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RECOVERY RATE AND QUALITY OF EMBRYOS AS AFFECTED BY REPEATED SURGICAL COLLECTION FROM NEW ZEALAND WHITE AND BALADI RABBIT DOES.

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ABSTRACT

Total of 12 New Zealand white (NZW, n=6) and Baladi black (BB, n=6) rabbit does (6-7 mo of age and 3.25-3.50 kg LBW) as embryo donors and two fertile bucks one from each breed (7.5-8 mo of age and 3.5-4 kg LBW) for natural mating during the experimental period were used in this study were used to study the effect of repeated surgical collection on quality and recovery rate of embryos recovered from does in each breed induced to ovulate by GnRH at insemination. Ovulation was induced by an i.m. injection of 20 mg GnRH and embryos were surgically collected 72–76 h post-mating by ventral midline laparotomy. After each of repeated embryo collection for 3 times, the number of corpora lutea (CLs), normal (NF) and hemorrhagic (HF) follicles on the ovary and the number of normal (NE) and unviable (UVE) embryos recovered were recorded. Then ovulation rate (OR) and embryo recovery rate (ERR) were calculated. Results show that number/doe of NF (23.8 vs. 16.6), HF (6.6 vs. 4.2) and total follicles (TF, 30.4 vs. 20.8) was higher ($P<0.05$) and ovulation rate (OR) was lower (28.3 vs. 42.8%, $P<0.05$) in Baladi than in MZW does. Number of CLs was 8.9 and 8.6/doe in NZW and Baladi rabbits, respectively. Average number/doe of NF, HF and TF was not affected by collection number. Average number of CLs/doe and OR increased ($P<0.05$) by increasing collection number, being 7.6 and 27.1%, 8.8 and 35.6% and 10.0 and 41.3% with the 1st, 2nd and 3rd collection, respectively. Average number of UVE/doe (1.9 vs. 1.3), TE (7.5 vs. 5.7) and ERR (87.0 vs. 64.0%) was higher ($P<0.05$) in Baladi than in MZW does. The difference in number of NE/doe was insignificant between NZW (4.4) and Baladi does (5.6). Average number of NE and TE/doe and ERR was higher ($P<0.05$) with the 1st (6.2, 7.3 and 96.1%) and 2nd (6.8, 8.3 and 94.3%) collections than with the 3rd collection (1.9, 3.7 and 37%). Average number of UVE/doe was 1.1, 1.5 and 1.8 at three successive collections. Such results may reflect the save side of embryo surgical recovery for two collections on number of embryos/doe and embryo recovery rate as a results of increasing number of UVE and ERR by increasing collection number up to three times.

Keywords: Rabbit does, surgical collection, follicles, embryos, recovery rate.

INTRODUCTION

Rabbit is widely used for biomedical purposes in several areas. Genetic lines for high growth rate and litter size have been developed associated to reproductive technologies for increasing the productivity (Baselga, 2004 and Gondret *et al.*, 2005). Also, rabbit was used as model animals elucidate the procedures for preservation of rabbit embryos (Joly *et al.*, 1996).

The use of reproductive technologies requires the highest efficiency. A superovulation treatment is usually used in order to obtain the highest

number of gametes. In rabbits, both FSH (Mehaisen *et al.,* 2006 and Salvetti *et al.,* 2007) and eCG (Mehaisen *et al.,* 2005 and Mehaisen *et al.,* 2006) have been used to increase the ovulation rate. However, superovulation may cause some problems including increase in the number of hemorrhagic follicles or decrease in the quality of embryos (Mehaisen *et al.*, 2005 and Salvetti *et al.*, 2007).

On the other hand, when embryos are recovered from rabbits with high genetic value, the chance of recovering more than once from the same female is interesting for maximizing the number of embryos or oocytes collected (Medjdoub *et al.*, 2000 and Mehaisen *et al.*, 2004). Nevertheless, some studies have demonstrated that, when superovulation is induced more than once in the same animal, the response to treatment and gametes quality may be reduced in rabbits (Mehaisen *et al.*, 2006). This reduced response may be related to an increase in anti- FSH or anti-eCG sera antibodies (Boiti *et al.*, 1995 and Swanson *et al.*, 1996).

There are several methods of embryo recovery from different species including repeated surgical flushings, non-surgical method of embryo collection (Wulster-Radcliffe *et al.*, 1999), laparoscopic embryo collection and trans-cervical embryo collection (Holtz, 2005).

The Baladi Black rabbit is a local breed in Egypt, which have been good adaptation, diseases resistance as competitive new breeds (New Zealand and Californian) and can be used as genetic source, however, a limited population size and lower in litter size. Therefore, the objective of this study was to evaluate the effect of repeated surgical collection on quality and recovery rate of embryos recovered from New Zealand White (NZW) and the Baladi black induced to ovulate by GnRH at insemination.

MATERIALS AND METHODS

This study was carried out at the Laboratory of Physiology and Biotechnology belonging to the Animal Production Department, Faculty of Agriculture, Mansoura University during the period from October 2009 to April 2010.

Animals:

Total of 12 New Zealand white (NZW, n=6) and Baldi black (BB, n=6) rabbit does (6-7 mo of age and 3.25-3.50 kg LBW) as embryo donors and two fertile bucks, one NZW and one Baladi black (7.5-8 mo of age and 3.5-4.0 kg LBW) for natural mating during the experimental period were used in this study. Also, 5 NZW does (6 mo of age and 3.5 kg LBW) were used for collection of blood samples for preparation rabbit doe serum (RDS).

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

Induced ovulation:

A total of 36 induced-ovulatory treatments and embryo recoveries (12 does x 3 embryo collections) were performed in this study. Ovulation was

induced in all does by an i.m. injection of 20 mg GnRH (0.2 ml Receptal, Intervet, Salamanca, Spain), immediately after natural mating by the fertile buck of the same breed.

Preparation of rabbit doe serum:

Rabbit doe serum (RDS) was prepared from blood collected from 5 rabbit does. The collected blood was centrifuged two times at 3000 rpm for 15 minutes. Clear sera were aspirated by pasture pipette and placed in another 15 ml sterile centrifuge tubes. These tubes were placed into water bath at 56°C for 30 min and then left to cool. Thereafter, sera were placed into 1.5 ml eppendorf tubes and frozen until used.

Preparation of flushing medium:

Phosphate buffer saline (PBS) medium was prepared as flushing medium and the composition of this medium is shown in Table (1). The flushing medium was adjusted at pH of 7.2-7.3 and osmolarity of 280-300 mOsmol/kg. The medium was filtrated by 0.22-µm millipore filter (milieux GV, millipore, Cooperation Bedford MOA) according to Shamiah (2004).

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	Streptomycin	100 mg
KCl	0.2	Sodium Penicillin G	100,000 IU
$NaHPO_4$	2.17		

Embryo recovery:

Embryos were surgically collected 72–76 h post-mating by ventral midline laparotomy. Food was withheld for 24 hours for diet and 12 h for water prior to surgical collection of embryos. Does were i.m. injected with 0.3 mg Debocaine/kg LBW (Each 1 ml contains lidocaine HCL, anhydrous 20 mg Sodium chloride, 6 mg methylparaben, and 1 mg was added as preservative, M.O.H. Reg. No. 21748/2002) plus 0.1 mg/kg LBW Atropine (1 mg/1 ml., Atropine sulphate, CID Co., Egypt).

Does were left for 15 min for analgesia before they were injected i.v. with 22 mg Ketamine HCl 2%/kg LBW (50 mg/ml, Amoun Pharmaceutical Co., Egypt) for anesthesia (Shamiah, 2004). Local anaesthesia was also induced by subcutaneous administration of 10 mg lignocaine hydrochloride (Xiloca´ına; Ovejero, Le´n, Espa´a).

Embryos were recovered from each uterine (right or left) horn and flushed by injection of pre-warmed (38°C) phosphate-buffered saline (PBS), containing 10% heat-inactivated RDS (55°C for 30 min), antibiotics (penicillin and streptomycin) and 0.3 *mM* sodium pyruvate. Thereafter, the flushing medium (PBS) was collected through the fimbrial end of the oviduct by fin plastic capillary attach with 1 ml syringe and its collection through a (Foley catheter (6 FG 2.7 mm, Norta, Malaysia) inserted anterior to the body of the uterus. Thus a total volume of 8-10 ml PBS was flushed through each uterine horn. The flushings were collected in sterile plastic Petri dishes. The collected embryos were washed three time with PBS, counted, evaluated under a stereo microscope at a magnification of 20–40 X and classified according to

their morphological appearance to normal (viable without any imperfections and spherical in shape) and unviable (fair and poor) embryos (Forcada and L`pez, 2000).

Immediately after the surgical collection and before closure, the reproductive tract was washed with a 2.5% heparin solution in saline in order to minimize the post-operative development of abdominal adhesions. At the end of the laparotomy, the females were treated with an intramuscular injection of 5000 IU penicillin G (Terramicina®, Pfizer Salud Animal, Madrid, Spain).

After embryo recovery, number of corpora lutea and follicles (normal and hemorrhagic) presented on the ovarian surface and the number of embryos recovered from oviduct on each side (right or left) was recorded.

Each doe was ovulated and subjected to surgical embryo recovery for three occasions at intervals of at least 50 days. Animals were only eliminated from the study if anatomical problems (adhesions, lesions, etc.) prevented embryo recovery, although data obtained for item previously were included in the analyses.

Statistical analysis:

The obtained data were statistical analyzed using General Linear Model Procedures of SAS (1990). The significant differences were performed using Duncan Range Test (Duncan, 1955).The percentages values were adjust to arcsine transformed before performing the analysis of variance. Means were presented after being recalculated from transformed values to percentages.

RESULTS AND DISCUSSION

Ovarian activity and ovulation rate:

Effect of rabbit breed:

Results in Table (2) show that average number of normal follicles (NF, 23.8 vs. 16.6/doe), hemorrhagic follicles (HF, 6.6 vs. 4.2/doe) and total follicles (TF, 30.4 vs. 20.8/doe) was significantly ($P<0.05$) higher in Baladi than in NZW does. However, ovulation rate (OR) was significantly ($P<0.05$) higher in NZW than in Baladi does (42.8 vs. 28.3%) as a result of nearly similarity between both breeds in CLs number. Yet, the differences in average number of CLs (8.9 and 8.6/doe, respectively) were not significant in NZW and Baladi does.

In agreement with the present results to induce ovulation by GnRH, El-Keraby *et al.* (1991) found insignificant differences in number of CLs between Baladi black and NZW does, being 7.8 and 9.0/doe, respectively.

Table (2): Effect of breed on average number of corpora lutea and follicles per doe, and ovulation rate.

Breed	Average number/doe				Ovulation Rate (%)
	Corpora lutea	Normal follicles	Hemorrhagic follicles	Total follicles	
NZW	8.9±0.50	16.6±1.31^{B}	4.2±0.72^{B}	20.8±0.94^{B}	42.8±0.71^{A}
Baladi black	8.6±0.59	23.8±1.55^{A}	6.6±0.85^{A}	30.4±1.27^{A}	28.3±0.89^{B}

$^{A-B}$: **Means denoted within the same column with different superscripts are significantly different at $P<0.05$.**

However, in the literature, slight differences in number of ovulation sites was found between R and V line rabbits, being 15.3 and 15.9/doe (Mehaisen, 2005), 14.3 and 13.8 (Viudes-de-Castro *et al.*, 1995), and 13.5 and 13.2 (Vicente *et al.*, 2003), respectively. In rabbit, Besenfelder *et al.* (2002) found that he number of ovulation sites varied between the different rabbit breeds (Chinchilla, Vienna White, and Belgian Hare) responded to the eCG application. In cattle, sheep and goats the number of CLs recorded following superovulation differ between breeds (Goel and Agrawal, 2005 and Ammoun *et al.*, 2006).

In similarity with presence of hemorrhagic follicles in our study, many authors found that superovulation may cause some problems including increase in the number of hemorrhagic follicles or decrease in the quality of embryos (Kauffman *et al.*, 1998; Mehaisen *et al.*, 2005 and Salvetti *et al.*, 2007). In this respect, Mehaisen *et al.* (2005) found that the number of hemorrhagic follicles was 13.8 vs. 3.8 and 3.8 for three doses (0, 50 and 200 IU) of eCG. Also, ovulation could be induced in relatively immature, atretic and mature follicles, when hCG is used to induce ovulation to recover embryos (Adams, 1982 and Bourdage and Halbert, 1988).

In agreement with the significant differences in OR of both breeds in our study, Bolet *et al.* (2000) found that the response to superovulation treatment differed among rabbit breeds. Using different types of hormonal administration to induce ovulation (superovulation), recovery rate was 43.2 and 40.3% (Mehaisen *et al.*, 2005), 63 and 82% (Viudes-de-Castro *et al.*, 1995), and 77 and 74% (Vicente *et al.*, 2003) for the R and V line rabbits, respectively.

Effect of repeated collection:

Results in Table (3) show that average number of follicles (NF, HF and TF) per doe was not affected significantly by collection number, although there was a tendency of reduction in number of follicles by increasing number of collection. However, number of CLs and consequently OR significantly ($P<0.05$) increased by increasing collection number, being the lowest with the 1st collection (7.6/doe and 27.1%), the modest with the 2nd collection (8.8/doe and 35.6%) and the highest with the 3rd collection (10.0/doe and 41.3%). The differences were significant ($P<0.05$) only between the 1st and 3rd collection.

Table (3): Effect of collection number on average number of corpora lutea and follicles per doe, and ovulation rate.

Collection number	Average number/doe				Ovulation rate (%)
	Corpora lutea	Normal follicles	Hemorrhagic follicles	Total follicles	
1	7.6±0.63^{B}	22.7±1.65	5.3±0.91	28.0±1.31	27.1±0.94^{B}
2	8.8±0.71^{AB}	18.9±1.87	5.8±1.03	24.7±1.54	35.6±1.13^{AB}
3	10.0±0.67^{A}	19.2±1.76	5.0±0.97	24.2±1.34	41.3±0.87^{A}

$^{A-B}$: Means denoted within the same column with different superscripts are significantly different at $P<0.05$.

Such results may reflect the save side of embryo surgical recovery up to three collections on number of follicles and ovulation rate. Besenfelder and Brem (2002) clearly demonstrate that for repeated flushing in rabbits,

follicle development was stimulated by application of eCG. In agreement with our study, the same authors found no difference regarding the ovarian response during the two collection procedures in term of number of corpora lutea (10.4 vs. 9.4/doe).

Effect of interaction between breed and collection number:

Analysis of variance revealed that the effect of interaction between breed and collection number was significant only on number of HF/doe. This effect was reflected in different trend of change in number of HF with increasing collection number in each breed. In NZW does, number of HF showed marked increase by increasing collection number, while an opposite trend was observed in Baladi does (Table 4 and Fig. 1).

Table (4): The interaction effect of breed with collection number on average number of corpora lutea and follicles per doe, and ovulation rate.

Breed	Collection number	Average number/doe				Ovulation rate (%)
		Corpora lutea	Normal follicles	Hemorrhagic follicles	Total follicles	
NZW	1	7.5±0.82	18.0±2.05	2.0±1.17	20.0±1.54	37.5±1.40
	2	9.0±0.82	15.3±2.15	4.5±1.19	19.8±1.45	45.5±1.24
	3	10.3±0.95	16.7±2.49	6.0±1.37	22.7±2.01	45.4±1.56
Baladi black	1	7.7±0.95	27.3±2.49	8.7±1.37	36.0±2.00	21.4±1.35
	2	9.5±1.17	22.5±3.05	7.0±1.68	29.5±2.74	28.8±1.87
	3	9.7±0.95	21.7±2.49	4.0±1.37	25.7±2.64	37.7±1.74

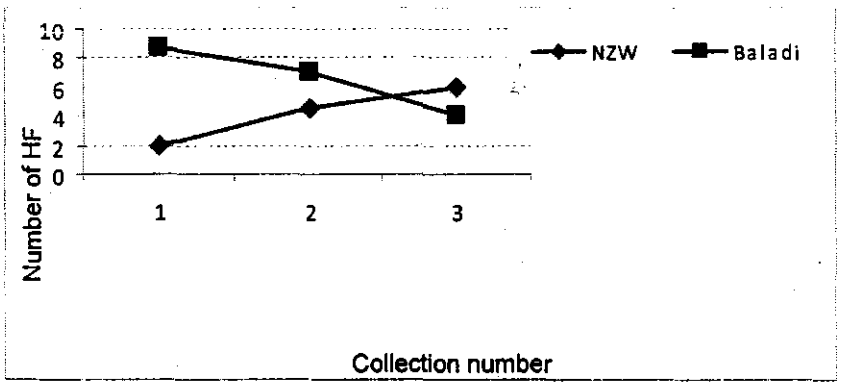

Fig. (1): Change in number of hemorrhagic follicles in NZW and Baladi does by increasing collection number.

Embryo recovery rate:

Effect of rabbit breed:

Results in Table (5) show that average number of unviable embryos (UVE, 1.9 vs. 1.3/doe), total embryos (TE, 7.5 vs. 5.7/doe) and embryo recovery rate (ERR, 87.0 vs. 64.0%) was significantly ($P<0.05$) higher in Baladi than in NZW does. However, the difference in number of normal

embryos (NE) was insignificant between NZW (4.4/doe) and Baladi does (5.6/doe).

Table (5): Effect of breed on average number of embryos per doe and embryo recovery rate.

Breed	Average number of embryos/doe			Embryo recovery Rate (%)
	Normal	Unviable	Total	
NZW	4.4±0.97	1.3±0.12^{B}	5.7±0.51^{B}	64.0±0.54^{B}
Baladi black	5.6±1.15	1.9±0.15^{A}	7.5±0.67^{A}	87.2±0.68^{A}

$^{A-B}$: Means denoted within the same column with different superscripts are significantly different at P<0.05.

In accordance with the present results, El-Keraby *et al.* (1991) found that number of embryos and recovery rate of embryos collected from oviduct after induction of ovulation by GnRH was higher for Baladi black than NZW does. When embryos were collected by flushing the recovery rates of embryos collected from does were higher from Baladi black than NZW does (36.7 vs. 10%). While, overall recovery rate of embryos was higher for NZW than Baladi black does (88.8 vs. 71.8%). Generally, number of transferable embryos recovered following superovulation significantly differs between different breeds of cattle, sheep and goats (Goel and Agrawal, 2005 and Ammoun *et al.*, 2006).

Effect of repeated collection:

Results in Table (6) show that average number of NE and TE per doe and ERR was significantly (P<0.05) higher with the 1st and 2nd collection than with the 3rd collection. It is of interest to note that the lowest number of NE and TE as well as ERR were obtained with the 3rd collection (1.9 and 3.7/doe and 37%). The corresponding values were 6.2 and 7.3/doe and 96.1% with the 1st collection and 6.8 and 8.3/doe and 94.3% with the 2nd collection. However, number of UVE/doe was not affected significantly (P<0.05) by collection number, being 1.1, 1.5 and 1.8/doe at three successive collections.

Table (6): Effect of collection number on average number of embryos per doe and embryo recovery rate.

Collection number	Average number of embryos/doe			Embryo recovery Rate (%)
	Normal	Unviable	Total	
1	6.2±0.22^{A}	1.1±0.16	7.3±0.17^{A}	96.1±0.41^{A}
2	6.8±1.30^{A}	1.5±0.17	8.3±0.71^{A}	94.3±0.63^{AB}
3	1.9±1.38^{B}	1.8±0.18	3.7±0.74^{B}	37.0±0.69^{c}

$^{A-B}$: Means denoted within the same column with different superscripts are significantly different at P<0.05.

Such results may reflect the save side of embryo surgical recovery for two collections on number of embryos/doe and embryo recovery rate as a results of increasing number of UVE and ERR by increasing collection number up to three times.

In rabbit, Besenfelder and Brem (2002) clearly demonstrated that flushing oviducts and uterine horns allows a highly efficient collection of all

pre-implantation stages of rabbit embryos. For repeated two flushings, the collection of embryos showed analogous results, being greater (11.6 vs. 11.5) than those obtained in our study. However, they reported that the embryo recovery rates range from 65-69% following surgical embryo recovery for three times. This is comparable to recovery rates reported in rabbits ranging from 60 to 78.7% following Laparoscopic embryo flushing (Baril *et al.*, 1989), which were lower than the recovery rate obtained for the 1st and 2nd collection in the present study.

In accordance with the obtained results on rabbits, repeated superovulation with FSH in goats has been reported to reduce the number of embryo recovered, as well the number of transferable embryos (Baril *et al.*, 1989 and Beckers *et al.*, 1990). Similar observations have been reported in other species (Al-Kamali *et al.*, 1985). Explanation regarding the reduction in the response of females to repeated superovulation was that gonadotrophin antibodies are formed following successive superovulation (Holtz, 2005). In cattle and rabbits anti- gonadotrophins following repeated superovulation have been indicated to neutralize the follicular stimulatory effect of the hormone used for superovulation (Jainudeen *et al.*, 1966 and Maurer *et al.*, 1968). Moreover, an increase in the immune response to egg following repeated treatment was observed in goats and cattle (Drion *et al.*, 2001). The anti-egg antibodies produced in goats have been indicated to have a negative effect on reproduction. This was confirmed by Roy *et al.* (1999), where the high concentration of anti-egg antibodies was correlated with a decrease in fertility.

The tendency of increasing number of unviable embryos by increasing collection number was observed by Mehaisen *et al.*, 2006), who demonstrated that, when superovulation is induced more than once in the same animal, the response to treatment and gametes quality may be reduced in rabbits. Also, Torres and Sevellec (1987) have documented the detrimental effects of repeated surgical recoveries of embryos on the fertility of the donors. However, Wildt and Goodrowe (1989) suggested that extremely careful surgical procedures may allow two embryo recovery operations before tissue adhesions would be inhibitory.

Effect of interaction between breed and collection number:

Analysis of variance revealed that the effect of interaction between breed and collection number on number of embryos/doe and ERR was not significant, reflecting similar trend of changes in This effect was reflected in different trend of change in number of embryos/doe and ERR with increasing collection number in each breed (Table 7).

Regarding the differences between both rabbit breeds, genotype has been indicated in several studies to be a factor to be taken into consideration in superovulation programmes (Baril *et al.*, 1989). Breed has been indicated as a major factor contributing to the variation recorded in the ovarian response to superovulation (Ammoun *et al.*, 2006).

Table (7): The interaction effect of breed with collection number on average number of embryos per doe and embryo recovery rate.

Breed	Collection number	Average number of embryos/doe			Embryo recovery rate (%)
		Normal	Unviable	Total	
NZW	1	5.8±1.60	1.0±0.21	6.8±1.42	90.7±1.14
	2	4.7±1.85	1.3±0.24	6.0±1.14	66.7±0.98
	3	2.8±1.60	1.5±0.21	4.3±1.35	41.7±1.05
Baladi black	1	6.7±1.85	1.0±0.24	7.7±1.57	100.0±1.28
	2	8.0±1.85	0.7±0.24	8.7±1.27	91.5±1.15
	3	1.0±2.26	2.0±0.30	3.0±1.25	30.9±1.21

It is of interest to note that the breed effect has been associated with the different prolificacy of each breed, where a high prolific breed has been reported to respond better to exogenous gonadotrophins (Bindon *et al.*, 1986). Genotype has been recognized to have a major effect on ovarian follicular development even though gonadotrophin treatment increases the follicular development in all breeds; the numbers of ovarian follicles which are recruited to ovulate differ in the different breeds (Ammoun *et al.*, 2006).

Based on the foregoing results, the current study may reflect the save side of embryo surgical recovery for two collections on number of embryos/doe and embryo recovery rate as a results of increasing number of UVE and ERR by increasing collection number up to three times.

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تأثير الجمع الجراحى المتكرر على معدل استرداد وجودة أجنه أرانب النيوزيلاندى الأبيض والبلدى الأسود

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إستخدم فى هذا البحث عدد١٢ انثى أرانب (٦ نيوزيلاندى ابيض و٦ بلدى اسود) ، تتراوح أعمارها من٦-٧ شهر وأوزانها من ٣,٢٥ -٣,٥٠ كجم واستخدمت كمعطية للأجنة وكذلك ذكر من كل سلاله (٧,٥-٨ شهر ووزن ٣,٥-٤ كجم) للتلقيح الطبيعى ، وذلك لدراسة تأثير تكرار الجمع الجراحى على السلالتين ومعدل إسترداد ألاجنة من إناث أرانب المعامله بهرمون GnRH للحث على التبويض.و تم إستحداث التبويض بواسطة ٢٠ مجم GnRH وتم جمع الأجنه جراحيا بعد ٧٢-٧٦ ساعه من التلقيح الطبيعى وبعد الأنتهاء من الجمع حتى ٣ مرات تم عد الأجسام الصفراء – الحويصلات – الأجنه وحساب معدل التبويض ومعدل الأسترداد للأجنه

وقد اظهرت النتائج ان:

١. كان عدد الحويصلات الطبيعية والنزيفية والعدد الكلى للحويصلات أكبر معنويا فى البلدى عن النيوزيلاندى (٢٣,٨ مقابل ١٦,٦ ، ٦,٦ مقابل ٤,٢ ، ٣٠,٤ مقابل ٢٠,٨ لكل إنثى على الترتيب و كان معدل التبويض أعلى معنويا فى النيوزيلاندى عن البلدى (٤٢,٨% مقابل ٢٨,٣ %).

٢. كان عدد الاجسام الصفراء ٨,٩ و ٨,٦ لكل إنثى فى النيوزيلاندى والبلدى على الترتيب و لم يتأثر متوسط عدد الحويصلات الطبيعية والنزيفيه والعدد الكلى للحويصلات معنويا بعدد مرات الجمع الجراحى.

٣. زاد عدد الأجسام الصفراء لكل إنثى ومعدل التبويض معنويا بزيادة عدد مرات الجمع وكانت ٧,٦، ٢٧,١% - ٨,٨ ، ٣٥,٦% - ١٠، ٤١,١% فى المرة الأولى والثانية والثالثه للجمع على الترتيب.

٤. زاد عدد البويضات الشاذة (١,٩ مقابل ١,٣ لكل إنثى) والعدد الكلى للبويضات (٧,٥ مقابل ٥,٧ لكل إنثى ومعدل إسترداد الأجنه (٨٧ مقابل ٦٤%) فى البلدى عن النيوزيلاندى. ولم يختلف عدد البويضات الطبيعية بين السلالتين.

٥. زاد عدد البويضات الطبيعية والعدد الكلى للبويضات لكل إنثى وكذالك معدل الأسترداد معنويا فى المرة الأولى للجمع وتناقص فى المرة الثالثه معنويا.

وتوصى الدراسه بأمكانية الجمع الجراحى للأجنة من الأرانب النيوزيلاندى والبلدى الأسود لمرتين على الأقل دون التأثير على جودة الأجنة ومعدل إستردادها.

قام بتحكيم البحث

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