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Multiple Natural and Hormonal Methods Improve Reproductive and Productive Performance of Naturally Mated Multiparous Rabbit Does

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Abstract

This experiment illustrates the impact of various natural and hormonal methods on the reproductive and productive performance of naturally mated multiparous rabbit does. 180 multiparous does were randomly and equally allotted on six experimental treatments (1) DLS (doe litter separation); (2) DG (does gathering), (3) CC (cage change), (4) OP100 (estradiol 27 µg and progesterone 270 µg/kg body weight), (5) OP300 (estradiol 80 µg and progesterone 800 µg/kg body weight), and (6) control group, the experiment was repeated on three-time points (2, 7, and 14 days postpartum). All used treatments, either natural or hormonal, induced significant positive effects on receptivity and fertility compared with control treatment, with the superiority of the natural methods to hormonal one. Although DLS group significantly decreased average fryer weaning weight, OP300 group increased it significantly compared with the control group. All experimental treatments achieved significantly higher weaning numbers than the control. It's better to apply these methods on days 2 and 7 postpartum than on day 14 postpartum. It concluded that the CC and DG methods are effective in improving multiparous rabbit production. However, these treatments must only be applied to healthy herds as animal contact may serve as a source of contamination. Moreover, estrogen and progesterone can be used as good hormonal methods for improvement of rabbit production although further studies are necessary to determine how it affects the other blood hormones and whether prolonged usage of these hormones can impair ovarian function and trigger an immunological response.

Keywords: Rabbit; Natural and hormonal methods; Productive performance

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1. Introduction

Based on the mechanism of ovulation rabbit females classified with ferret, cat, koala, llama, camel, alpaca as induced ovulators which do not have a well-defined sexual cycle, and their ovulation is induced by coitus **(Rebollar et al., 1992)**. Moreover, the usage of lactating rabbit does at the beginning of the nursing phase is required by the intense and semi-intensive reproductive rhythms employed in rabbit production systems. However, the lactation period depressed females' sexual receptivity and fertility **(Ubilla and Rebollar, 1995)** by depressing follicle development **(Kermabon et al., 1994)**, oocyte quality **(Garcıa-Garcıa et al., 2012)**, ovulation rate, fertilization, and embryo development **(Fortun-Lamothe and Bolet, 1995)**. For these reasons, rabbit does under high production rhythms were subjected to a variety of methods have been utilized to improve female receptivity and reproductive outcomes. Hormones are sometimes used to synchronize oestrus for a systematic breeding system; however, natural approaches have also been created as alternatives to hormones (biostimulation). These techniques enable novel production models like "cycling production," in which all the does from the same batch are inseminated on the same day regardless of their level of sexual receptivity.

Although the sexual receptive behavior is correlated with more pre-ovulatory follicles on the rabbit ovary **(Kermabon et al., 1994)** and consequently with a higher concentration of plasma estradiol **(Rebollar et al., 1992)**, few experiments evaluated the influence of esteroidal and progesterone on estrous induction and reproductive performance of induced ovulators. Estradiol stimulated both chinning and sexual receptivity in the female rabbit **(Hudson et al., 1990, Hoffman and González-Mariscal, 2006, 2007and Hoffman et al., 2009)**. Moreover, **Hawk and Cooper, (1976)** showed that exogenous estradiol boosted sperm counts in uterine and oviduct horns as early as two hours after hormone delivery or one hour after mating. **Sawyer et al., (1950)** observed that administering estrogen and progesterone together for two days daily in rabbits, but not estrogen or progesterone alone, caused ovulation in 40% of synchronized does. Moreover, **Goodman et al., (1998)** concluded that estrogen has a luteotrophic effect on rabbit luteal cells by inhibiting apoptosis. Conversely, prostaglandin F2 alpha's luteolytic pathways may block estradiol signaling in luteal cells **(Maranesi et al., 2010)**. **Bianchi et al., (2020)** studied how estradiol-17β affected ovulation and luteal development when administered to llamas. They discovered that administering increased dose of estradiol-17β (0.6, 1, and 1.6 mg/llama) raised the ovulatory rate incrementally (0/4,1/4, and6/6, respectively).

Biostimulation is a natural method used in animal production to improve reproductive parameters. It is based on changing external environmental stimuli (such as visual, olfactory, pheromone, tactile, auditory, social, and nutritional stimuli, among many others that have not yet been identified) that elicit particular behavioral and endocrine responses **(Theau-Clément et al., 1998, Choudhary and Lal-Kamboj, 2019)**. Some of the methods that have been tried including feeding control **(Quintela et al., 2001)**, control lighting, and changing does to another cage **(revised by Theau-Clement, 2008)**, and female-female interaction **(González, 2005)**. Lactation has previously mentioned, negatively influences sexual receptivity and fertility **(Ubilla and Rebollar, 1995)**. In light of this, lactation control appears to be a very efficient way to promote ovarian activity before AI. Doe-litter separation on specific days of lactation before insemination was as effective as equine chorionic Gonadotrophin (eCG) treatment, especially for the first four inseminations applied at 4 days postpartum **(Rebollar et al., 2006)**. It causes a drop in plasma prolactin levels that may encourage the development of follicular waves and strong steroidogenesis activity **(Ubilla et al., 2000, Rebollar et al., 2008)**. It can be performed for 24–48 hours in early lactation **(Bonanno et al., 2002)** or nursing with a short-controlled suckling in a 48-hours period of doe-litter separation **(Bonanno et al., 2004)**. Another approach involves using a 2-day controlled nursing period before insemination and allowing the litter to nurse for 10 minutes after 24 hours of separation **(Rebollar et al., 2008)**. Receptivity and fertility rates are increased significantly in all methods.

This study prefers natural mating since prior research on rabbits found that physical coital stimulation via action potential transmission from the spinal cord is the main cause of hypothalamic secretion of GnRH into the portal arteries in induced ovulators **(Kaynard et al., 1990)**. Accordingly, the current study aimed to evaluate the influence of different biostimulation methods (cage change, dams gathering, and dam litter separation) and hormonal methods (different doses of estradiol and progesterone) compared with the control group on the reproductive and productive performance of naturally mated multiparous rabbit does in different postpartum days.

2. Materials and Methods

2.1. Animals and feeding ration

The National Board of Agriculture's guidelines and general recommendations for using animals in research were followed in conducting this study. This work was carried out in a commercial rabbit farm in Badr Center, Buhaira Governorate, Egypt, and lasted about six months from September to February. An open-sided house with the electric exhausted fan was used, and the house temperature was maintained between 18 ˚C and 22˚C. A total of 210 New Zealand rabbits were used, including 180 multiparous females weighing about 3.5–4 kg and 30 breeder males weighing 5–6 kg. Males and females were

individually housed in a standard dimension wired metallic cage with external nest boxes in female cages. Does that were nursing or pregnant were fed ad libitum, whereas those that weren't restricted to 150 g of commercial food per day until one week before mating when they were also fed ad libitum. According to **De Blas and Mateos, (1998)**, the rations satisfied the nutrient requirement of the does providing 17.36% crude protein, 12.37% crude fiber, and metabolizable energy 2257 kcal/ kg diet (**Table 1**). Fresh and clean water was available *ad libitum*.

2.2. Reproductive management

Does were transferred to the rabbit bucks' cages for the natural mating process and kept under examination until natural mating was completed. They were kept with a lighting program of 16 hours light/8 hours dark; light intensity was 70 lux. Sexual receptivity was confirmed by determining the color of the vulva (pale, pink, red) after treatment application and before natural mating **(Quintela et al., 2001)**. At 11–14 days after mating, all does were pregnancy diagnosed by abdominal palpation. Parturitions took place mainly on day 31 post-mating and the kits were weaned after one month. To decrease statistical error, the same farm employees always carried out natural mating and the associated handling.

2.3. Experimental design

Does were labeled and randomly distributed in six experimental groups. Each group was composed of ~30 multiparous does, where the experiment was repeated on the same animals on three different periods postpartum (the day 2, the day 7 and the day 14 postpartum). The experimental groups were: (1) The doe litter separation group (DLS) where does were separated from their litters and prevented from suckling by closing nest box for 24 hours before breeding, allow suckling immediately after successful mating; (2) the does gathering treatment (DG) where dams of this treatment were placed together (6 does/cage) for two hours before mating;(3) the cage change treatment (CC) where the doe was transferred to different cage for two hours before natural mating, (4) estradiol 27 µg and progesterone 270 µg/kg (OP100) where does were injected intramuscular with 0.55 ml from mixture of lutofolone® (estradiol benzoate 2 mg and progesterone 20 mg, Misr Company for Pharmaceuticals, Cairo, Egypt) diluted with sesame oil (1/2 ml lutofolone® + 5 ml sesame oil) two hours before natural mating; (5) estradiol 80 µg and progesterone 800 µg/kg (OP300) where does were injected intramuscular with 1.65 ml from mixture of lutofolone® (estradiol benzoate 2 mg and progesterone 20 mg) diluted with sesame oil (1/2 ml lutofolone® + 5 ml sesame oil) two hours before natural mating, and (6) control group its does were at the same physiological condition of treated does but did not receive any treatment.

2.4. Reproductive traits

a. Vulva color (pale, pink, red) in each treatment after treatment application.
b. Receptivity percentage indicated the percentage of does accepted mating.
c. Fertility percentage measured the percentage of females became pregnant.
d. The unfertile receipt percentage determined the percentage of females accepted mating but failed to be pregnant.

2.5. Productive traits

1. Prolificacy measured average number of born kits/litter.
2. Birth weight measured the average born kits weight/ litter.
3. Weaned number measured the average weaned kits number/doe.
4. The average fryer weaning weight measured the average fryer weaning weight/ doe.
5. Doe feed intake measured the average weekly feed intake of doe during the first five weeks after treatment establishment.

2.6. Statistical analysis

The reproductive traits (vulva color, receptivity, fertility, and unfertile receipt) were expressed as a proportion, and the productive traits (prolificacy, birth weight, weaned number, average fryer weight, and doe feed intake) expressed as an absolute number were analyzed two-way analysis of variance by **SAS (2002)**, Proc GLM where differences (LSD) between means were tested according to Duncan's multiple range test **(Duncan, 1955)** using the following model:

$$Xijk = \mu + Ai + Bj + eijk$$

Where:
Xijk = An individual observation.
µ = Overall mean.
Ai = Effect of i^{th} treatment (CC, DG, DLS, OP100 and OP300)
Bj = Effect of j^{th} time of treatment application postpartum (day 2, 7, and 14 postpartum).
Eijk = Random error.

Table 1. Composition and chemical analysis of the basal diet

Ingredient	%	Chemical analysis (% as DM):	%
Berseem hay (*Trifolium alexandrinum*)	30.05	Dry matter (DM)	85.81
Barley grain	24.60	Crude protein (CP)	17.36
Wheat brain	21.50	Organic matter (OM)	91.42
Soybean meal (44% CP)	17.50	Crude fiber (CF)	12.37
Molasses	3.00	Ether extract (EE)	2.229
Limestone	0.95	Metabolizable energy (ME, kcal/kg)	2257
Di-calcium phosphate	1.60	Calcium	1.243
Sodium chloride	0.30	Phosphorus	0.808
Mineral-vitamin premix	0.30	Methionine	0.454
DL-Methionine	0.20	Lysine	0.862

Mineral–vitamin premix provided the following per kilogram of diet: Vitamin A, 150,000 UI; Vitamin E, 100 mg; Vitamin K3, 21 mg; Vitamin B1, 10 mg; Vitamin B2, 40 mg; Vitamin B6, 15 mg; pantothenic acid, 100 mg; Vitamin B12, 0.1 mg; niacin, 200 mg; folic acid, 10 mg; biotin, 0.5 mg; choline chloride, 5000 mg; Fe, 0.3 mg; Mn, 600 mg; Cu, 50 mg; Co, 2 mg; Se, 1 mg; and Zn, 450 mg.

3. Results

3.1. Reproductive performance

Vulva Color: The control group had the significantly highest ($P < 0.0001$) pale vulva percentage followed by DLS group and the significantly lowest percentage observed for the two hormonal treatments, however there were un statistical differences between CC, DG groups and the other treated groups. On the contrary, the control groups reported the significantly lowest pink and red vulva percentages (**Table 2**), the hormonal treatments (OP100 and OP300) and CC treatments had the significantly highest pink vulva percentage ($P= 0.0089$), however, DLS and DG groups did not differ significantly in pink vulva percentage from control group although these groups had significantly higher red vulva percentage ($P= 0.0281$) compared with the control group. OP100 group reported significantly higher red vulva color percentage compared with the control; however, neither OP300 nor CC treatments differ statistically in red vulva percentage from the control treatment.

Influence of the day of treatment application on vulva color included in the **Table 2**, wherever only the pale vulva percentage statistically influenced with this independent factor as it was significantly higher on the day 14 than the day 2 and 7 postpartum (0.0008).

Receptivity: **Table 2** data illustrated low receptivity percentage of this farm as the does of control treatment achieved only 0.65 receptivity. Application of different experimental treatments, either natural or hormonal, induced significantly ($P= 0.0355$) high improvement of receptivity percentage compared with control, with the highest values reported for CC and DG treatments and the lower improvement achieved by OP300 treatment. The influence of time point on receptivity was not significant.

Fertility: The fertility percentages illustrated in **Table 2** revealed that does in either natural or hormonal treatments of the experiment greatly improved compared with the very low fertility percentage of the control group (0.47). Wherever the significantly highest fertility percentage ($P= 0.0003$) resulted from DG treatment (0.90) followed by CC and DLS treatments, however, the hormonal groups induced lower improvement compared with natural groups. Moreover, fertility percentage did not influence significantly with time point.

Unfertile receipt: Unfertile receipt doe is the doe, although it accepted the buck, gave negative result with pregnancy diagnosis. Data in **Table 2** showed that the control and hormonal groups had significantly higher ($P= 0.0287$) unfertile receipt percentage compared with all-natural treatments. Concerning the impact of the day of treatments application on the unfertile receipt percentage, the differences between treatments were not significant.

3.2. Productive performance

Prolificacy and births weight: Data in **Table 3** include prolificacy means the litter size (no of kits born/litter) and birth weight means weight of all born kits/ litter. Neither prolificacy nor birth weight was significantly influenced by the type of treatment or day of treatment application. However, numerically all natural groups had better values than the control and the hormonal groups and application of treatments on

the day 7 postpartum achieved better results than on day 2 and day 14 postpartum.

Weaned number and weight: The number of weaned fryers and the average fryer weaning weight per doe were calculated after one month of the suckling period and data summarized in the **Table 3**. All treated groups achieved higher weaning numbers over the control group, although the differences were significant only with DG and DLS groups (P= 0.0351). On the other hand, DLS treatment reported significantly decreased average fryer weaning weight than the control group. However, the hormonal treatments induced the highest average weaning weight followed by CC treatment compared with the control treatment (the difference was significant only with OP300 treatment P= 0.0012).

Concerning the influence of the day of treatment application on the weaned number and average fryer weaning weight (**Table 3**), the differences between the data were not significant.

Doe feed intake: Doe feed intake during the first five weeks of the experiment was estimated to evaluate the influence of the different experimental treatments on doe's appetite. **Table 4** illustrated that the does feed intake increased with week progress regardless of the treatment; additionally, the feed intake of all treatments was approximately the same during the first week. From the second week to the fourth-week, feed intake values of control and DLS groups were significantly lower than all other experimental groups (P < 0.0001).

Influence of the day of treatment application on does feed intake explained in **Table 4**; in all weeks, the feed intake when the experiment applied on day 14 postpartum was higher than when applied on day 2 postpartum, wherever the feed intake values on day 7 postpartum were intermediate.

Table 2. Means ± standard error of vulva color and reproductive traits considering experimental groups and the day of treatment application

	Vulva color			Reproductive traits		
Item	Pale	Pink	Red	Receptivity	Fertility	Unfertile receipt
			Experimental group			
CC	0.15 ± 0.05 ^{bc}	0.70 ± 0.08 ^{a}	0.14 ± 0.08 ^{bc}	0.92 ± 0.04 ^{a}	0.84 ± 0.05 ^{ab}	0.08 ± 0.04 ^{b}
DG	0.15 ± 0.05 ^{bc}	0.49 ± 0.05 ^{ab}	0.31 ± 0.01 ^{ab}	0.92 ± 0.04 ^{a}	0.90 ± 0.05 ^{a}	0.06 ± 0.04 ^{b}
DLS	0.26 ± 0.07 ^{b}	0.39 ± 0.09 ^{b}	0.34 ± 0.06 ^{a}	0.84 ± 0.08 ^{a}	0.77 ± 0.07 ^{ab}	0.07 ± 0.04 ^{b}
OP100	0.10 ± 0.05 ^{c}	0.63 ± 0.09 ^{a}	0.27 ± 0.09 ^{ab}	0.84 ± 0.05 ^{a}	0.67 ± 0.06 ^{b}	0.21 ± 0.05 ^{a}
OP300	0.11 ± 0.05 ^{c}	0.72 ± 0.10 ^{a}	0.17 ± 0.07 ^{abc}	0.82 ± 0.08 ^{ab}	0.66 ± 0.08 ^{b}	0.15 ± 0.05 ^{ab}
Control	0.55 ± 0.08 ^{a}	0.37 ± 0.07 ^{b}	0.08 ± 0.04 ^{c}	0.65 ± 0.06 ^{b}	0.47 ± 0.05 ^{c}	0.22 ± 0.05 ^{a}
P-value	<.0001	0.0089	0.0281	0.0355	0.0003	0.0287
			The day of treatments application postpartum			
2	0.16 ± 0.05 ^{b}	0.58 ± 0.06	0.24 ± 0.05	0.83 ± 0.04	0.76 ± 0.05	0.11 ± 0.03
7	0.16 ± 0.05 ^{b}	0.56 ± 0.07	0.27 ± 0.05	0.86 ± 0.05	0.71 ± 0.05	0.14 ± 0.03
14	0.34 ± 0.06 ^{a}	0.51 ± 0.07	0.15 ± 0.04	0.82 ± 0.05	0.69 ± 0.06	0.15 ± 0.03
P-value	0.0008	0.6466	0.1367	0.7787	0.5731	0.7014

Means within the same column within the same category have different superscripts that are significantly different.
CC= cage change group
DG= does gathering group
DLS=dam litter separation group
OP100= estradiol 27 µg and progesterone 270 µg/kg group
OP300= estradiol 80 µg and progesterone 800 µg/kg group.

Table 3. Means ± standard error of productive traits considering the experimental group and the day of treatments application postpartum

Item	Prolificacy	Birth Weight	Weaned No	Fryer weaned weight
		Experimental group		
CC	6.58 ± 0.26	321.58 ± 10.57	6.32 ± 0.22 ^{abc}	479.52 ± 7.82 ^{ab}
DG	6.91 ± 0.20	333.64 ± 7.23	6.59 ± 0.16 ^{ab}	460.87 ± 5.80 ^{bc}
DLS	7.15 ± 0.22	340.25 ± 9.05	6.85 ± 0.20 ^{a}	442.42 ± 6.30 ^{c}
OP100	6.56 ± 0.28	315.28 ± 10.74	6.22 ± 0.24 ^{bc}	476.55 ± 10.66 ^{ab}
OP300	6.53 ± 0.26	319.41 ± 9.78	6.18 ± 0.21 ^{bc}	485.77 ± 7.46 ^{a}
Control	6.33 ± 0.26	317.92 ± 8.22	5.92 ± 0.19 ^{c}	455.56 ± 9.53 ^{b}
P value	0.2166	0.343	0.0351	0.0012
		The day of treatments application postpartum		
2	6.74 ± 0.15	326.05 ± 5.22	6.37 ± 0.12	467.46 ± 4.92
7	6.94 ± 0.17	335.14 ± 6.22	6.64 ± 0.16	460.06 ± 5.93
14	6.44 ± 0.20	315.29 ± 8.40	6.15 ± 0.16	472.80 ± 7.15
P value	0.1346	0.1288	0.0626	0.3715

Means within the same column within the same category have different superscripts that are significantly different.
CC= cage change group
DG= does gathering group
DLS=dam litter separation group
OP100= (estradiol 27 µg and progesterone 270 µg/kg group)
OP300= (estradiol 80 µg and progesterone 800 µg/kg group)

Table 4. Means ± standard error of weekly feed intake within the first five weeks after establishment of treatment considering the experimental group and the day of treatments application postpartum.

Level	FI1	FI2	FI3	F14	FI5
			Treatment		
CC	1086.05 ± 9.62	1338.16 ± 9.64 ^{a}	1525.26 ± 15.19 ^{ab}	1705.26 ± 18.79 ^{ab}	1911.84 ± 15.82
DG	1076.36 ± 13.03	1350.00 ± 10.29 ^{a}	1513.18 ± 13.99 ^{bc}	1686.36 ± 17.95 ^{b}	1866.36 ± 18.30
DLS	1076.00 ± 12.66	1284.00 ± 20.12 ^{b}	1443.75 ± 22.86 ^{d}	1600.50 ± 21.98 ^{c}	1854.00 ± 20.65
OP100	1065.28 ± 9.38	1341.11 ± 9.82 ^{a}	1532.22 ± 12.93 ^{ab}	1693.33 ± 19.75 ^{ab}	1920.83 ± 15.34
OP300	1070.88 ± 11.52	1334.71 ± 6.52 ^{a}	1562.06 ±10.68^{a}	1745.59 ± 14.09 ^{a}	1930.59 ± 13.32
Control	1096.67 ± 18.15	1225.83 ± 28.30 ^{c}	1461.25 ± 40.11 ^{cd}	1630.00 ± 49.36 ^{bc}	1876.67 ±6 1.88
P value	0.6106	<0.0001	0.0001	0.0001	0.1039
			The day of treatments application postpartum		
2	1066.84 ± 6.59	1296.97 ± 12.57 ^{b}	1481.45 ± 15.34 ^{b}	1642.50 ± 17.47^{b}	1871.97 ± 16.60
7	1079.31 ± 9.76	1316.67 ± 12.66 ^{ab}	1513.47 ± 15.57 ^{ab}	1696.25 ± 17.87^{a}	1893.33 ± 18.95
14	1087.65 ± 9.30	1342.94 ± 8.90 ^{a}	1530.44 ± 11.81 ^{a}	1698.38 ± 16.01^{a}	1914.26 ± 14.92
P value	0.2188	0.0183	0.0343	0.0163	0.2055

Means within the same column within the same category have different superscripts are significantly different.
CC= cage change group
DG= does gathering group
DLS=dam litter separation group
OP100= (estradiol 27 µg and progesterone 270 µg/kg group)
OP300= (estradiol 80 µg and progesterone 800 µg/kg group)

4. Discussion

This study was applied on multiparous rabbits, excluding nulliparous and primiparous females due to their unstable reproductive parameters. Therefore**, Fernández, (2010)** indicated in his Ph.D. thesis that nulliparous females have the best fertility rates, while primiparous females show the lowest values for this parameter. Vulva color was significantly influenced by different experimental treatments, wherever all treatments, either natural or hormonal, significantly decreased pale vulva percentage compared with the control treatment, with the highest effect induced by hormonal and DLS treatments, and therefore all treatments significantly increased the percentages of pink and/or red vulva color percentage on the control group. When a dam-litter separation was applied in experimental group (doe gatherings 8/cage, 15 minutes before insemination) and control groups, **Duperray et al. (1999)** found that the frequency of red and purple vulva were higher in the experimental group. **Ilès et al., (2013)** reported a higher percentage of red, pink, or turgid vulva in DLS than control group, but the control does have the highest percentage of white vulva. Mating acceptance was most when the vulva was red, pink, or turgid; it was lowest when it was white.

All experimental treatments, either natural (CC. DG, and DLS) or hormonal (OP100 and OP300) significantly improved reproductive performance traits compared with control treatments with the superiority of the natural methods over the hormonal one. On the contrary, **Villamayor et al., (2022)** showed that the bio stimulation methods employed in their experiment did not significantly improve any of the analyzed parameters related these results to the high reproductive performance of the treated farm and recommended to test biostimulation efficiency in farms with low fertility rates (50–60%). Thus, our high positive results may be related to the low reproductive performance of the treated farm indicated from control group data, previous studies on the regulation of rabbit reproduction have demonstrated that hormonal or biostimulation techniques enhance reproductive performance in females with conception rates around 50 to 60 % of the average **(Szendr˝o et al., 2012)**.

Rebollar et al., (1995) evidenced that a change of cage can improve fertility, similarly**, Luzi and Crimella, (1998)** discovered that changing the cage 48 hours before fertilization enhanced fertility (+14%) in non-nulliparous does compared with the control group. **Duperray et al., (1999)** discovered that applying doe gatherings (8/cage, 15 minutes before insemination) with a dam-litter separation had a positive effect on rabbit doe receptivity and significantly increased fertility (+ 6.1 percent). However, the positive effect on fertility is evident on nulliparous, multiparous lactating, and non-lactating does but not in primiparous rabbit does. Moreover, **Tůmová et al., (2005)** noted that using group housing increased the receptivity percentage in nulliparous females.

Similar DLS results obtained by **Pavois et al., (1994), Maertens, (1998), and Theau-Clément and Mercier, (1999)** who found that 24 hours dam-litter separation improved sexual receptivity and fertility of 11 days lactating does. Additionally, **Ladyková et al., (2008)** compared the effect of hormonal treatment and group housing system of does on vulva coloration before artificial insemination and showed the increased receptivity percentage of both treatments with better results obtained from cage change treatment. On the contrary, fertility was improved by +17 percent (47.4 vs. 64.2 percent) on 3-day lactation does by **Alvario et al. (1998)** as opposed to not improving fertility at all when the stimulation was applied to 10-day lactating does.

Productive performance traits including prolificacy (litter size), birth weight, weaning number, average fryer weaning weight, and average doe feed intake within the first five weeks of experiments were estimated during the different experimental periods. Litter size and weight did not differ statistically between treatments; similarly, **Duperray et al., (1999)** found that at birth, the size and the weight of the litter are not modified by the treatment, neither doe gatherings nor dam-litter separation. **(Maertens, 1998, Bonanno et al., 1999a; b, 2000 and 2004)** found that dam-litter separation does not generally influence litter size nor the mortality of kits. There were little previous data about the further productive performance traits of dams and their litter subjected to the different estrus induction methods; therefore, our experiment was interested to measure some of them. The weaning number was high in all treated groups than in the control one. However, differences were significant with DLS and DG treatments only (P=0.0012), the weaning number positive results despite the non-significant prolificacy differences reflecting the higher kits mortality induced in the control group. Average fryer weaning weight was significantly higher in OP300 group and significantly lower in the DLS group compared with control group. Moreover, the lowest feed intake values were reported for DLS treatments, and the highest was for OP300 compared with the other treated groups (there was no significant differences between DLS and control group). A similar result found by **Maertens, (1998)** who reported that in mother- litter separation group the weight of litter after application of suckling prevention at day 11postpartum was lower compared with flushing and pregnant mare serum (PMS) groups, however decrease weight sustained during the further lactation period, and the young of these mother had a lower weaning weight 40-47g. Wherever related these results to the large interval between 2 suckling (40 hours), which may be decreased the milk yield of these mothers during the further lactation period and thus partly responsible for the reduced weight of the young, however, the feed intake of mother + young in this group was significantly lower between the day 8-11 postpartum, and the day 21 to weaning could also be responsible for the lower weaning weight. Additionally, the pregnant mare serum (PMS) group had significantly high feed intake capacity results. The author also concluded that the decreased feed intake of the DLS group during the period of suckling prevention might be the cause of increasing receptivity and fertility of this treatment. Because these does produce less milk (one nursing inhabited) which may be led to improving energy balance than free suckling. Although, **Theau-Clément and Mercier, (1999) and Szendrö et al., (1999)** reported marked fall in kits weight after 24 hours and 48 hours of dam litter separation (24 hours separation; - 6%- and 48-hours separation - 13%), when rabbits are weighed immediately after suckling, they did not follow the further weaning weight. On the contrary, **Bonanno et al., (2004)** discovered that dividing the 48 hours DLS into two subsequent 24 hours periods boosted fertility like the 48 hours DLS and prevented a decrease in litter growth rate brought on by a reduction in milk intake. Therefore, a viable alternative strategy for preventing the slowing down of the litter growth rate and limiting any potential negative effects on rabbit welfare without reducing fertility might be the brief interruption in the continuous DLS lasting 48 hours that results from the controlled suckling.

The rabbit does' production influenced by many factors, one of them is the age at first insemination **(Rommers et al., 2002 and Bonanno et al., 2004)**. Our results illustrated that better reproductive and productive performance obtained when the treatments applied at the day 2 and 7 postpartum compared with day 14 postpartum. **Ubilla and Rebollar, (1995)** observed high plasma estradiol-17β concentration that reflects maturity of ovarian follicles on days 1, 5-7, and 23-30 of the postpartum periods, so does insemination at these periods will induce high conception rates. Moreover, the effects of reducing the re-mating interval after parturition evaluated **Awojobi et al., (2011)** concluded that reducing the re-mating interval after parturition enhanced sexual activity where fertility was comparable in does re-mated 1-9 and 21-28 days after parturition compared with 10-20 days. The increased level of weekly feed intake observed in this experiment agreed with **Pascual et al., (2003)** related it to the usage of female body reserves to provide kits needs at the beginning of gestation (the first 21 days). Additionally, multiparous does requires feed for milk production for their suckling kits.

The effects of estradiol and progesterone on rabbits does reproduction and production performance rarely applied. Although, there were old researches concluded the efficiency of these hormones on the reproduction of induced estrus animal species. **Sawyer et al., (1950)** reported an improvement of ovulation in 40% of synchronized does from the administration of a combination of estrogen and progesterone daily for two days, but not estrogen or progesterone independently. Moreover, in California voles, **Milligan, (1978)** found that administration of estradiol-17β or estradiol benzoate caused ovulation in up to 28% of treated females. Recently, **Bianchi et al., (2021)** reported that administering llamas with increasing estradiol-17β concentrations caused an incremental increase in ovulation. The ovulatory response, corpus luteum development, and plasma progesterone profile agreed with the hypothesis that estradiol induces ovulation in this species even though LH concentrations were not recorded. They also concluded that estradiol might be stimulating neural pathways in camelids, causing a rise in GnRH and LH.

5. Conclusions

All used treatments, natural (CC, DG, and DLS) or hormonal (OP100 and OP300), induced a significant positive effect on measured reproductive performance traits, with the superiority of the natural methods on hormonal one. Considering productive performance traits, CC and DG methods were better as natural methods than the DLS method; nevertheless, these biostimulation methods have to be used only on healthy herds since the contact between animals could represent a source of contamination. Moreover, estrogen and progesterone can be effective hormonal methods in improving rabbit's reproductive and productive performance. However further research is required to evaluate its effect on other blood hormone levels, the feedback mechanism from its supplementation and if repeated use of this hormone can induce an immune response and affect ovary function. Finally, the natural or hormonal rabbit reproduction stimulation methods should be involved in farms of bad performance to induce a good response.

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