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In Vitro Development of Vitrified Rabbit's Embryos as Affected by some Nutritional and Hormonal Treatments

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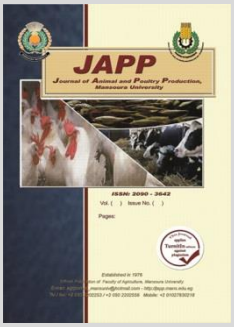

ABSTRACT

The purpose of the study was to measure the effect of 1-nutritional stages on reproductive performance in NZW rabbit doe. Total of 21 adult NZW rabbit were divided into 3groups (n=7). In the first group does were given 100%NRC (Control,G1), In the second and third groups does were given 110(G2) or 120%(G3) from NRC for 3week before naturally mating. Weight, litter size and possibility rate at weaning and birth were recorded. 2-flushing (110% from NRC) (G1) and (superovulation, G2) using PMSG on ovarian activity, (recovery, freezability and *in vitro* development of rabbit embryos (n=4/group). Embryos were collected after 56h after naturally mating. Embryos freezing by vitrification and survival was assessed by *in vitro* culture. Results revealed that, 1-Total litter size and alive born at birth and litter size at weaning was much higher (P>0.05) in (Group2) than in other groups. 2-Mean number of corpus lutea and large follicles were much higher (P<0.05) in (Group2) than in (Group1) (8.5 and 6.25) also (6.25 and 4.5), respectively. Survival rate and normal embryos post-vitrification in (Group1) was higher than in (Group2) by 1.48% too 6.99% respectively. The percentage of hatched blastocyst stage in (Group1) was significantly (P<0.05) higher than in (Group2) (83.33 vs. 71.74%, respectively). However, expanded blastocyst stage showed opposite of this trend. Conclusion, improving reproductive performance for rabbit does in G2 (110% from NRC) than in other groups. Also the potential *in vitro* growthing of rabbit embryos from flushing group post-vitrified was significant higher than in hormonal group.

Keywords: Rabbit, flushing, vitrified embryo, survival rate

INTRODUCTION

Many authors have reported that when induced superovulation several time in the same animal, perhaps reduced the response of embryos quality and ovarian function in rabbits (Mehaisen *et al.*, 2006). This reduced answer may be related to an increase in anti-Fertile Stimulating Hormone or anti- equine chorionic gonadotropin sera antibodies (Boiti *et al.*, 1995; Swanson *et al.*, 1996).

Peinado *et al.* (1995) treated NZW does (18 week old, atestruswith25 IU PMSG, then does were injected either with 50 international unit of HCG or LHRH at 48 hour later, and embryos were collected 72 h post mating. They found that quality of embryos in the LHRH vs was higher. Human chorionic gonadotropin group, respectively. Also, Mehaisen *et al.* (2005) found that management of 200 IU equine chorionic gonadotropin significantly the number of ordinary embryos recovered was decreased per doe as compared to 50IU (5.8 vs. 8.2).

Nutrition affects procreative performance through 2 ways; straight by supplying certain nutrients which are necessary for the ovulation, embryo survival, oocyte development and conception; and indirectly through its effect on some metabolites and hormones (Robinson *et al.* 2006). Improved ovulation and fertility rates by increasing protein and nutritional energy before and after mating have been benefited by some researchers (Al-Haboby *et al.* 1999 and Daghigh Kia *et al.* 2012).

This work was planned to study:- 1- Effect of nutritional levels (100, 110 and 120 % NRC for rabbit nutrition)on reproductive performance in NZW rabbits. 2- Comparing the best nutritional level with the effect of hormonal treatment on ovarian activity, embryo recovery and *in vitro* development of vitrified rabbit's embryos.

MATERIALS AND METHODS

This work was implemented at private farm of rabbit, in co-operation between Sakha Animal Production Experimental Station, belonging to (APRI) the Animal Production Research Institute, Agricultural Research Center, Department of Agriculture and Animal Production Management, Faculty of Agriculture, Tanta University, through the period from November 2018 to April 2019.

Animals and the experimental groups:

First experimen:

Total of 21 adult NZW rabbit does having LBW of 3024-3070g and about 6.5 months of age were split into 3 similar groups (7 animals in group). Animals were permitted to acclimatize for seven days in their own cages. Does in the first group were given 100% of a basal diet according to rabbit does requirements of NRC(1977) (Control, G1) Does in the 2nd and 3rd groups were given 110 % (G2) and120 % (G3) of the same basal diet, as flushing for 3 weeks before mating. At mating, fertile males from the same type were used for natural insemination of does. After kindling, The litter size at weaning and birth (total and live), viability rate at weaning and birth, middling live weight at birth of rabbits, and litter weight at weaning and birth were recorded. Feed chemical composition Table 1.

Second experiment:

According to the best results in the first experiment, does in G2 fed 110 % from NRC (1977), showed the nest reproductive

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performance. Total of 8 adult NWZ rabbit does having LBW of 3050 -3150 g and at six months of oldness were divided into two analogous groups (4 animals in group) and permitted to acclimatize for seven days in their own cages. In the first group does were given 110 % NRC diet for rabbit nutrition (Control, G1), while does in the second group were given 100 % NRC diet and superovulated with hormonal treatment (group, G2).

Chemical composition and components of experimental diets.

Components	%	Calculated analysis 1%	%
Barely grains	32.80	OM%	89.76
Clover hay	27.41	CP%	17.26
Wheat bran	17.10	CF%	12.92
Soybean meal (44% CP)	16.24	EE%	2.16
Molasses	3.00	NFE%	57.42
Di calcium phosphate	2.00	Ash%	10.24
Sodium Chloride (NaCl)	0.30	DE(kcal/kg)	2518.7
Vit.& min. Mix*	0.30	Calcium	1.13
Lime stone	0.40	Total phosphorus	0.80
DL-Methionine	0.40	Methonine	0.64
Anticoccidia(Diclazuril)	0.05	Lysine	0.86
Molasses	3.00		
Total	100		

***Each1.5 kg of vitamins and minerals mixture contains: Vit. A 10 million IU, Vit.B1 a thousand mg, Vit.B2 5 thousands mg, Vit.D3 2 million IU, Vit E 10 thousands mg, Vit. K 31000 mg, Pantothenic acid 10.000mg; Nicotinic acid, 30.000g; Vit. B_6 15000mg; Vit. B_{12} 10 mg, Folic acid 1.0g, Biotin 50 mg, Cu 4.000 mg, choline chloride 200mg, Mn 60.000 mg, Fe 30.000 mg, , Co 0.1 g, Se 0.1 g, Zn 50.000 mg, Iodine 0.3 g and Antioxidant 10g. [1] according to Feed composition for animal and poultry feedstuff used in Egypt (2001).**

All bucks and does were kept under the same condition and management in the place, being individually housed in cages metallic (40x50x60cm) provided with water nipple for drinking and feeders in each cage.

Superovulation treatment:

Superovulation was carried out by intramuscular injection of each female (in G1) with 100 IU PMSG (Folligon, Inter vit International BV. Boxmeer-Holland)) in legs, followed by 0.8 µg GnRH (Receptal, Hoechst) in ear vein at the time of natural mating for control (G1) and superovulation (G2) groups to encourage ovulation.

Embryos were collected from 4 does from oviduct and uterine horns in each group (G1 and G2) after 56 h from mating, where the ovarian structure of each female including number of large, small and haemorrhagic follicles and *corpora lutea* was recorded.

Embryo recovery:

Preparation of flushing medium:

Phosphate buffer saline (PBS) medium was Set up according to Gordon (1994) as shown in Table (1).

Table 1. Configuration of phosphate buffer saline (PBS) medium.

Component	g / l	Ingredient	g/l
CaCl2.2H2O	0.133	Kh_2PO_4	1.0
NaCl	8.0	Sodium pyruvate	0.036
$NaHPO_4$	2.17	Sodium penicillin G	100,000 IU
KCl	0.2	Streptomycin	100 mg
MgSO4.7H2O	0.120	Glucose	1.0

About 2mg from bovine serum albumin (BSA) was added to 1ml of Phosphate buffer saline (2%). The prepared medium was adjusted to pH value of 7.2 - 7.4 using ph-meter and to Osmosis level of 280-300 mOsmol/kg using osmo-meter. Then, the medium was filtered by 0.22 µm milli-pore filter (milieux GV, milli-pore, Co-operation Bedford MOA).

Embryos were grouped from does superovulated and control groups after 56 hrs of mating. Embryos recovery rates were computed as (number of grouped embryo / number of CL)x100.

Embryos were recovered from uterine horns and oviducts by flushing with phosphate buffer solution (PBS) with 4% bovine serum albumin (BSA) at room temperature (20-25°C). Thereafter, the recovered embryos were counted and examined for normal and abnormal morphological measures using an inverted microscope.

Vitrification procedures:

The basal medium used for vitrification was PBS supplemented with antibiotics (100 IU/ml penicillin and 100 µg/ml streptomycin) and 20% fetal calf serum (FCS). The vitrification procedures used throughout this experiment were according to Vicente *et al.* (1999) with some modification.

Vitrification solutions:

The basal medium was supplemented with 12.5% (v: v) dimethyl-sulfoxide (DMSO), 0.5 M sucrose and 12.5% (v:v) ethylene glycol (EG) and considered as vitrification solution No. 1 (VS1). While, the basal medium was supplemented with 20% (v: v) DMSO, 0.5 M sucrose and 20% (v: v) EG was considered as VS2.

Vitrification was Implemented in 2 steps: in the first step, embryos were pipetting into vitrification solution1 in Sterile disposable petri dish for two min, the embryos were absorbent into VS2 for 30 s (2nd step). Embryos with 3µl loading into a column located between 2 columns of VS2 detached by air bubbles into the center of 0.25ml plastic pollination straws (IVM L' Aigle, France) using a delicate glass capillary pipette. The straws were plunged immediately into LN2 (-196°C) after heat-sealing.

Thawing and culture of vitrified embryos:

After storage of embryos for at least three weeks in LN_2, vitrified embryos were warmed by holding the straw for six second in air and then agitating them in water bath at 20 °C for at least ten second. The contents of each straw were expelled into Petri-dish. To remove the intracellular cryoprotectants, embryos were transported into a Petri dish containing PBS with 4% BSA and 0.33 M sucrose for one min, followed by serial dilutions in PBS and 0.175 M sucrose solution for two min. The embryos were washed 3 times in PBS solution for five min per time to remove cryo-protectants at room temperature (Vanderzwalmen *et al.*, 2003).

Post-thawing scalable embryos were *in vitro* cultured in 100µl drops of tissue culture medium-199 (TCM 199) (Sigma) complemented with 6% BSA and 50µg /ml of Gentamicin sulphate under oil for 72 h, at 38.5°C, higher dampness and 5% CO_2 in air. Number of normal (viable), unviable and total embryos and then recovery rate was calculated as the following:

Embryo survival was assessed by their ability to grow to the expanded and hatched blastocyst stages following *in vitro* culture.

Statistical analysis:

Data for effects nutritional levels (1st experiment) wfere tatistically analyzed by one way analysis. In the 2nd experiment, data were analyzed by factorial design (2x2) for test the effect of treatment (hormonal and nutrition), ovarian site (right and left) or their interaction. The significant differences between group means (only in 1st experiment) were performed using Duncan Range Test (Duncan, 1955).

Chi-square test was used to analysis for embryos recovery, survival and *in vitro* development. The statistical analysis was done by computer program of SAS (2000). The percentages standards were adjust to arcsine transformed before performance the analysis of variance. Means were offered after being re-calculated from converted values to percentages.

RESULTS AND DISCUSSION

The first experiment:

The main effects of dietary level of Litter size and litter weight of New Zealand rabbit does:

Data in Table 2 showed litter size (total and live) at birth and weaning, and litter weight at birth and weaning substantially (P<0.05) increased in (G2) than in other groups.

Average kit weight and viability rate at birth weren't affected substantially by the nutritional plane. Table (2).

Table 2. Impact of nutrition level on reproductive performance in rabbit does.

Parameter	Treatment		
	Group 1	Group 2	Group 3
	Litter size		
Total at birth (n)	6.75±0.48^{b}	8.75±0.25^{a}	6.5±0.46^{b}
Alive at birth (n)	6.25±0.25^{b}	8.25±0.25^{a}	6.25±0.48^{b}
At weaning(n)	5.75±0.48^{b}	7.75±0.25^{a}	5.75±0.25^{b}
	Average body weight (g)		
At birth	43.5±1.55	42.25±1.31	45.50±1.19
At weaning	399.67±0.96	396.25±7.47	403.25±10.89
	Litter weight (g)		
At birth	270.75±2.56^{b}	348.0±9.5^{a}	282.75±15.3^{b}
At weaning(g)	2296.0±186.6^{c}	3070.3±93.9^{a}	2354.0±100.1^{b}
	Viability rate		
At birth	93.30±3.88	94.44±3.21	96.88±3.13
At weaning	95.83±4.17	94.1±3.42	92.86±4.12

Means denoted within the similar row with different superscripts are substantially different at (P<0.05).

Nutrition may impact reproductive performance by number of mechanisms including centralization effects on gonadotropin secretion (Booth et al., 1994) and local effects on ovarian job (Cosgrove and Foxcroft, 1996). Concentrate supplementation (300g) for two weeks before breeding ewes was beneficial for improving the reproductive efficiency, Islam *et al*., (2007).

The present results are in accord with Martınez-Paredes et al. (2012), who recommended that the introduction of a short flushing around first AI is used for good fertility rates at the 1st parturition in multiparous rabbits. Increasing litter size of does in G2 (110% NRC) may be attributed to that a short flushing period of 7 days could be good bio-stimulation technique for oestrus synchronisation and development of receptivity in multiparous constrained fed multiparous NZW rabbit does after a long break period (Manal, 2010).

Biostimulation using flushing can change doe power status before mating (Theau-Clément, 2000). Generally, many authors recorded positive effects of restricted and ensuing higher nutrient supply (a few days previous to AI) on reproduction in multiparous rabbits (Eiben et al.,2001; Gómez et al., 2004; Bonannoet al., 2004). Flushing diet along with nutrients have a positive effect on breeding rate, which is due to an increase in egg-laying and follicle growth rate (Ahmad Fazel and Daghighkiam 2014).

The type of feed offered and configuration of feed may be more important than the amount (Smith, 1984) in dynamic flushing influence response of animals. The protein: energy percentage of the pre-mating diet was conveyed to be more critical to get a reproductive response (Croker *et al*. 1985). Therefore, improving reproductive performance of does in G2 than in G3 may be due to that high dietary protein (in 120% NRC diet), resulting in high concentrations of urea nitrogen in plasma and milk has been related with reduced fertility (Ferguson et al., 1993). This result was confirmed in a study by (Butler *et al.*1996).

Second experiment:

Ovarian responses to flushing and hormonal treatments.

Effect of flushing and hormonal treatment on ovarian activity of rabbit is presented in (Table 3). Analysis of contrast exposed that the mean number of corpora lutea (CL) and large follicles per ovary in hormonal group was significantly (P<0.05) higher than in flushing group (8.5 vs. 6.25) and (6.25 vs.4.5), respectively. However, the mean number of small follicles showed an oppositetrend. But the number of bleeding follicles/ovary was higher in hormonal group than in flushing group without significant differences.

Results in Table (3) revealed insignificant differences between site of the ovary (right and left) in the mean numeral of small, large and bleeding follicles. While, the mean number of CLs in right was substantially (P<0.05) higher than left site (8.38 vs. 6.38).

Table 3. Influence of treatment (flushing and hormonal) and ovarian side on ovarian structures of rabbit.

Item	Ovarian structure			
	CL/ovary	LF/ovary	SF/ovary	BF/ovary
		Treatment:		
Flushing	6.25±0.49^{b}	4.50±0.42^{b}	10.13±0.61^{a}	0.50±0.19
Hormonal	8.50±0.42^{a}	6.25±0.31^{a}	8.25±0.37^{b}	1.13±0.23
		Ovarian site:		
Right	8.38±0.5^{a}	5.88±0.44	9.75±0.65	0.75±0.25
Left	6.38±0.5^{b}	4.88±0.48	8.63±0.5	0.88±0.23

$^{a \text{ and } b}$ **Means indicated within the same column for each variable with different superscripts are substantially different at (P<0.05).**

CL: Corpus luteum, LF: Large follicles, SF: Small follicles, BF: Bleeding follicles.

The effect of inter-action between side of the ovary and treatment on ovarian activity is presented in Fig. (1). Data revealed that no substantial (P<0.05) effect of inter-action between site of ovary and treatment on ovarian structures. These results reflected the same trend effect of nutrition and hormonal on ovarian site function.

Accordding to the present results, Bonhoff and Adams (1985) cleared that rabbit females treated with HCG or LHRH showed higher number of CLs, while no CLs were found in the controls.

Lee *et al.* (1991) noted that the number of ovulation score averaged 19.2/female rabbit super-ovulated with PMSG. Gosalvez *et al.* (1994) observed that more CLs/ ovulating female (11.0 vs. 7.92, P<0.05) and number of antral follicles (> 0.6mm) for rabbit female injected with one hundred IU saline solution or PMSG, followed by 20 mg LHRH (Fertagyl) two days later. Gravance (1994) cleared that the ovaries of rabbit females Deal with 36 IU of HCG and 72 IU of PMSG in a single intra-muscular injection had significantly higher number of CLs than those treated with physiological saline (control). Fukunari*et al.* (1990) observed that when ovarian were grouped 72 hour after treatment of immature Japanese white rabbit with 50 IU PMSG or from untreated does, the number of large follicle was substantially higher in treated does than un-treated. Follicles number was higher in PMSG treated rabbit than in untreated. The total number of antral follicles and number of healthy antral follicle (>700 µm in diameter) were higher in PMSG–treated than control females.

El- Gaafary *et al.* (1994) found that the number of matured follicles on the female ovaries (16/ovary) increased in NZW rabbits injected with hCG than in the control does. The mean number of anovulted haemorrhagic follicles found in the ovary of does treated with PMSG increased especially when high of dosage was employed. VeriniSupplizi *et al.*(1994) reported that the number of haemorrhagic follicles (HFs) for100 IU PMSG treated does was significantly reduced by the administration of monoclonal anti-PMSG. These finding suggest that the effect of PMSG is due overstimulation of ovarian follicles owing to its long half-life. However, it cannot exclude that 0.8 µg

GnRH following 10 IU PMSG can be insufficient to trigger ovulation of all stimulated follicles.

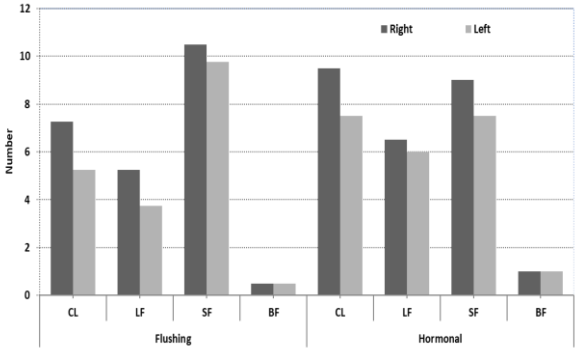

Fig. 1. The influence of inter-action between treatment and site of ovary on ovarian activity.

Recovery rate and viability of embryos:

Results offered in Table (4) demonstrated that percentage of viable embryos were higher in flushing group (95.74%, P<0.05) than in hormonal group (87.1%). Also, embryo recovery rate was higher flushing group than in hormonal group, but the differences were not significant. The highest percentage of viable embryos in flushing group was associated with the lowest unviable embryos (4.26%, P<0.05) as compared to hormonal group (12.9%). These results referred positive influence of flushing treatment on viability of retrieve embryos.

El-Keraby *et al*., (1991). found that the recovery rates of embryos grouped from does treated with 0.2 or 0.4 ml GnRH ranged from 88.8 to 90% in treated vs 90% in the control groups, in comparable with the present results. Higher embryo recovery in rabbits was informed when PMSG was injected intramuscularly (Besenfelder *et al*., 2000) or when follicle stimulating hormone (FSH) was used for superovulation (Joly, 1997).

Table 4. Effect of treatments (flushing and hormonal) on recovery rate (RR) and viability of rabbit embryos.

Treatment	Number		Recovery rate (%)	Viable Embryos		Unviable Embryo	
	CL	Embryos		N	%	n	%
Flushing	50	47	94.0	45	95.74[a]	2	4.26[b]
Hormonal	68	62	91.18	54	87.1[b]	8	12.9[a]

[a and b:] **Means indicated within the same column for each effect with different super-scripts are substantially different at P<0.05.**

The retort to super-ovulation treatment not adopted only on the type of hormone and the method of administration, but also varies among rabbit breeds (Bolet *et al*., 2000). Using different kinds of hormonal management to induce ovulation (superovulation) for the V and R line rabbits, (Mehaisen *et al*., 2005) reviewed lower recovery rate (43.2 and 40.3%), respectively as compared to 63 and 82% in the study of (Viudes-de-Castro *et al*., 1995), 77 and 74%, in the study of (Vicente *et al*., 2003) for V and R lines, respectively. These differences may be due to the influence of the super-ovulation treatment that was used.

Improvement of foodstuffs supply can affect the hypothalamus pituitary axis thereby effecting to gonadotropins, change estrogen, progesterone, insulin, growth hormones concentrations and some metabolites amount (such as glucose) leading to higher oocyte quality and ovulation rate (Scaramuzzi *et al.*, 2006). Thomas *et al.* (1984). informed that an increase in nutrient consumption, particularly protein, effectively increases levels of hepatic steroid metabolizing enzymes. Decreases of steroids higher clearance rate is associated with an increase in gonadotropins which can improve ovulation rate.

Survival rate and quality of vitrified embryos:

Total survival rate, normal, and abnormal embryos post-vitrification is presented in Table (5). The percentage of total survival rate and normal embryos post-vitrification was higher in flushing group than in hormonal group by (1.48%) and (6.99%) respectively, but without significant differences.

Table 5. Effect of treatments (flushing and hormonal) on survival rate and quality of vitrified/thawed embryos.

Treatment	Vitrified embryos (N)	Post-vitrification		Normal embryos		Abnormal embryos	
		N	%	n	%	N	%
Flushing	45	44	97.78	42	95.45	2	4.56
Hormonal	54	52	96.3	46	88.46	6	11.54

It is of attention to note that the percentage of post-vitrified abnormal embryos in flushing group was lower by 7.02% than in hormonal group, but this difference was not significant. These outcomes indicated an impact of flushing group therapy on quality of post-vitrified embryos.

A primary problem in the cryo-preservation actions was the proportion of intact embryos (homogeneous cell mass and zona pellucida and mucin coat without damage) after thawing or devitrification (Vicente et al., 2003).

Vitrified embryos *in vitro* culture:

Outcomes of *in vitro* post-thawing-culture for 72 h presented in Table (6) revealed that post-vitrified embryos recovered from does in flushing group showed substantially (P<0.05) higher percentage of embryos than in hormonal group (83.33 vs. 71.74% respectively). However, the percentage of expanded blastocysts was substantially (P<0.05) lower in flushing group than in hormonal group (15.22 and 9.53%, respectively).

Table 6. Effect of treatments (flushing and hormonal) on *in vitro* development of embryos post-vitrified/ thawing for 72h.

Treatment	Survival Embryos (N)	Embryonic stage of *in vitro* culture for 72 h							
		Early Blastocyst		Expanded blastocyst		Hatched blastocyst		Degenerated embryos	
		N.	%	N.	%	N.	%	N.	%
Flushing	42	1	2.38	4	9.53[b]	35	83.33[a]	2	4.76
Hormonal	46	2	4.35	7	15.22[a]	33	71.74[b]	4	8.7

[a and b:] **Means indicated within the same column for each effect with different super-scripts are substantially different at (P<0.05).**

These outcomes indicated further beneficial influence of flushing treatment on quality of post-vitrified embryos. It is of attention to note that the percentage of post-vitrified *in vitro* culture early blastocysts and degenerated embryos was lower in flushing group (2.38 vs.4.35%) and (4.76 vs. 8.7%) than in hormonal group, but this differences were not significant.

The viability of frozen-thawed embryos can be evaluated by several methods, such as morphological note after thawing and *in vitro* culture. But the best mode to confirm their capacity for more development is by transferring them into a pseudo-pregnant recipient (Techakumphu and Heyman, 1987).

The lower development of embryos recovered from does treated by hormones was reported by Stradaioli *et al.* (1997), who suggested that embryos recovered from PMSG treated does were less viable rather than delayed in development capacity compared with the control ones. Also, Fujimoto *et al*. (1974) suggested that the low embryo development rate in 100 IU PMSG treated does could be due to the high incidence of chromosome abnormalities and the low mitotic index. Moreover, Stradaioli*et al*. (1997) reported that the decreased embryo viability and embryo development in PMSG treated groups after 48 and 72 hours of *in vitro* culture may be due to a negative influence on oocytes maturation and early embryo development induced by gonatrophin.

A direct effect of PMSG on the ovary must also to be taken into consideration. Exogenous gonadotropins administrated at higher doses from 40 to 150 IU, may induce ovarian overstimulation resulting in abnormal follicular steroid production and several environmental changes of reproductive tract (Foote and Elligton, 1988). Furthermore, these hormonal treatments may alter follicular growth, cause failure to ovulate (Hyttel *et al*., 1991), interference of ovum transport mechanisms (Greenwald 1961), and delayed zygote development (Kennelly and Foote 1965).

In a study has shown that, embryos from rabbit eCG-treated had a lower antioxidant defense after the motherly eCG therapy and such oxidative events may be involved in the reduced prolificy and fertility rates noted in females when eCG management is used repeated and successive times (Arias-Alvarez *et al*., 2013).

These results indicated that the reproductive performance for rabbit does in group 2 (110% from NRC) was significantly higher than in hormonal group. *In vitro* also the potential development of rabbit embryos from flushing group post-vitrified was significant higher than hormonal group.

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تأثير المعامله الهرمونيه والغذائيه علي تطور اجنه الارانب المزججه معمليا

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تهدف التجربة الاولى الي 1- معرفه تأثيرمستويات التغذيه على الاداء التناسلى لاناث الارانب النيوزلاندى. استخدم فى هذه التجربة 21ارنبة نيوزلاندي قسمت الى 3مجاميع الاولى (7/مجموعه) اعطيت عليقة 100% (كنترول) و110 و120% من المقررات الغذائية للارانب للمجموعة الثانية والثالثة لمده21 يوم قبل التلقيح. تم قياس عدد ووزن المواليد عند الميلاد والفطام واظهرت النتائج: ان عدد المواليد فى البطن والولادات الحية عندالميلاد وعدد المواليد فى البطن عندالفطام كانت اعلى بمعنوية $P<0.05$ لاناث الارانب فى المجموعة الثانية عنها فى المجاميع الاخرى. 2 -تهدف التجربة الثانية الي معرفه أثر الدفع الغذائي (110% من المقررات الغذائيةG1) والمعاملة الهرمونية (تبويض متعدد G2) باستخدامPMSG علي النشاط المبيضي ومعدل استرداد الاجنه والقدرة التجميديه وتطورها معمليا. (4 اناث/مجموعة). تم جمع الأجنة بعد 56ساعه من التزاوج الطبيعي، تم حفظ الأجنة بالتبريد بطريقه التزجيج وتم تقييم حيوية الأجنة من خلال تطورها في المعمل .واظهرت النتائج :-2- ان عدد الاجسام الصفراء والحويصلات المبيضية الكبيرة كانت اعلي معنويا في المجموعة الهرمونية عن مجموعه الدفع الغذائي(8.8 مقابل 6.25),(4.5 مقابل 6.25) علي التوالي. كان معدل بقاء الاجنه الطبيعيه بعد التزجيج اعلي في مجموعه الدفع الغذائي عن المجموعة الهرمونية (1.48%) ، (6.99%) علي التوالي ولكن دون معنويه. كانت النسبه المئويه لمرحله البلاستوسيست في مجموعه الدفع الغذائي اعلي عن المجموعة الهرمونية ($P<0.05$) (83.33 مقابل 71.74%) علي التوالي، بينما اظهرت مرحله البلاستوسيست الممتدة عكس ذلك . تشير هذه الدراسة الي ان الاداء التناسلى لاناث الارانب فى المجموعة الثانية (110%من المقررات الغذائية) اعلى بمعنوية عن المجاميع الاخرى. وان القدرة التطورية في المعمل لأجنه الارانب من مجموعه الدفع الغذائي بعد التزجيج اعلي عن المجموعة الهرمونية.