litter size at birth improved significantly ($P< 0.01$ or 0.05) by $PGF_{2\alpha}$ injection (**Kirrella *et al.*, 1995; Dorra *et al.*, 1997; McNitt *et al.*, 1997 and Gogol, 2009)**. On the other hand, **Lavara *et al.* (2002)** observed no significant differences were found in litter size at birth and live-born kits when used synthetic $PGF_{2\alpha}$ analogue (cloprostenol) compared to control. Also, **Pimenta *et al.* (1996)** obtained the same results with using natural $PGF_{2\alpha}$.

Abdo *et al.* (1979) indicated that $PGF_{2\alpha}$ causes a significant increase in the number of red blood cells and red blood cells of Najdi sheep. On the other hand, $PGF_{2\alpha}$ did not affect significantly the other hematological parameters. The $PGF_{2\alpha}$ could be effective in removing the progesterone block as reported by **Boiti *et al.* (2006)**.

The present investigation aimed to study the effects of $PGF_{2\alpha}$ injection and age of doe on some reproductive, hematological parameters, and progesterone level of rabbit does.

Materials and Methods

Animals and treatments:

Forty eight V-line rabbit does which used in this study have low reproductive performance which represented by a small litter size (less than 5 kits per litter at birth), low conception rate (less than 50%) and does refusal to be mated by males. The does randomly allotted into three equal groups, 16 does of each. The first group acts as negative control and the second group was considered as positive control (each doe was injected intramuscularly with 0.25 ml saline solution, 0.9% NaCl), however, group 3 treated with cloprostenol; prostaglandin synthetic analogue $PGF_{2\alpha}$ (each doe was injected intramuscularly with 62.5 µg Cloprostenol (0.25 ml estroPLAN; Parnell Laboratories (Aust) PTY.LTD.). Does were mated naturally by fertile bucks 72 hrs post- injection. The does were stratified by age (18 and 30 months). Mean live body weight of does was 3044±58.98g. Does were allowed to kindle for two parities consecutively.

Parameters of doe and its offspring:

The traits study were vulva color (Pale = 0, pink =1, purple = 2, congested (dark) red = 3), sexual receptivity (determined by the willingness of the doe to mate combined with signs of estrus such as; swelling of the vulva, exposition of the rear quarters and lordosis. It was scored on the scale of 0 = none, 1 = slight, 2 = moderate, 3 = strong),Conception rate, gestation period and litter size (total, alive and dead) were recorded at different periods (birth, 21 days of age and weaning; 28 days of age) also, mortality rate calculated at different periods (birth, 21 days of age and weaning). Litters weights were recorded at different periods (birth, 21 days of age and weaning).

Hematological parameters:

Blood samples (3 ml) were taken twice in the morning from four does of each group. The first sample was taken pre-mating and the second sample was taken at 14 days post mating from pregnant does. Xylol was applied on ear to increase blood flow (**Hoppe *et al*., 1969**) and heparin used as anticoagulant. Blood samples were divided into two parts, the first part, was used to determine the hematological parameters that red blood cells (RBCs) count and platelets (PLT) count according to **Perkins (2009)**, also hematocrit value (Ht %) according to **Bauer (1970)** and hemoglobin (Hb) as stated by **Van Kampen and Zillstra (1983)**. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from the parameters of RBCs count, Ht% and Hb% (**Perkins 2009**). White blood cells (WBCs) count and the differential count of sub-classes of WBCs were determined as reported by **Ewuola and Egbunike (2008)**. The second part was centrifuged (15 min, 3,000 rpm) and plasma was removed and stored frozen at -20 C^{o} until the time of plasma progesterone determination by radioimmunoassay technique (**Abraham *et al*., 1971**).

Statistical analysis:

Data of parameters of the doe and its offspring were analyzed using General Linear Models (GLM) procedure of SPSS software (**SPSS, 2008**), two way analyses of variance with the $PGF_{2\alpha}$ injection treatment and age of doe as main effects for as follows: $Y_{ijk}=\mu+ T_i + A_j + (TA)_{ij} +e_{ijk}$

where: Y_{ijk}: the observation on the the ijk^{th} parameters of the doe and its offspring, μ: overall mean, T_i: the fixed effect of the i^{th} $PGF_{2\alpha}$ injection treatment (i: 1 to 3), A_j: the fixed effect of the j^{th} age of doe (j: 1 and 2), $(TA)_{ij}$: Interaction of $PGF_{2\alpha}$ injection treatment by age of doe and e_{ijk}: random error term.

Treatment means indicating significant differences ($P\leq0.05$) were tested using Duncan's multiple range test **(Duncan, 1955)**.

Results

The does that injected with $PGF_{2\alpha}$ had highest sexual receptivity and conception rate compared to the other groups as shown in Figures (1 and 2). The rate of pregnancy increased approximately double of control.

Results in Table (1) indicated that $PGF_{2\alpha}$ injection caused highly significant ($P\leq0.001$) increase in vulva color (VC), litter size at {birth (LSB), 21 days of age (LS_{21}) and weaning (LSW)} compared to the other groups. The gestation period (GP) reduced significantly ($P\leq0.01$) with $PGF_{2\alpha}$ injection as shown in Table (1). The influence of $PGF_{2\alpha}$ injection was insignificant ($P>0.05$) on mortality rate at 21 days of age (MR_{21}%) and total preweaning mortality (TPWM %) but mortality rate at birth (MRB %) was significantly ($P\leq0.05$) high in $PGF_{2\alpha}$ group.

Does at 18 months of age had significant ($P\leq0.01$) increase in LSB and had significant ($P\leq0.05$) increase in each of MRB%, LS_{21}, LSW and TPWM% compared to does at 30 months of age as shown in Table (1). However, age of doe showed insignificant effects ($P\leq0.05$) on VC, GP and MR_{21}%. The results refer to decrease in litter size (LSB, LS_{21} and LSW) with increasing maternal age.

The $PGF_{2\alpha}$ injection caused highly significant ($P\leq0.001$) increase of litter weight at birth (LWB), litter weight at 21 days of age (LW_{21}) and litter weight at weaning (LWW) as shown in Table (2). There no significant differences were found in LWB, LW_{21} and LWW between the two ages as shown in Table (2).

Results in Table (3) showed that $PGF_{2\alpha}$ injection treatment had highly significant increase ($P\leq0.001$) on RBCs count and highly significant decrease on MCV and significant decrease ($P\leq0.05$) on MCH, while it had insignificant ($P>0.05$) effect on Hb%, Ht%, PLT count and MCHC% of rabbit does.

Results presented in Table (4) shown that $PGF_{2\alpha}$ injection caused highly significant increase in WBCs count ($9.36x10^3/\mu L$) of rabbit does compared to other groups. While insignificant effects ($P>0.05$) were found of $PGF_{2\alpha}$ injection treatment on neturophils%, lymphocytes%, monocytes%, eosinophils% and neutrophils/ lymphocytes (N/L) ratio.

The effect of $PGF_{2\alpha}$ injection was insignificant ($P>0.05$) on progesterone level as shown in Table (4). However does at 30 months have higher levels of progesterone (3.67 ng/ml) than does at 18 months (3.26 ng/ml) as shown in Table (4).

Discussion

Sexual receptivity can be evaluated more accurately by the color of the vulva and its turgid (**IRRG, 2005**). The results of the present study indicated that vulva color increased by using $PGF_{2\alpha}$ injection. This indicator may have some relevance in explaining the mating acceptance post-injection $PGF_{2\alpha}$ from one hand. Prostaglandin $F_{2\alpha}$ injection could have very

rapid and dramatic effects on steroid synthesis in the lutein cell whereas normal luteolysis would seem to involve more gradual regression of the gland. This may have some relevance in explaining the mating acceptance post-injection $PGF_{2\alpha}$ from the other hand. Similarly, **Dragan *et al.* (1996)** reported that sexual receptivity of rabbit doe injected with PG analogues is better (85 to 95%) than the control (67.5%). Also, **Hassanein (2000)** found that the percentage of mating acceptance was 100% when does injected by 2.5 mg of Lutalyse® ($PGF_{2\alpha}$ analogue) 48 hours before natural mating with fertile bucks. The rate of pregnancy in the present study increased approximately double of control. This result enables to say that the $PGF_{2\alpha}$ improved conception rate of does. **Theau-Clement and Roustan (1992)** have studied the relation between receptivity and each of ovulation and fertilization. They found that the results in the case of natural mating were 100% and 88%, respectively for receptive females but were 13 and 10%, respectively for non-receptive females.

Shorter gestation period by the effect of $PGF_{2\alpha}$ injection in this study may be attributed to the greater number of LSB (8.46) (Table 1) as compared to positive and negative control (4.43 and 4.94, respectively). Also, the gestation period decreased linearly with the increase of LSB (**Afifi and Emara, 1984** and **Hilmy, 1991)**. The significant effects of $PGF_{2\alpha}$ on litter size agree with those obtained by **Abd El-Glil (1993)** and **Kirrella *et al.* (1995**). These results may be attributed to the $PGF_{2\alpha}$ causes regression of the corpus luteum and the cycle repeats itself (**Niswender *et al.*, 2000**), also secretion of luteinizing hormone (LH) (**Carlson *et al.*, 1977**) and, as a result of the increase of LH, ovulation of the mature follicle occurs followed by various sequential processes lead to increase LSB.

The decrease in LSB with increasing maternal age has been attributed to uterine ageing (**Adams, 1970**), inadequate environment (**Maurer and Foote, 1971**), reduced rate of uterine blood flow (**Larson and Foote, 1972**) and may be the appearance of amorphous material beneath the basal lamina of the endometrial epithelium with advancing age. This could impair communication between the luminal epithelium and the endometrial stroma, which plays an important role in implantation and thus, the decrease in reproductive efficiency (**Shimizu and Yamada, 2000**). In agreement with these findings, **Aumann *et al.* (1984)** reported a highly negative significant effect of doe's age on LSB.

Increasing the LWB, LW_{21} and LWW with $PGF_{2\alpha}$ injection treatment may be due to increase LSB, LS_{21} and LSW. Similarly, **Abd El-Glil (1993)** revealed that $PGF_{2\alpha}$ injection significantly affected on LWB. Also, the same author concluded that LWB increased by the increase in LSB. In addition to, differences between $PGF_{2\alpha}$ treatments on LW_{21} were statistically significant and concluded that there were a positive correlation between LS_{21} and LW_{21} from one hand and LSB from the other hand, also $PGF_{2\alpha}$ injection caused increasing LS_{21} and LW_{21} which resultant from improvement of does fertility. Similar trend of doe age effects on LWB were recorded by **Kumar *et al.* (2005)**.

The increase of RBCs count with $PGF_{2\alpha}$ injection treatment was consistent with that obtained by **Abdo *et al.* (1979)**, who indicated that $PGF_{2\alpha}$ injection of Najdi sheep causes a significant increase in the number of RBCs. This may be indicated to $PGF_{2\alpha}$ effect on RBCs synthesis. These changes of RBCs count are known to be associated with an increase in the oxygen carrying capacity of the blood accompanied with an increase in the respiration rate (**El-Banna *et al.*, 2005)**. The significant decrease in MCV with $PGF_{2\alpha}$ injection treatment compared to the negative or positive control despite the $PGF_{2\alpha}$ injection

increased the RBCs count as shown in Table (3), these results indicated that the $PGF_{2\alpha}$ injection have role directly or indirectly of increasing RBCs synthesis which have small size (reticulocyte). Furthermore, findings of MCH may be attributed to increase RBCs count of does received $PGF_{2\alpha}$ injection and the relative stability of hemoglobin which in turn decreased quotient. Insignificant effects of $PGF_{2\alpha}$ injection on progesterone may be attributed to the difference of time between injection and sampling of blood. The significant increase in WBCs count with $PGF_{2\alpha}$ injection treatment compared to the negative or positive control as shown in Table (4). This may be indicated to $PGF_{2\alpha}$ effect on WBCs synthesis. Similar trend of $PGF_{2\alpha}$ injection effects on WBCs count were recorded by **Abdo *et al.* (1979)**.

CONCLUSIONS

Obtained results support that in rabbit does having low reproductive performance which may be output due to increase plasma progesterone concentration or/and advance age, the $PGF_{2\alpha}$ could be effective in removing the progesterone block and improving the most of reproductive parameters studied.

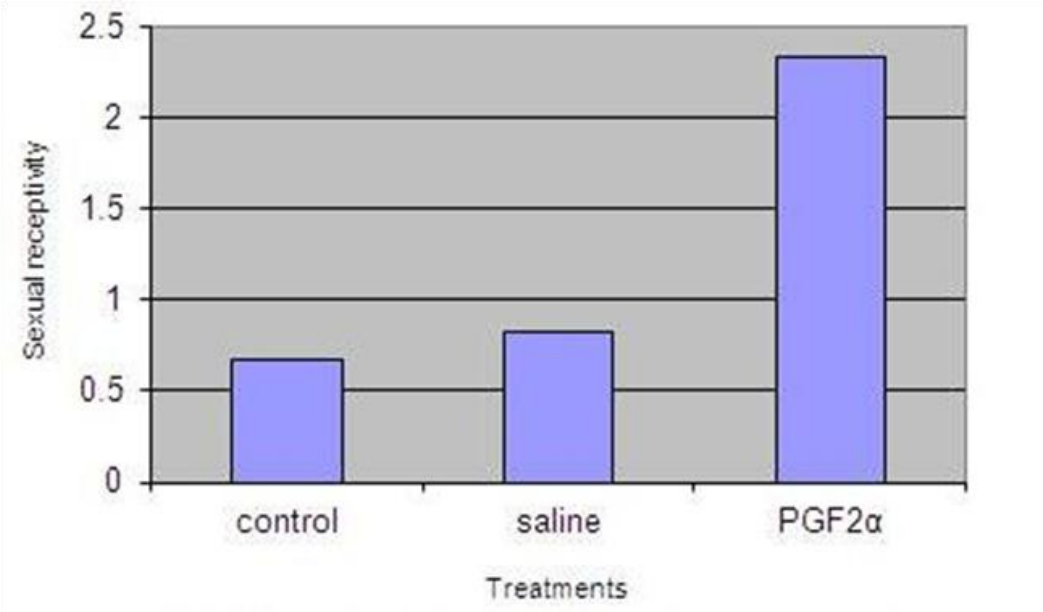

Figure (1): Effect of PGF2α injection on sexual receptivity of rabbit does (0 = none, 1 = slight, 2 = moderate, 3 = strong)

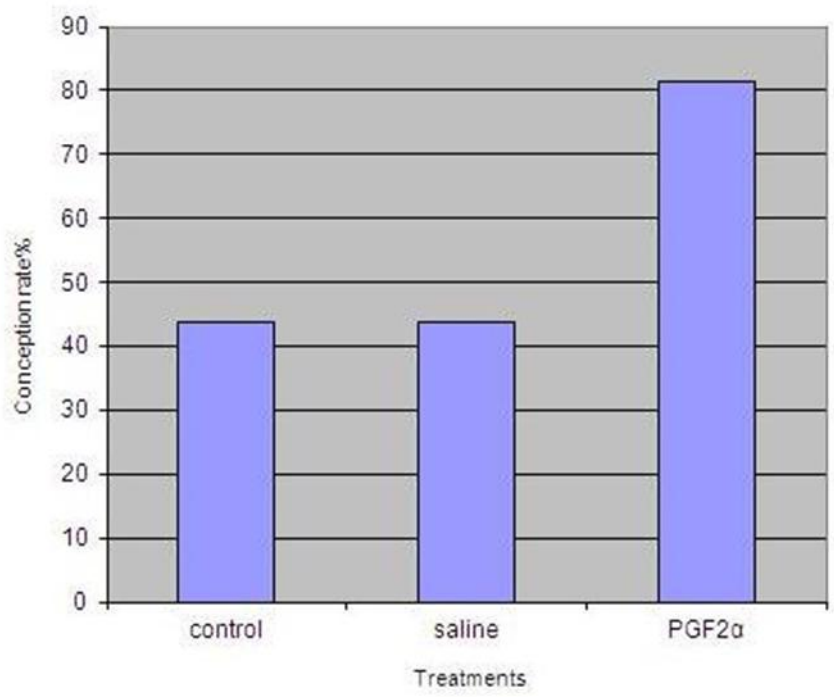

Figure (2): Effect of PGF2α injection on conception rate of rabbit does

Table (1): Effects of $PGF_{2\alpha}$ injection and age of rabbit does on vulva color, gestation period, litter size and mortality rate.

Items	Vulva color	Gestation period	Litter size				Mortality %		
			Birth		21 days	Weaning	Birth	21 days	TPWM%
			Total	Alive					
Treatments									
Control	0.69 [b]	32.14 [a]	4.43 [b]	4.29 [b]	4.29 [b]	4.00 [b]	2.86 [a]	0.00	3.34
Saline	0.88 [b]	31.86 [a]	4.94 [b]	4.83 [b]	4.79 [b]	4.70 [b]	0.00 [b]	0.66	2.21
$PGF_{2\alpha}$	2.34 [a]	30.96 [b]	8.46 [a]	8.19 [a]	7.88 [a]	7.65 [a]	3.08 [a]	3.03	5.59
SE	0.14	0.15	0.26	0.35	0.32	0.23	2.36	0.98	1.08
Sig	***	**	***	***	***	***	*	NS	NS
Age(months)									
18	1.35	31.48	7.29 [a]	6.86 [a]	6.55 [a]	6.32 [a]	6.55 [a]	3.35	6.19 [a]
30	1.25	31.82	5.33 [b]	5.03 [b]	5.01 [b]	4.98 [b]	4.52 [b]	0.28	0.93 [b]
SE	0.11	0.16	0.27	0.36	0.33	0.24	2.46	1.03	1.12
Sig	NS	NS	**	**	*	*	*	NS	*

Means in the same column within each effect bearing different small superscript differ significantly at P≤0.05.

TPWM%= total preweaning mortality;

NS=insignificant *=significant at P≤0.05 **=significant at P≤0.01
***=significant at P≤0.001

Table (2): Effects of $PGF_{2\alpha}$ injection and age of rabbit does on litter weight (g)

Treatments	Litter weight (g)		
	Birth	**21 days**	**Weaning**
Control	221.16 [b]	1509.70 [b]	2232.40 [ab]
Saline	205.43 [b]	1000.14 [b]	2060.70 [b]
$PGF_{2\alpha}$	419.04 [a]	2087.23 [a]	3997.90 [a]
SE	11.70	51.81	117.62
Sig	***	***	***
Age (months)			
18	295.88	1453.46	2733.80
30	248.94	1236.88	2577.70
SE	12.50	53.99	122.56
Sig	**NS**	**NS**	**NS**

Means in the same column within each effect bearing different superscript differ significantly at P≤0.05.

NS = insignificant *** = significant at P≤0.001

Table (3): Effects of $PGF_{2\alpha}$ injection and age of rabbit does on hematological parameters

Items	RBCs $X10^6/\mu L$	Hb g/dL	Ht %	PLT $X10^3/\mu L$	MCV FL	MCH Pg	MCHC %
Treatments							
Control	3.92 [b]	13.8	50.8	49.0	131 [a]	36.00 [a]	27.3
Saline	3.86 [b]	13.8	50.1	46.0	129 [a]	36.00 [a]	27.9
$PGF_{2\alpha}$	4.36 [a]	13.9	49.5	47.0	116 [b]	32.00 [b]	27.7
SE	0.09	0.20	0.47	1.40	3.29	0.84	0.50
Sig	***	**NS**	**NS**	**NS**	***	*	**NS**
Age(months)							
18	4.01	13.8	49.8	48.0	126	34.70	27.7
30	4.08	13.8	50.4	47.0	125	34.30	27.5
SE	0.07	0.16	0.39	1.2	2.69	0.68	0.41
Sig	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Means in the same column within each item bearing different small superscript differ significantly at P≤0.05.

RBCs = red blood cells count; Hb = hemoglobin; Ht= hematocrit , PLT = platelets count, MCV= mean corpuscular volume; MCH= mean corpuscular hemoglobin and MCHC= mean corpuscular hemoglobin concentration.

NS = insignificant * = significant at P≤0.05 *** = significant at P≤0.001

Table (4): Effects of $PGF_{2\alpha}$ injection and age of rabbit does on white blood cells count and differentiation and progesterone level.

Items	WBCs $X10^3/\mu L$	Neutrophils %	Lymphocytes %	Monocytes %	Eosinophils %	N/L ratio	Progesterone ng/ml
Treatments							
Control	7.88 [b]	42.3	51.4	3.88	2.42	0.82	3.40
Saline	8.47 [ab]	41.9	51.7	3.78	2.60	0.81	3.43
$PGF_{2\alpha}$	9.36 [a]	43.9	51.1	3.00	2.05	0.86	3.57
SE	0.32	1.86	1.82	0.20	0.20	0.09	0.13
Sig	**	NS	NS	NS	NS	NS	NS
Age (months)							
18	8.61	42.8	50.8	4.08	2.33	0.84	3.26 [b]
30	8.53	42.5	51.6	3.20	2.71	0.82	3.67 [a]
SE	0.26	1.52	1.49	0.16	0.15	0.08	0.11
Sig	NS	NS	NS	NS	NS	NS	**

Means in the same column within each effect bearing different small superscript differ significantly at P≤0.05.

WBCs= white blood cells count

N/L= Neutrophils/ Lymphocytes

NS = insignificant

** = significant at P≤0.01

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الملخص العربي

تأثير حقن البروستاجلاندين إف ٢ الفا وعمر اناث الارانب على قياسات التناسل والدم ومستوى هرمون البروجستيرون

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تم تحليل بيانات ٩٦ ارنب انثى (في لاين) لتقييم تأثير حقن البروستاجلاندين اف ٢ الفا وعمر الانثى على بعض قياسات التناسل والدم ومستوى هرمون البروجستيرون لإناث الارانب .واظهرت مجموعة الاناث المحقونة بالبروستاجلاندين زيادة معنوية في لون فتحة الحيا، وقبول التزاوج، معدل الحمل، حجم البطن عند الميلاد وعند ٢١ يوم من الميلاد وعند الفطام، ، وزن البطن عند الميلاد وعند ٢١ يوم من الميلاد وعند الفطام، عدد كرات الدم الحمراء والبيضاء، في حين انه كان بهذة المجموعة نقص معنوي في مدة الحمل، متوسط حجم كرة الدم ومتوسط كمية الهيموجلوبين بكرة الدم مقارنة بالكنترول السلبي والايجابي.وجدت زيادة معنوية في حجم البطن عند الميلاد وعند ٢١ يوم من الميلاد وعند الفطام للاناث عند عمر ١٨ شهر مقارنة بالاناث عند ٣٠ شهر، في حين ان اناث الارنب عند عمر ٣٠ شهر كانت اعلى في مستوى هرمون البروجستيرون عن الاناث عند عمر ١٨ شهر . ويمكن التوصية بان اناث الارانب منخفضة الاداء التناسلي الراجع لارتفاع مستوي هرمون البروجستيرون في بلازما الدم او /و تقدم العمر يمكن ان يكون استخدام البروستاجلاندين ($F_{٢\alpha}$) فعال في ازالة حاجزالبروجستيرون وتحسين معظم القياسات التناسلية المدروسة.