RECOVERY RATE OF FOLLICULAR AND OVIDUCTAL OOCYTES OF DOE RABBITS SUPER-INDUCED TO OVULATION BY HORMONAL TREATMENTS

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ABSTRACT

This study was carried out, at the in vitro fertilization (IVF) Laboratory, International Livestock Management Training Center (ILMTC), Sakha, Kafr El-Sheikh Governorate, belonging to the Animal Production Research Institute, Ministry of Agriculture, Agricultural Research Center, on 35 New Zealand white (NZW) rabbit does (5-6 mo of age and 3.0-3.5 kg LBW) to evaluate the effect of different hormonal treatments, using PMSG and hCG or GnRH on recovery rate of follicular and oviductal oocytes. Does in the1st group (G1) were i.m. injected with GnRH and slaughtered after 12 h from GnRH-injection (control group). However, does in G2, G3 and G4 were i.m. injected with 75 IU of PMSG analogue (Poligon). Rabbit does were injected 48 h later by 150 IU of hCG (G2), 48 h later by 0.02 ml GnRH analogue (Receptal, G3) and 72 h later by GnRH injection (G4). Does in all groups were slaughtered 12 h from the last injection for oocyte collection. Mature oocytes were collected from oviduct. Number of corpora lutea (CLs) and follicles more than 1 mm in diameter were recorded on each of left and right ovary of each doe. Oocytes were collected from ovaries using slicing technique. Follicular and oviductal oocytes were classified into different categories. Results revealed significant ($P<0.05$) differences in number of oocytes recovered from ovaries or oviducts per right and left side or per doe and in number of corpora lutea on the ovaries between experimental groups, being the highest in G3. The highest recovery rate from ovaries was obtained in G3 (91.2%) as compared to 85.7% in G1, 83.3% in G2 and 81%% in G4. The highest recovery rate from oviduct was obtained in G4 (92%) as compared to 82.8% in G3 and 82.4% in G1. H6wever, no oocytes were recovered from oviduct of G2. Total recovery rate of oocytes from ovaries and oviducts was the highest in G3 (87%), followed by GG4 (86.5%) and G1 (84.4%), and the lowest in G2 (82.3%). Percentage of compact oocytes recovered from the ovaries was the highest in G3, ranked the second in G2 and the third in G4, being 89.6, 86.0 and 81.3%, respectively. H6wever, G1 showed the lowest percentage (55.6). In all groups, frequency distribution of oocytes at M II was the

highest as compared to those at M I or degenerated oocytes. Percentage of oocytes at M II was lower in G3 and G4 than in G1, being 83, 78 and 86%, respectively.

According to the obtained results, treatment of rabbit does to stimulate over-ovulation by PMSG analogue (75 IU) followed 48 h later by GnRH analogue (0.02 ml) and slaughtered 12 h from the last injection yielded the highest number of compact oocytes (immature) recovered from the ovarian follicles and acceptable number of mature oocytes from oviduct.

Keywords: Rabbits, oocytes, ovarian characteristics, recovery rate, oocyte categories.

INTRODUCTION

Recent advanees in artificial insemination (AI) and embryo transfer (ET) teehnology have allowed progress towards increasing the number of offspring produced from genetically superior animals. The appliеation of bioteehnology in (AI) and (ET) in rabbit had a very limited suecess in Egypt. For most species of mammals, AI could be achieved and pre-implantation embryos can be produced routinely in the laboratory.

Superovulation is considered to be an efficient eeonomic method for producing additional embryos or oocytes from females of high genetic merit. The exogenous hormones as stimuli for ovulation induction in higher number of follicles included both follicle stimulating hormone (FSH) and equine chorionic gonadotrophin, eCG **(Schmidt et al., 1992 and Joly et al., 1996)**, human choronic gonadotrophin, hCG **(Carney and Foote, 1990)** and gonadotrophin releasing hormone, GnRH **(El-Keraby et al., 1991)**.

Conflicted superovulatory responses to hormonal treatment were reported in relation with type of hormonal treatment, being higher to eCG than to FSH treatment **(Kauffman et al. (1998)** or to FSH than eCG treatment **(Rebollar et al. (2000)**. This may be related to variation in breed and timing of hormonal treatment.

Some authors invcstigated the effeet of superovulation treatments on ovarian characteristies and rccovery rate of embryos **(Fahim, 2008)** or oocytes **(Daader et al., 2003)** in rabbits, reporting some differences in the obtained results. Therefore, this work was designed to study the effect of different hormonal protocols, using Pregnant Mars Serum Gonagotrophin (PMSG) to superinduce follicular development and hCG (Human Chorionic Gonagotrophin) or gonadotropin-releasing hormone (GnRH) to induee ovulation, on recovery rate of follicular and oviductal oocytes.

MATERIALS AND METHODS

This study was carried out at the in vitro fertilization (IVF) Laboratory, International Livestock Management Training Center (ILMTC), Sakha, Kafr El-Sheikh Governorate, belonging to the Animal Production Research Institute, Ministry of Agriculture, Agricultural Research Center, Egypt and Poultry Production Department, Faculty of Agriculture, Mansoura University, during the period from **September, 2007 to May, 2008**.

Animals:

Total of 35 New Zealand white (NZW) rabbit does having 5-6 months of age and 3.0-3.5 kg live body weight were used in this study. Rabbit does were subjected as donors of oocytes. All females were kept under the same condition of feeding and management in the station, being individually housed in metal cages provided with feed source and nibble for water in each cage. Does were fed ad. libitum on a commereial pelleted concentrate diet.

Protocols of super-inducing ovulation:

Rabbit does in the1st group were intramuscularly injected with GnRH and slaughtered after 12 h from GnRH-injection (control group, G1). However, all rabbit does in treatment groups (G2, G3 and G4) were stimulated over-ovulation by intramuscular injection with 75 IU of PMSG analogue (Folligon Intervet International B.V., Boxmeer, Holand). Rabbit does in G2 were injected 48 h later by 150 IU of hCG, in G3 followed 48 h later by 0.02 ml GnRH analogue (Receptal, IntervetInternational B.V., Boxmeer, Holand) and in G4 followed 72 h by GnRH injection. Does in treatment groups (G2, G3 and G4) were slaughtered 12 h from the last injection for oocyte collection.

Collection of follicular oocytes:

After slaughtering, ovaries were removed, washed with PBS and submerged in a glass Petri dishes containing PBS and immediately transported to laboratory. Number of visible follicles (1mm in diameter) was counted on each of left and right ovary of each doe.

Ooeytes were collected using slicing technique into Petri dishes containing 4 ml of harvesting medium. Oocytes were transferred into small Petri dishes containing 2 ml medium for washing and searching oocytes to evaluate different categories using stereomicroseopy.

Collection of oviductal oocytes:

The genital tract was washed once in fresh Dulbecco's phosphate buffer saline (DPBS) medium (Gibco, Grand Island, New York, USA) and each side of oviduct and 2 cm of uterine horn were disseeted from the surrounding tissue. The oocytes were collected by using direct flushing of the oviduct as described previously by **Techakumphu (1986)**. A blunt-ended (18-gauge) needle was inserted through the uterine end to the infundibulum and flushed with 10 ml of DPBS medium plus 2 mg/ml bovine serum albumin (BSA). The flushing was performed twice and the media were recovered in a 35-mm Petri dish. The oocytes were immediately searched under a 10x stereomicroscope. Oocyte recovery rate was compared with the number of corpora lutea on each ovary.

The cumulus cells around the oocytes were removed by pipetting, and then oocytes were loaded on slide, placed into fixation solution (3 ethanol : 1 glacial acetic acid) overnight. Thereafter, ooeytes were stained with 1% orcein in 45% acetic acid and examined for maturation under phase-contrast microscopy.

Oocyte evaluation:

Oocytes recovered from oviduct were classified according to in vivo maturation into three categories: 1) oocytes at metaphase I stage (with chromosomes condensed in pairs and without deteeted polar body, M I), 2) oocytes

at metaphase _I stage (one large group of chromosome formed an equatorial plate and the remaining chromosome are highly condensed or had extruded a polar body. M II) and 3) degenerated oocytes (vacuolated or had scattered or highly condensed chromatin. Degen.).

Oocytes recovered from ovarian follicles were morphologically evaluated for cumulus-oocyte complex (COC) using inverted microscope and classified according to the method described by **Leibfried and First (1979) and Madison et al. (1992)** into 4 categories : 1) oocytes with 1-4 layers of cumulus cells with dark spots of degenerated cumulus cells (Expanded cumulus), 2) oocytes with ≥5 layers cumulus cells and had a compact and bright cumulus investment (Compact cumulus), 3) oocytes with less compact and obviously darker cumulus investment (Partial denuded) and 4) oocytes with completely denuded both cumulus and corona cells and covered by zona pellucida only (Denuded).

The oocyte yields from the right and left ovaries or oviducts were recorded. The recovery rate was determined as number of oocyte proportional to each of the total vesicular follicles (for ovaries) or number of CLs (for oviduct).

Statistical analysis:

Data were analyzed using computer programm of **SAS (2000)** according to Snedecor and **Cochran (1982)** and the significant differences among treatment means were preformed using Duncan Range Test **(Duncan, 1955)**.

RESULTS AND SISCUSSION

Ovarian characteristics of does superovulated with different protocols:

Number of follicles and corpora lutea (CLs) on the surface of right and left side recorded immediately after slaughter were affected significantly (P<0.05) by hormonal treatment (Table 1). Results revealed that number of follicles on the right or left ovaries, total number/doe and mean number/ovary significantly (P<0.05) increased only in G2 and G3 and did not differ significantly in G4 as compared to the control group (G1). Generally, number of follicles was greater on the right than left ovaries and does in G3 showed the highest values on both sides (Table 1).

The differences in number of follicles between G3 and G4 may suggest pronounced effect of timing GnRH injection for inducing ovulation (after 48 h of PMSG in G3 and 72 h in G4). The earlier injection of GnRH in G3 significantly (P<0.05) increased the number of follicles on the ovarian surface than the later injection with GnRH in G4. On the other hand, the observed tendency of greater number of follicles in G3 than in G2 may be associated with type of hormones injected at the same time (hCG in G2 and GnRH in G3 after PMSG injection).

In agreement with the obtained results, **Bonhoff and Adams (1985)** found an increasing degree and number of follicular development when rabbit does were treated with hCG or LHRH. **Fukunari et al. (1990)** found that total the number of follicles was significantly higher in immature Japanese White rabbits treated with 50 IU PMSG. Also, **Gosalaves et**

al. (1994) reported that does treated with PMSG had a greater number of antral follicles than those injected with saline solution followed by 20 mg LHRH (Fertagyl) 2 days later. Similar results were reported **El-Gaafary et al. (1994)**, who found that the number of follicles on ovaries of NZW rabbits increased to 16 follicles/ovary when doe rabbits were injected with 50 IU of HCG. In supporting the previous results, **Daader et al. (2003)** reported similar results to that obtained in this study on rabbit does treated with PMSG and hCG. Generally, number of follieles was affected by type **(Peinado et al., 1995)**, dose of hormones **(Mehaisen et al., 2005)** and timing of treatment and slaughter.

The most interesting results is number of CLs per right, left, ovary and doe on the ovarian surface of experimental does was significantly ($P<0.05$) the highest in G3, unaffected in G4 and significantly ($P<0.05$) the lowest in G2 as compared to control (G1). Number of CLs was greater on the right than left ovaries of all groups, being the greatest in G3 (Table 1).

A lower response for ovulation in term of low number of CLs/doe was observed in this study, although **Fahim (2008)** found higher number in NZW does treated with PMSG (150 IU) followed by 72 h later with hCG (75 IU) and slaughtered 24 or 72 h of mating. Also, the number of ovulation points averaged 19.2/female rabbit treated with PMSG **(Lee et al., 1991)** and ranged from 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)**. Furthermore, the recent studies showed that the numbers of CLs were higher ($P<0.05$) in GnRH treated mice than controls **(Kanter, 2002)**. In rabbits, **Mehalsen, et al. (2005)** recorded a higher number of ovulation sites (15.3 and 15.9/doe) as compared 13.5 and 13.2/doe **(Vicente et al., 2003)** for R and V lines, respectively.

The marked reduction in CLs number in this study may be in relation to timing the ovulation after the injection with hormone of follicular growth and/or mating process (LH surge) and time of slaughter. This can be explained on the basis that rabbit does were slaughtered 12 h after the 2nd injection and ovulation in rabbits occurs 10-12 hours postcopulation after some other stimulation **(Hafez, 1970)**, but this period was not enough for CL formation after ovulation. In this respect, **Peinado et al. (1995)** used similar protocol to that of G2 in this study and found that the number of CLs/doe slightly increased after 72 than 14 h of mating, being 13 and 12/doe, respectively). Similar findings were obtained by **Daader et al. (2003)** of rabbit does treated with PMSG and hCG and slaughtered 12 h later.

Oocyte recovery:

The obtained results presented in Table (2) show that only does in G3 yielded significantly ($P<0.05$) higher number of oocytes on the right and left ovaries and total number of oocytes per doe (18.25, 13.0 and 31.25) than that of the control group (G1, 5.25, 3.75 and 9.00), respectively. Also, the corresponding oocyte yields were significantly ($P<0.05$) higher in G2 than in G1 and did not differ significantly from that in G3. However, oocyte yields were higher in G4 than that in the control, but the differences were not significant.

Oocyte recovery rate from ovaries was higher (91.2%) only for G3 than in G1 (85.7%), G2 (82.3%) and G4 (81.0%, Table 2). In agreement with the present results, **Daader et al. (2003)** found that the mean number of oocytes presented on left and right ovaries was significantly higher in does treated with PMSG and hCG than in untreated does, being higher on right than left sides. These findings suggested that the effect of PMSG may be due to an over stimulation of ovarian follicle owing to its long half-life.

The differences regard to oocyte yields from oviducts were significant, being higher in G3 than in G1 and G4. However, the differences in oocyte yields between G1 and G4 were not significant (Table 2). The obtained number of recovered oocytes/doe in G3 and G4 in our study (31 and 16, respectively) was greater than that obtained by **Al-Hasani et al. (1984)**, who recorded that the average number of oocytes recovered from ovarian follicles by puncture was 11.7 oocytes/doe during different times after p-LH injection. Also, lower number of embryos were recovered from oviduct/doe was observed by **Fahim (2008)** in rabbit does treated with PMSG and hCG.

It is of interest to observe that no oocyte were recovered from oviduct of does in G2, indicating no incidence of ovulation for the hormonal treatment used in this group. On the other hand, the highest recovery rate from oviduct was obtained in G4 (92.0%) as compared to 81.1% in G3 and 82.4% in G1, although this group yielded the lowest number of oocytes recovered from oviducts (Table 2).

As compared to the results of G2, lower embryo recovery rate (67.7 and 53%) was obtained for rabbit does treated with PMSG and hCG, which were slaughtered after 24 and 72 of mating, respectively **(Fahim, 2008)** despite that **Bourdage and Halbert (1988)** showed that the use of 50 IU hCG provoked an alteration in oviductal motility causing an accelerated transit of the embryos. They claimed that this acceleration could be responsible for the higher number of embryos found in the uteri of does treated with hCG. Generally, oocyte yield from oviduct of does in G3 and G4 and even in the control group (G1) as compared to the results of other authors may be attributed to absence of mating process. Rabbits are ovulatory inducer and ovulation is depending on mechanical stimulation during mating.

Total oocyte recovery rate from ovaries and oviducts per doe was the highest for G3 (87%), followed by G4 (86.5) and G1 (84%), while G2 showed the lowest total recovery rate (82.3%, Table 2). The present results indicated a confliction in the results of response to hormonal treatments recorded by several authors. In this respect, lower recovery rates of embryos collected from does treated with 0.2 or 0.4 ml GnRH were obtained by **El-keraby et al. (1991)**. However, in PMSG (Besenfelder et al., 2000) or FSH **(Joly, 1997)** injected rabbits, higher recovery rates were recorded. Also, **Mehaisen, et al. (2005)** evaluated the effect of different doses of eCG administered subcutaneously (0, 50 and 200 IU) and the hormonal induction of ovulation (GnRH or hCG) on recovery rate in R and V line-rabbit does. Administration of 200 IU of eCG significantly decreased recovery rate (28.8 vs. 47.7

and 48.7) as compared to 50 IU and 0 IU eCG, respectively.

Evaluation of recovered oocytes:

Oocytes recovered from ovaries:

Examination of oocytes recovered from the ovaries of doe rabbits in all groups reveals that all oocytes were immature and were evaluated into compact, denuded, partial denuded and expanded oocytes (Fig. 1, 2, 3 and 4, respectively).

Data in Table (3) clearly revealed that frequency distribution of compacted oocytes was the highest in all groups as compared to the other oocyte categories. Percentage of compacted oocytes was pronouncedly affected by hormonal treatment, being the highest in G3, ranked the second in G2 and the third in G4 (89.6, 86.0 and 81.3%, respectively).

While the control group (G1) showed the lowest percentage of compacted oocytes. The highest frequency distributions of denuded (31%) and partial denuded (8.4%) oocytes were obtained from the control group (G1) and the highest percentage of expanded oocytes (6.3%) was obtained from does in G4 (Table 3).

The observed increase in frequency distribution of compact oocytes in treated groups (G2, G3 and G4) as compared to the control (G1) may be associated with increasing the number of antral follicles with a wide diameter in treated than the control **(Gosalves et al., 1994)**. There was a higher correlation between follicle quality and the distribution of different oocyte categories **(Wani, et al., 2000)**. Moreover, **Daader et al. (2003)** found that percentage of oocytes with even ooplasm granulation was higher in does treated with PMSG and hCG than control group (88.7 vs. 38.9%). This indicated that the hormonal treatment improves the ovarian activity of does as compared to untreated ones. These results were supported by several authors, who reported that oocyte with strongly expanded cumulus investment and oocytes enclosed only by the corona radiated are known to have a very low efficieney in in vitro embryo production **(Crosby et al., 1981 and Stalgmiller and Moor, 1984)**.

Oocytes recovered from oviducts:

Examination of oocytes recovered from oviduct show that all oocytes were in metaphase I and II stages or degenerated oocytes (Fig. 5, 6 and 7, respectively).

Results presented in Table (4) revealed that frequency distribution of oocytes at metaphase II was the highest in all groups as compared to those at metaphase I or degenerated oocytes. Percentage of oocytes at metaphase II was lower in treated groups (G3 and G4) than in G1 (83 and 78% vs. 86%). Percentage of oocytes at metaphase I stage was higher in G1 and G4 and than in G3 (7.1 and 8.6 vs. 3.3%). However, the lowest frequency distribution was for degenerated oocytes, being higher for treated groups (13% in each of G3 and G4) than in G1 (7.1%).

Total recovered oocytes:

When frequency distribution was calculated on the basis of different categories of oocytes recovered from ovaries and oviducts, results in Table (5) cleared that the highest distribution was for ooeytes at metaphase II

stage in G1 and for compact oocytes in G3 and G4.

These results indicated that the hormonal treatments used in this study are stimuli for super-follicular growth. Frequency distribution of compact oocytes (immature and valid to in vitro maturation) was the highest in G2 (86%), but no mature oocytes were obtained in this group. However on the basis of compact oocytes (immature) recovered from ovaries and those at metaphase II from oviduct, it could be concluded that the highest frequency for both categories was recorded for G3 (88.3%) versus 80.6% in G4 and 69.3% for the control group (G1, Table 5).

According to the obtained results, treatment of rabbit does to stimulate over-ovulation by PMSG analogue (75 IU) followed 48 h later by GnRH analogue (0.02 ml) and slaughtered 12 h from the last injection yielded the highest number of compact oocytes (immature) recovered from the ovarian follicles and acceptable number of mature oocytes from oviduct.

Table (1): Number of follicles and corpora lutea of ovarian surface of experimental NZW does.

Item	G1	G2	G3	G4
Mean number of follicles:				
No./right ovary	6.25±0.25^{c}	15.50±4.17^{ab}	20.75±2.52^{a}	11.25±0.75^{bc}
No./left ovary	4.50±0.64^{b}	12.75±3.03^{a}	13.50±1.55^{a}	8.50±0.50^{ab}
Total No./ovary	5.25±0.32^{c}	14 13±3.55^{ab}	17.13±1.34^{a}	9.88±0.59^{bc}
Total No./doe	10.50±0.64^{c}	28.25±7.11^{ab}	34.25±2.68^{a}	19.75±1.18^{bc}
Mean number of corpora lutea (CLs):				
No./right ovary	4.75±0.62^{b}	1.50±0.64^{c}	10.0±0.70^{a}	3.75±0.47^{b}
No./left ovary	3.75±1.03^{b}	1.00±0.40^{c}	7.50±0.85^{a}	2 50±0 64^{bc}
Total No./ovary	4.25±0.77^{b}	1.25±0.32^{c}	8.75±0.42^{a}	3.13±0.51^{b}
Total No./doe	8.50±1.55^{b}	2.50±0.64^{c}	17.5±0.62^{a}	6.25±1.03^{b}

$^{a, b \text{ and } c}$: Means within the same row with different superscripts are significantly different at P<0.05.

Table (2): Number and recovery rate of oocytes recovered from ovaries and oviducts of experimental NZW does.

Item	G1	G2	G3	G4
Number of oocytes recovered from ovary:				
No./right ovary	5.25±0.47^{c}	13.25±1.76^{a}	18.25±1.18^{a}	9.25±0.75^{b}
No./left ovary	3.75±0.47^{c}	10.00±2.34^{ab}	13.00±1.77^{a}	6.75±0.85^{bc}
Total No./doe	9.00±0.40^{c}	23 25±3.08^{ab}	31.25±2.39^{a}	16.00±1.58^{bc}
Recovery rate (%)	85.7	82.3	91.2	81.0
Number of oocytes recovered from oviduct:				
No./right ovary	3.75±1.03^{b}	-	8.50±0.91^{a}	3 25±0.250^{b}
No./left ovary	3.25±0.75^{b}	-	6.00±0.64^{a}	2.50±0.645^{b}
Total No./doe	7.00±1.73^{b}	-	14.50±0.95^{a}	5.75±0.750^{b}
Recovery rate (%)	82.3	-	82.8	92.0
Total recovered oocytes from ovary and oviduct:				
Total No./doe	16.0	23.25	45.75	21.75
Recovery rate (%)	84.0	82.3	87.0	86.5

$^{a, b \text{ and } c}$: Means within the same row with different superscripts are significantly different at P<0.05.

Table (3): Categories of oocytes recovered from ovaries of experimental NZW does.

Group	N	Compact		Denuded		P. denuded		Expanded	
		n	%	N	%	n	%	n	%
G 1	36	20	55.6	11	31.0	3	8.4	2	5.5
G 2	93	80	86.0	8	8.60	2	2.2	3	3.2
G 3	125	112	89.6	5	4.00	4	3.2	4	3.2
G 4	64	52	81.3	3	4.70	5	7.8	4	6.3

N : Total number of oocytes n: Number of oocytes within each category

Table (4): Frequency distribution of oocytes recovered from oviduct of experimental NZW does at different stage of in vivo maturation

Item	N	M I		M II		Degenerated	
		n	%	n	%	n	%
G 1	28	2	7.14	24	86	2	7.1
G 2	-	-	-	-	-	-	-
G 3	30	1	3.33	25	83	4	13
G 4	23	2	8.60	18	78	3	13

N : Total number of oocytes n: Number of oocytes within each category

Table (5): Categories of oocytes recovered from ovaries and oviducts of experimental NZW does.

Gr.	N	Category of oocytes recovered from ovaryies and oviducts													
		Compact		Den.		P. Den.		Extended		M I		M II		Degn.	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%
G1	64	20	31.3	11	17.2	3	4.7	2	3.12	2	3.1	24	38	2	3
G2	93	80	86.0	8	8.60	2	2.2	3	3.20	-	-	-	-	-	-
G3	155	112	72.3	5	3.23	4	2.6	4	2.58	1	0.7	25	16	4	3
G4	87	52	59.6	3	3.50	5	5.5	4	4.50	2	2.9	18	21	3	4

N: Total number of oocytes n: Number of oocytes within each category

Fig.(1):
Compact oocyte with ≥5 layers cumulus cells and had a compact and bright cumulus investment

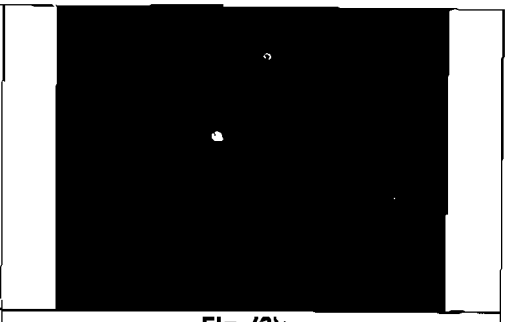

Fig. (2):
Denuded oocyte completely denuded both cumulus and corona cells and covered by zona pellucida only.

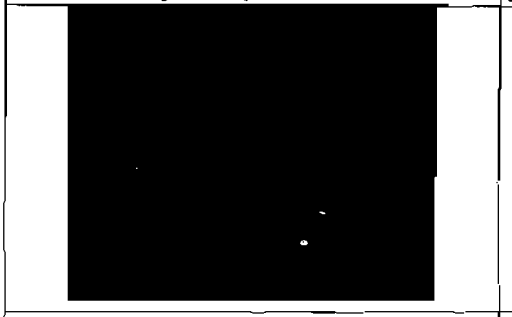

Fig. (3):
Partial denuded with less compact and obviously darker cumulus investment

Fig. (4):
Expanded oocyte oocytes with 1-4 layers of cumulus cells with dark spots of degenerated cumulus cells

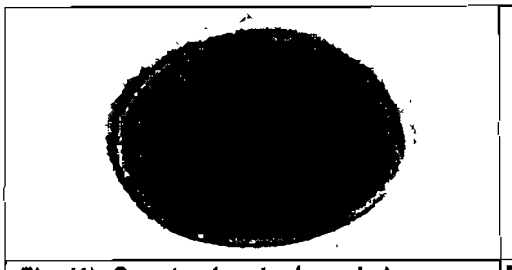

Fig. (1): Oocyte at metaphase I stage

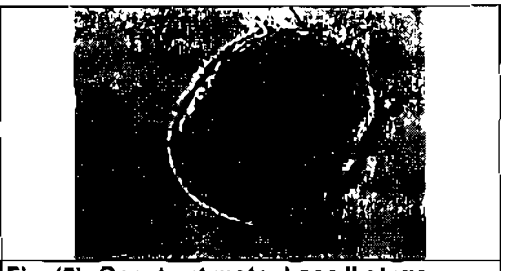

Fig. (2): Oocyte at metaphase II stage

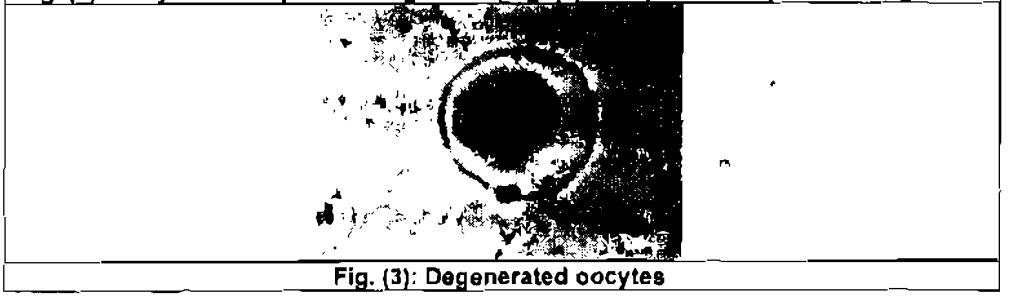

Fig. (3): Degenerated oocytes

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الملخص العربى

معدل استرداد البويضات من المبيض وقناة المبيض فى الأرانب المستحثة للتبويض بمعاملات هرمونية

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تم إجراء هذه الدراسة فى معمل التكنولوجيا الحيوية - سخا التابع لمعهد بحوث الإنتاج الحيوانى على عدد ٣٥ أنثى نيوزيلاندى بيضاء متوسط عمرها ٥ر٥ شهور ومتوسط وزن حى ٢٥ر٣كجم وذلك لدراسة تأثير الطرق المختلفة لحدوث عملية التبويض الفائق وذلك باستخدام هرمونات (hCG. GnRH - RNSG) على معدل الحصول على البويضات الناضجة من قناة المبيض وعلى الصفات المبيضية والصفات المورفولوجية لبويضات الحويصلات المبيضية.

حيث تم حقن إناث المجموعة الأولى بحوالى ٢٠ر٠ ريسبتال (إحدى صور GnRH) ثم الذبح بعد ١٢ ساعة من الحقن (كنترول) بينما حقن إناث المجموعة الثانية بـ ٧٥ وحدة دولية من الفولجون والمشابه لهرمون (PMSG) لمدة ٤٨ ساعة ثم الحقن بـ ١٥٠ وحدة دولية من هرمون التبويض (hCG) ثم ذبح الإناث بعد ١٢ ساعة من آخر حقنة، كما تم حقن إناث المجموعة الثالثة بـ ٧٥ وحدة دولية من الفولجون لمدة ٤٨ ساعة ثم الحقن بحوالى ٢٠ر٠ ريسبتال ثم ذبح الإناث بعد ١٢ ساعة من آخر حقنة، ثم تم حقن إناث المجموعة الرابعة بـ٧٥ وحدة دولية من الفولجون لمدة ٧٢ ساعة ثم الحقن بحوالى ٢٠ر٠ ريسبتال ثم ذبح الإناث بعد ١٢ ساعة من آخر حقنة، تم جمع البويضات من الإناث المذبوحة من قناة المبيض بطريقة الغسيل ثم تثبيتها وصبغها وذلك لمعرفة نسبة البويضات التى وصلت لمرحلة النضج، وكذلك تم أخذ القياسات المبيضية والتى تتضمن (عدد الحويصلات المرئية - عدد الأجسام الصفراء وكذلك تم الحصول على البويضات (غير ناضجة) من الحويصلات المبيضية بطريقة التشريح للمبيض، وتم وصفها مورفولوجيا وذلك لكلا المبيضين الأيمن والأيسر، وأهم النتائج :

١- اختلف عدد الحويصلات المبيضية وعدد الأجسام الصفراء الموجودة على المبيضين الأيمن أو الأيسر لكل أم ومتوسط العدد لكل مبيض معنوياً فى المجموعات التجريبية وكانت الأكبر معنوياً (عند مستوى معنوية ٥٪) فى المجموعة الثالثة، ولقد وجد أن عدد الأجسام الصفراء فى كل المجموعات أكبر على المبيض الأيمن عن الأيسر.

٢- كان معدل استرداد البويضات من المبيض أعلى فى المجموعة الثالثة (٢ر٩١٪) مقارنة بالمجموعة الأولى والثانية والرابعة (٧ر٨٥ - ٣ر٨٢ و ٠ر٨١ على التوالى)، وكان معدل الاسترداد للبويضات من قناة المبيض أعلى فى المجموعة الرابعة (٩٢٪) مقارنة بالمجموعة الثالثة والأولى (٨ر٨٢ و ٤ر٨٢٪ على التوالى)، ولم يسترد أى بويضات من قناة المبيض للمجموعة الثانية ، بينما كان معدل الاسترداد الكلى للبويضات المتحصل عليها من المبايض وقناة المبيض أعلى فى المجموعة الثالثة (٨٧٪) يعقبها المجموعة الرابعة (٥ر٨٦٪) والمجموعة الأولى (٤ر٨٤٪) بينما سجلت المجموعة الثانية أقل نسبة (٣ر٨٢٪).

٣- وجد أن النسبة المئوية للبويضات الجيدة المستردة من المبيض فى المجموعة الثالثة - الثانية والرابعة (٦ر٨٩ - ٠ر٨٦ و ٣ر٨١٪ على التوالى) أعلى من المجموعة الأولى (٦ر٥٥٪)، وكانت النسبة المئوية للبويضات التى وصلت إلى مرحلة M II المستردة من قناة المبيض عالية فى كل المجاميع مقارنة بالبويضات التى وصلت إلى مرحلة MI أو البويضات المضمحلة، وكان معدل التوزيع التكرارى للبويضات الناضجة (مرحلة MII) منخفضة فى المجموعة الثالثة والرابعة عن المجموعة الأولى (٨٣ - ٧٨ و ٨٦٪ على الترتيب).

حقن إناث الأرانب بـ ٧٥ وحدة دولية من الفوليجون لمدة ٤٨ ساعة ثم الحقن بحوالى ٢ ٠ر٠ ريسبتال ثم ذبح الإناث بعد ١٢ ساعة من آخر حقنة أعطى أحسن النتائج من حيث عدد الحويصلات المبيضية وعدد الأجسام الصفراء الموجودة على المبيض - أعلى معدل استرداد البويضات الجيدة من المبيض ومعدل مناسب من قناة المبيض.