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EFFECT OF SUPEROVULATION ON OVULATORY RESPONSE, EMBRYO RECOVERY AND EMBRYO MEASUREMENTS IN BALADI RED RABBIT DOES

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ABSTRACT

Total of 24 rabbit does (5-7 mo of age, 3-4 kg LBW and 1-2 parities were used to study the effect of superovulation by PMSG on ovarian characteristics, quality and measurements of embryos at different stages (pronucli, morula and blastocyst) of Baladi Red (BR) rabbit does. Also, 3 fertile BR bucks were used for natural mating. All does and bucks were kept under the same conditions of feeding and management**.** Does in the 1st group (n=12) were injected with 20 mg GnRH/doe (0.2 ml Receptal) immediately after natural mating (control, G1), while does in the 2nd group (n=12) were superovulated by injection of 40 IU/kg LBW from PMSG (Foligon), followed by 0. 2 ml receptal immediately after natural mating (treatment, G2). Does in G1 and G2 were sub-divided into 3 sub-groups, 4 does in each. Ovarian characteristics were determined and embryos were recovered by flushing from each treated doe in each sub-group slaughtered after 40-46 h of mating for collection of embryos at pronucli stage (1-16 cell embryos), after 60-64 h of mating for embryo collection at morula stage and after 70-72 h of mating for those at blastocyst stage. Embryos were recovered from each uterine horn and oviduct per doe by flushing and morphologically measured for thickness of mucin coat (MC), *zona pellucid*a (ZP) and interzonal (IZ), as well as total diameter of embryos (TDE) with or without MC at different stages. Results show that average ovarian weight (right and left) and ovarian weight relative to LBW were higher (P<0.05) in G2 than in G1 (0.26 and 0.22; 0.15 vs. 0.33 and 0.27 g/doe; 0.19 g/kg LBW, respectively). Ovulatory response in terms of average number of normal follicles (large and small), hemorrhagic follicles and total follicles and average number of corpora lutea (CLs) were greater (P<0.05) in G2 than in G1 (17.75, 26.62, 0.5, 45.17 and 13.58 vs. 28.83, 32.0, 1.83, 62.67 and 22.0/doe, respectively). Ovulation rate was 74.83 and 77.15% (P>0.05) in G1 and G2, respectively. Number of embryos (viable, unviable and total) was greater (P<0.05), percentage of viable embryos was lower (P>0.05) and recovery rate of unviable embryos was higher (P<0.05) in G2 than in G1. Embryo measures including thickness of MC and ZP as well as diameter of IZ and TDE with or without MC were higher (P<0.05) in G2 than in G1, regardless embryonic stage**.** All embryo measures showed gradual increase (P<0.05) by increasing embryonic stage, except thickness of ZP, which showed an opposite trend (P<0.05), regardless treatment. These changes must keep in mind during cryopreservation (type of used cryoprotectants and freezing device) and embryo transfer to increase successful rates.

Keywords: Rabbit, superovulation, PMSG, ovarian characteristics, embryos.

INTRODUCTION

The rabbit is an important agricultural species and a useful model animal for biomedical research (Fan *et al.,* 2003). Rabbit is considered as a

preferred laboratory species for the development of several reproductive technologies, such as *in vitro* maturation and *in vitro* fertilization (Chang 1959), embryonic stem cells (Graves and Moreadith 1993), transgenesis (Hammer *et al.,* 1985) and animal cloning (Yang *et al.,* 1985).

Superovulation is usually used in order to obtain the highest possible number of oocytes or embryos, particularly with high genetic value (Mehaisen *et al*., 2004). The response to superovulation treatments is highly variable and in relation with genetics, age, breeding, parity, physiological status of the animals (Takagi *et al*., 2001), but the hormonal preparations used are very important too. Effect of superovulation on thickness of mucin coat and *zona pellucida*, diameter of mass cells and embryo was reported (Fahim 1999).

Generally, superovulation may induce alteration in embryos, doing them more sensitive to cryoconservation process than non superovulated embryos (Mehaisen *et al*., 2005&2006). Cracked *zona pellucida* or mucin coat are related to suboptimal cryopreservation procedures. In several species as human or rabbits, cracked *zona pellucida* or mucin coat respectively is enough to reduce drastically *in vivo* development of cryopreserved embryos (Kasai *et al*., 1996). However, Viudes *et al.* (2010) found that *in vitro* developmental ability of undamaged embryos was not affected by superovulation treatment nor vitrification media in the current experiment. However, further studies of the *in vivo* viability of cryopreserved-superovulated rabbit embryos with dextran addition to the vitrification media must be done.

Superovulation in rabbits using FSH or eCG have been applied to increase the folliculogenesis and ovulation rate, however, may cause an increase in the number of hemorrhagic follicles or a decrease in the quality of embryos (Mehaisen *et al.,* 2005 , 2006 and Salvetti *et al.,* 2007). Using gonadotropines like eCG (Besenfelder, 1991) or FSH (Joly *et al.,* 1998; Rebollar *et al*., 2000) was reported in rabbits. In this respect, Moor *et al*. (1984) indicated that eCG has a more important half life than FSH (several days versus few hours). Several authors have underlined that the use of eCG presents numerous disagreements as chromosomal abnormalities in blastocyst (Fujimoto *et al* 1974), alteration of ova transport in the genital tract (Adams, 1965). However, informations about the effect of superovulation in rabbit does by PMSG in the literature are scare.

Baladi Red rabbit is a local Egyptian strain which has good adaptation ability and disease resistance compared with foreign breeds and can be used as a genetic resource.

The objective of this study was to evaluate the effect of superovulation by PMSG in Baladi Red rabbit does on ovarian characteristics, quality and measurements of embryos at different stages (pronucli, morula and blastocyst stages).

MATERIALS AND METHODS

This study was carried out at the Physiology Laboratory , Animal Production Department, Faculty of Agriculture, Mansoura University.

Animals:

A total of 24 Baladi Red (BR) rabbit does, of approximately 5-7 months of age, 3-4 kg live body weight (LBW) and within 1^{st}-2^{nd} parity were used in this study as donors of embryos. In addition, 3 fertile BR bucks, around 9 months of age and averaged 3.75 kg LBW were used for natural mating.

All does and bucks were kept under the same conditions of feeding and management in a private rabbit farm. All animals were individually housed in metal cages (40 x 50 x 60 cm) provided with feeders and water nibble in each cage. Does and bucks were fed *ad. libitum* on a commercial pelleted diet.

Experimental design:

Rabbit does in the 1^{st} group (n=12) were injected with 20 mg GnRH/doe (0.2 ml Receptal, Intervet, Salamanca, Spain), immediately after natural mating by the fertile BR buck and were considered as a control group (G1), while does in the 2^{nd} group (n=12) were superovulated by intramuscular injection of 40 IU/kg live body weight from PMSG (Foligon, Intervet International B.V., Boxmeer, Holland), followed by 0.2 ml GnRH analogue (Receptal) immediately after natural mating and served as treated group (G2).

Rabbit does in both control and treated groups (G1 and G2) were sub-divided into 3 sub-groups, 4 does in each. Ovarian characteristics were measured and embryos were recovered by flushing from each doe in each sub-group slaughtered after 40-46 h of mating for collection of embryos at pronucli stage (1-16 cell embryos), after 60-64 h from mating for embryo collection at morula stage and after 70-72 h from mating for those at blastocyst stage.

Experimental procedures:

Ovarian characteristics:

Regardless slaughter time of embryo collection in each sub-group, pre-slaughter weight of each doe in each group was recorded and immediately after slaughtering, ovaries were removed, washed by distilled water and dried by cleaning paper. Ovaries were collected and excised, submerged in a flacon plastic tissue culture dishes (60 x 15 mm) containing saline solution at 38.5°C.

Ovarian measurements including ovarian weight (right and left ovaries), number of corpora lutea (CLs), and small (less than 1 mm in diameter), large follicles (more than 1 mm in diameter) and hemorrhagic follicles, were recorded per/doe. Then, relative ovarian weight (ROW) to LBW, total number of follicles (TNF) and ovulation rate (OR) were calculated as the following: ***ROW = {ovary weight (g)/LBW of doe (kg)} x 100***

TNF = Number of small + large + hemorrhagic follicles

OR (%) = (Number of CLs/number of large + hemorrhagic follicles) x 100

Embryo recovery:

Preparation of flushing medium:

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

Table (1): Composition of phosphate buffer saline (PBS) medium.

Ingredient	g/l	Ingredient	g/l
$CaCl_2$. $2H_2O$	0.133	Kh_2PO_4	1.0
$MgSO_4$. $7H_2O$	0.120	Glucose	1.0
NaCl	8.0	Sodium pyruvate	0.036
KCl	0.2	Streptomycin	100 mg
$NaHPO_4$	2.17	Sodium penicillin G	100,000 I.U

About 2 mg from bovine serum albumin (BSA) was added to one ml of PBS (2%). The prepared medium was adjusted to pH value of 7.2-7.4 using pH-meter and to osmolarity level of 280-300 mOsmol/kg using osmometer.Then, the medium was filtered by 0.22 µm millipore filter (milieux GV, millepore, Cooperation Bedford MOA).

Embryo collection:

Embryos were recovered from each uterine horn and oviduct per doe by flushing using PBS. The flushings were collected in sterile plastic Petri dishes and embryos were washed three times with PBS, counted and evaluated for viable and un-viable embryos under a stereomicroscope at a magnification of 20–40 x.

Immediately after embryo recovery, embryos were morphologically measured for thickness of mucin coat, *zona pellucid*a and interzonal, as well as total diameter of embryos at different stages using eye piece in micrometer.

Statistical analysis:

Data obtainted from this study were statistically analyzed using a software package (SAS, 2000). Group differences in ovarian characteristics, ovulation rate, number and recovery rate of fresh embryos were performed using T-test to evaluate the effect of hormonal treatment. However, data of embryo measurements were subjected to analysis of variance using factorial design according to the following model: $Y_{ijk} = U + A_i + B_j + AB_{ij} + e_{ijk}$ where: Y_{ijk} = observed values, U= overall mean, A_i = treatment, B_j = embryonic stage, AB_{ij}= Interaction due to treatment x embryonic stage and e_{ijk}= Random error.

The significant differences among means were tested using Duncan's Multiple Range Test (1955). The percentage values were subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentages.

RESULTS AND DISCUSSION

Ovarian characteristics:

Regardless slaughter time in each group, average absolute ovarian weight (right and left) and ovarian weight relative to LBW in control group (G1) and superovulated one (G2) are presented in Table (2). These resulte showed significant (P<0.05) differences between both groups, being higher in G2 than in G1. The significant (P<0.05) increase in absolute ovarian weights in G2 was indicated also in term of significant (P<0.05) increase in ovarian weight relative to LBW in G2 than in G1.

Table (2): Ovarian characteristics of control and superovulated does.

Item	Control does	Superovulated does	Sign.
Average ovarian weight:			
Right ovaries/doe (g)	0.26±0.003	0.33±0.004	**
Left ovaries/doe (g)	0.22±0.003	0.27±0.004	**
Both ovaries/doe (g)	0.48±0.004	0.60±0.007	**
Relative to LBW (g/kg)	0.15±0.002	0.19±0.003	**

**** Significant at P<0.01.**

These results indicated that superovulation treatment by PMSG significantly (P<0.05) increased ovarian weights as compared to the control group. In agreement with the present results, El-Ratel (2008) and Fahim (2008) found that ovarian weight of rabbit does (NZW) superovulated by PMSG and GnRH were increased . Also, Fukunari *et al*. (1990) found that the ovarian weight of immature Japanese White rabbit does (4 months old) treated with PMSG (50 IU/doe) was significantly higher than that of untreated does after 72 h of mating. Moreover, Gosalves *et al.* (1994) recorded that rabbit does treated with PMSG had heavier ovaries (0.60 g) than those of does injected with the saline solution (0.18 g).

Generally, the observed increase in ovarian weight of superovulated does was associated with significant (P<0.05) increase in number of total follicles and corpora lutea on the ovarian surface of does in G2 as compared to those in G1 (Table 3). Similar findings were recorded in NZW does (Fahim, 2008) and California does (Gosalves *et al*., 1994).

On the other hand, ovarian weight of rabbit does was not affected by treatment with PMSG and hCG (Daader, *et al*., 2003), 100-150 IU PMSG (Kennelly and Foot, 1965) or 0.5 mg FSH (Younglai, 1984).

Ovulatory response:

Results in table (3) show that ovulatory response in terms of average number of normal follicles (large and small), hemorrhagic follicles and total follicles/doe were significantly (P<0.05) greater in G2 than in G1, regardless slaughter time in each group. However, ovulation rate was not significantly affected by treatment, being higher in G2 than in G1.

Table (3): Ovulatory response of control and superovulated does.

Item	Control does	uperovulated doe	Sign.
Ovulatory response (Number /doe):			
Large follicles/doe	17.75±0.372	28.83±0.505	*
Small follicles/doe	26.62±0.434	32.00±0.522	*
Hemorrhagic follicles/doe	0.50±0.151	1.83±0.271	*
Large and hemorrhagic follicles/doe	18.25±0.392	28.92±0.118	*
Total follicles/doe	45.17±0.626	62.67±0.791	*
Corpora lutea (CLs)/doe	13.58±.0.484	22.00±0.492	*
Ovulation rate (%)	74.83±3.038	77.15±2.771	NS

*** Significant at P<0.05. NS: Not significant.**

The present total number of follicles per doe was higher than that reported by Abdel-Khalek *et al* (2010), who found that number of follicles per doe was 30.4/doe in Baladi Black rabbits and 20.8/doe in NZW does.

In accordance with the results of the present study, increasing incidence of hemorrhagic follicles following superovulation treatment was recorded by Kauffman *et al*. (1998); Mehaisen *et al*. (2005) and Salvetti *et al*. (2007) and incidence of hemorrhagic follicles may be due to the stimulation of immature and atretic (Adams, 1982; Bourdage and Halbert, 1988).

The significant increase in number of CL in G2 than in G1 was mainly attributed to the effect of PMSG on increasing Luteinizing Hormone (LH) surge as compared to that occurred in the control does. The rabbit is a reflexively ovulating species in which sensory and neuroendocrine stimuli act together to induce a LH preovulatory surge (Dufy-Barbe *et al*., 1973) and determine the ovulatory response. A short mating bout including ejaculation induces genital somatosensory cues that contribute to the activation of GnRH neurons and the consequential generation of a preovulatory LH surge from the pituitary gland. Plasma LH levels start to rise within 3 min after mating and reach a plateau within 15 to 75 min (Jones *et al .,*1976).

The numbers of CLs were higher (P<0.05) in GnRH treated mice than controls (Ömer Coşkun *et al*., 2002). Similar trend was observed by El-keraby *et al*. (1991), who found that increasing GnRH does from 0.2 to 0.4 ml/doe increased number of CL/NZW doe. Also, Fahim (2008) found an increase in number of CL/ doe as a result of increasing time of slaughter after mating. In rabbits, the number of CLs ranged between 7.4 to 10.3 in different rabbit breeds treated with GnRH as compared to 6.6-8.0 in the controls (El-Keraby *et al*., 1991).

Moreover, Mehaisen (2005) recorded a higher number of ovulation sites (15.3 and 15.9) as compared to 13.5 and 13.2 (Vicente *et al*., 2003) for R and V lines (synthetic breeds), respectively.On the other hand, higher number of CLs (19.2/doe) for rabbits superovulated with PMSG was reported by Lee *et al.* (1991).

In agreement with the present results, several authors reported a range of ovulation rate between 40.3% and 82% among different superovulated rabbit breeds (Viudes-de-Castro *et al.,* 1995; Bolet *et al*., 2000; Vicente *et al.*, 2003; Mehaisen *et al.,* 2005).

Number and recovery rate of embryos:

Yield of embryos (viable and unviable)/doe collected from oviduct and fallopian tube, regardless slaughter time in each group, are presented in table (4). Results show that number of embryos (viable, unviable and total) was significantly (P<0.05) higher in G2 than in G1. However, the differences in recovery rate of viable and total embryos were not significant, but only recovery rate of unviable embryos was significantly (P<0.05) lower in G1 than in G2.

Table (4): Number and recovery rate of embryos in control and superovulated rabbit does.

Item	Control does	Superovulated does	Sign.
Number of recovered embryos/doe:			
Viable embryos	10.50±0.48	16.17±0.66	*
Unviable embryos	0.67±0.14	2.17±0.27	*
Total	11.17±0.52	18.25±0.79	*
Percentage of viable embryos	94.0	88.6	-
Embryo recovery rate (%):			
Viable	77.34±2.30	73.47±2.44	NS
Unviable	4.89±1.07	9.74±1.01	*
Total	82.24±2.54	82.87±2.88	NS

*** Significant at P<0.05. NS: Not significant.**

As affected by superovulation treatment, percentage of viable embryos decreased to 88.6% in G2 as compared to 94.0% in the control group (G1), indicating positive effect of superovulation treatment on number of viable embryos and negative effect on its percentage and insignificant effect on its recovery.

The present results are higher than that reported by Fahim (2008) in NZW does superovulated by PMSG and GnRH. The author found that number of embryos collected after 72 h of mating was 4.35/ovary and recovery rate was 66.7%. He concluded that number of embryos was nearly related to the number of CLs as indicated in the present study.

Embryo measurements:

Effect of treatment:

The effect of superovulation treatment on embryo measurements presented in Table (5). Embryo measures including thickness of mucin coat and *zona pellucid*a as well as diameter of interzonal and total embryo with or without mucin coat significantly (P<0.05) increased by superovulation treatment, regardless embryonic stage.

Table (5): Embryo measures as affected by superovulation protocol.

Embryo measure	Control does	Superovulated does	Sign.
Thickness (µm):			
Mucin coat (MC)	41.72±0.54	51.43±0.43	*
Zona pellucida (ZP)	12.08±0.24	16.97±0.56	*
Diameter (µm):			
Intrazonal (IZ)	132.24±0.70	137.12±1.40	*
Total without Mucin coat (TE)	144.22±0.65	154.10±0.53	*
Total with Mucin coat (TE MC)	186.04±0.87	205.53±0.70	*

*** Significant at P<0.05. NS: Not significant.**

Effect of embryonic stage:

The effect of embryonic stage on embryo measurements presented in Table (6) . Embryo measures including thickness of mucin coat as well as diameter of interzonal and total embryo with or without mucin coat showed significantly (P<0.05) gradual increase by increasing embryonic stage. However, thickness of *zona pellucid*a showed significantly (P<0.05) an opposite trend.

In accordance with the present results, Fahim (2008) found pronounced increase in mucin coat, diameter of interzonal and total diameter of embryo, while slight decrease had occurred in thickness of *zona pellucid*a by increasing time of embryo collection after 24 to 72 hours of mating. These changes in embryo measures may reflect pronounced developmental competence of embryos by *in vivo* culture within the fallopian tube of does

Table (6): Embryo measures as affected by embryonic stage.

	Embryonic stage		
	≤16 cell	**Morula**	**Blastocyst**
Thickness (µm):			
Mucin coat (MC)	34.46±0.642^{c}	51.703±0.591^{b}	53.57±0.569^{a}
Zona pellucida (ZP)	19.02±.0.285^{a}	14.30±0.262^{b}	10.25±0.252^{c}
Diameter (µm):			
Intrazonal (IZ)	113.93±0.834^{c}	140.53±0.767^{b}	149.60±0.738^{a}
Total without MC	132.95±0.780^{c}	154.84±0.718^{b}	159.849±0.691^{a}
Total with MC	167.41±1.037^{c}	206.54±0.954^{b}	213.41±0.919^{a}

a, b and c: Means denoted within the same row with different superscripts are significantly differed at P<0.05.

In rabbits is well known the essential role of mucin coat in the embryo development and implantation (Joung *et al*., 2004), therefore, the cryopreservation media and procedures must avoid damages on rabbit embryo coats.

Cracked *zona pellucida* or mucin coat are related to suboptimal cryopreservation procedures. In several species as human or rabbits, cracked *zona pellucida* or mucin coat respectively is enough to reduce drastically *in vivo* development of cryopreserved embryos (Kasai *et al*., 1996). Therefore, superovulation treatment reflected impact on increasing thickness of mucin coat for protecting embryos during *in vitro* culture or cryopreservation.

Analysis of variance revealed that the effect of interaction between treatment and embryonic stage on all embryo measures was not significant, reflecting thicker mucin coat and wider interzonal and total embryo with or without mucin coat with slight changes in thickness of *zona pellucid*a for embryos at pronucli, morula or blastocyst stages in superovulated than in control group (Fig. 1).

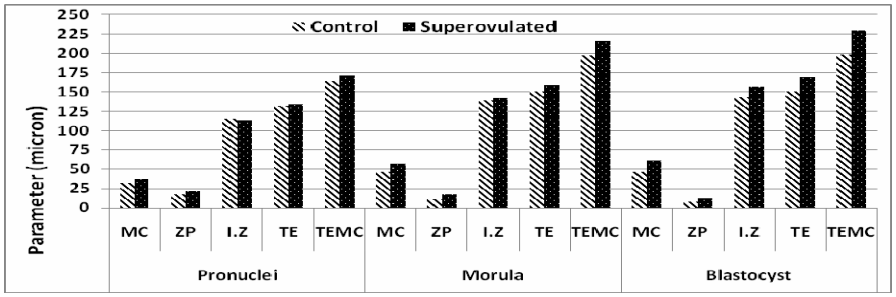

Fig. (1): Effect of interaction between treatment and embryonic stage on embryo measurements.

CONCLUSION

It was concluded that superovulation treatment of Baladi Red rabbit does with 40 IU/kg LBW from PMSG (Foligon), followed by 0.2 ml receptal immediately after natural mating increased ovarian weights, embryo yield and resulted in pronounced effects on embryo measurements at different embryonic stages. These changes must keep in mind during cryopreservation (type of used cryoprotectants and freezing device) and embryo transfer to increase successful rates.

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تأثير التبويض الفائق على الإستجابة المبيضية, معدل الإسترداد وقياسات الأجنة فى أرانب البلدى الأحمر.

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****قسم التكنولوجيا الحيوية- معهد بحوث الإنتاج الحيوانى- مركز البحوث الزراعية.**

استخدمت فى هذه الدراسة ٢٤ من أمهات الأرانب البلدى الأحمر ذات عمر من ٥-٧ شهور ووزن حى من ٣-٤ كجم بهدف دراسة تأثير التبويض الفائق بإستخدام هرمون PMSG على خصائص المبيض وجودة الأجنة وقياس قطرها فى المراحل الجنينية المختلفة (مرحلة١-١٦ خلية ومرحلة الموريولا ومرحلة البلاستوسيست).

وقد تم تقسيم الامهات الى مجموعتين :-

الاولى :- تم حقن الأمهات (١٢ أم) بـ٠.٢ مللى ريسبتال لكل أم بعد تلقيحها مباشرة بواسطة ذكور من نوع البلدى الأحمر. (الكنترول).

الثانية :- تم حقن الأمهات (١٢ أم) بـ (٤٠ وحدة دولية بهرمون PMSG لكل كجم) ثم حقنها بـ ٠.٢ مللى ريسبتال لكل أم مباشرة بعد تلقيحها طبيعيا .

بعد ذلك تم دراسة الخصائص المبيضية وعمل غسيل لقناتى المبيض لجمع الأجنة فى مراحلها الجنينية المختلفة فبعد ٤٠-٤٦ ساعة من التلقيح الطبيعى تم الحصول على أجنة فى مرحلة من١-١٦ خلية وبعد ٦٠-٦٤ ساعة من التلقيح الطبيعى تم الحصول على أجنة فى مرحلة الموريولا وبعد ٧٠-٧٢ ساعة من التلقيح الطبيعى تم الحصول على أجنة فى مرحلة البلاستوسيست. بعد ذلك تم قياس سمك كلا من طبقة الميوسين والطبقة الشفافة ومنطقة ما بين الطبقتين وقطر الجنين كاملا سواءا بطبقة الميوسين أو بدونها.

وتم الحصول على النتائج الأتية :-

كان متوسط وزن المبيضين الأيمن والأيسر ومتوسط الوزن النسبى للمبيض بالنسبة لوزن الجسم أعلى معنويا فى المجموعة الثانية عن المجموعة الأولى (كنترول) فكانت ٠.٢٦ و٠.٢٢ جرام لكل أم و٠.١٥جرام لكل كجم مقابل٠.٣٣و٠.٢٧ جرام لكل أم و٠.١٩جرام لكل كجم على التوالى). بالنسبة لإستجابة المبيض كان متوسط عدد الحويصلات المبيضية الطبيعية (الكبيرة والصغيرة) والحويصلات المدممة والعدد الكلى للحويصلات و متوسط عدد الأجسام الصفراء أعلى بمعنوية فى المجموعة الثانية عن مجموعة الكنترول فكانت ١٧.٧٥ و ٢٦.٦٢ و ٠.٥ و ٤٥.١٧ و ١٣.٥٨مقابل ٢٨.٨٣ و ٠.٣٢ و ١.٨٣ و ٦٢.٦٧ و ٠.٢٢ لكل أم على التوالى .وكان معدل التبويض فى المجموعة الأولى ٧٤.٨٣ مقابل ٧٧.١٥ فى المجموعة الثانية. كان عدد الأجنة (الحية أو الميتة أو عدد الاجنة الكلى) أعلى بمعنوية والنسبة المئوية للأجنة الحية أقل ومعدل الإسترداد للأجنة الميتة أعلى بمعنوية فى المجموعة الثانية عن المجموعة الأولى.وقد تضمنت قياسات الأجنة سمك كلا من طبقة الميوسين والطبقة الشفافة ومنطقة ما بين الطبقتين وقطر الجنين كاملا سواءا بطبقة الميوسين أو بدونها كانت أعلى بمعنوية فى المجموعة الثانية عن المجموعة الأولى بعض النظر عن المرحلة الجنينية.وقد أظهرت جميع قياسات الأجنة زيادة تدريجية مع زيادة المرحلة الجنينية بإستثناء سمك الطبقة الشفافة والتى اظهرت عكس ذلك بغض النظر عن نوع المعاملة .هذه التغيرات لابد من أخذها فى الإعتبارأثناء عملية التجميد (نوع المواد الواقية ووسيلة التجميد) وعميلة نقل الأجنة لزيادة معدلات النجاح.

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