

## Studies on embryo transfer in cattle with the help ultrasonography

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### Abstract

The efficacy of different superovulatory regimens for the production of high quality embryos suitable to be transferred to recipient synchronized cows using either eCG alone (different doses) or using eCG plus GnRH at the time of insemination of native and Holstein cows was studied with the help of a real-time B-mode linear array ultrasound scanner. A total of 30 non pregnant cows and heifers were sub-grouped and used in 5 trails for superovulation with Folligon using doses ranged from 1000 to 5000 IU which were injected a day 10 of the estrus cycle of those animals as a source of FSH hormone and GnRH in the first four groups of animals while the fifth group was injected with eCG plus GnRH. Ultrasonographic examination through trans-rectal scanning of ovaries of the superovulated cows was frequently used to follow up the procedure. The induced cows were closely observed for heat detection and they were inseminated using deep frozen semen of proven sires on three doses at 12 hrs interval starting 12 hrs after the onset of estrus. Eight cows were selected and used as recipients to which excellent embryos were transferred on the day of estrus of those cows and they were followed up by frequent trans-rectal ultrasonographic examination for pregnancy diagnosis on days 30, 60 and 90 afterwards till the time of delivery. The obtained results revealed that the appropriate superovulatory dose of eCG (Folligon) ranged from 2000 – 2500 IU and should be injected around the day 10 of the estrus cycle of synchronized cows. Moreover, 5 out of the 8 recipients were proved to be pregnant and ultrasonographic examination 30 days post-transfer and only four of them delivered full term alive calves.

### Introduction

Embryo transfer (ET) is a technical procedure by which fertilized ova are collected from a genetically superior female and then transferred to the reproductive tract of a lesser genetic quality recipient for incubation and subsequent birth. Nearly, all studies indicating that breeding schemes employing embryo transfer promised the opportunity of disseminating the genetics of high proven elite females than current AI progeny testing schemes. This conclusion was based on the argument that embryo transfer would lead to short generation interval (Mc Daniel and Cassell, 1981 and Smith, 1984). ET has proved to be a safe way of introducing new bloodlines into specific pathogen free herds, as a result of the embryos are most unlikely to transmit infectious diseases (Singh et al., 1982; Stringfellow et al., 1982; Bowen et al., 1987; Elizabeth, 1987 and Singh, 1987). Therefore, the present work was planned to evaluate the efficacy of several superovulatory regimens and to determine the most appropriate one for producing a large number of high quality embryos in native and Holstein cows under Egyptian conditions, compare the superovulatory effect of eCG alone with that of eCG plus GnRH at the time

insemination, evaluate the results of embryo transfer application in native cows under Egyptian conditions.

## Material and methods

### 1. Animals:

A total of 30 non lactating and non pregnant cows and heifers were used in 5 trials for superovulation. Ten of them were raised at the farm of Faculty of Veterinary medicine at Sadat City while, the others (20 cows) were reared at a private farm on Cairo-Alexandria desert road. The age of these animals ranged from 2 – 9 years. The cows had previously calved 100 – 180 days before the start of the experiments. The feeding regimens and system of management were almost similar in the two locations. Animals were raised free in the yard. They received clover and concentrates (3 – 5 Kg / animal / day) from November till April while, green corn, tbn and concentrates were supplied during the rest of the year. Rice straw was available continuously. Before the start of the experiments, all animals were examined by trans-rectal linear-array ultrasonography to exclude pregnancy or any abnormalities of the reproductive organs. Due to the low number of available cows for this study, all animals were used in more than one experiment. The animals were not used in consecutive attempts before the elapse of at least two normal estrus cycles or 45 – 60 days.

### 2. Products:

**Estrumate:** a synthetic  $\text{PGF}_{2\alpha}$  (Schering – Plough Animal Health), each ml contains 263  $\mu\text{g}$  Cloprostenol was used for synchronization in cattle at a dose of 2 ml administrated by IM injection for each cow. **Preloban:** (Intervet International B.V. Manufactured in the European Union (EU)). A clear, colorless aqueous solution for parenteral administration, each ml contains R-Cloprostenol sodium salt 0.075 mg active substance and Chlorocresol 1 mg preservative. Dose for estrus synchronization in cattle: 2 ml administrated by IM injection.

**Equine chorionic gonadotrophin (eCG):** (Folligon, Intervet International Boxmeer B.V., Holland). It is presented as a white freeze-dried crystalline plug, containing 1000 or 5000 IU pregnant mare serum gonadotrophin (eCG) per vial together with solvent used with deferent doses according to the experiment as showed in the experimental procedures. Gonadotrophin releasing hormone (GnRH): (Receptal, Intervet International Boxmeer B.V., Holland). One ml injection solution contains 0.0042 mg Buserelin acetate equivalent to 0.004 mg Buserelin, 10 mg benzyl alcohol. A single dose of 2.5 ml was administrated IM for each animal at the day of estrus.

**Flushing and holding medium:** Modified Dulbecco's phosphate buffered saline (PBS) was used for flushing of the uterine lumen of the cows. According to Seidel et al. (1980)

pH of the media should be near (7.25 – 7.45) while, osmolarity should be between 260 – 310. Just before flushing, 1% heat treated bovine serum was added to the prepared flushing medium. Holding medium consisted of the same ingredients of the flushing medium plus 20% heat treated bovine serum instead of 1%.

### 3. Equipments:

**The scanner:** A real time B and M-mode linear array ultrasound scanner (Scanner 480 – Vet – Scan, Pie Medical Co.) was used in this study.

Transducer: The scanner was provided with a transrectal linear transducer and 7.5 MHz) for endo-rectal scanning. The printer: Thermal paper video-printer (up-895 CE, Sony) was used for printing frozen images.

#### **4. Experimental procedures:**

Before each experiment all animals were scanned transrectally with linear-array ultrasonography to determine ovarian structures (follicle, corpus luteum (CL), cyst,.....etc). All animals carrying mature CL were injected with PG (Estrumate 2 ml or Preloban 2 ml). All animals were observed 3 times daily for 5 days after PGF<sub>2</sub> $\alpha$  injection to detect estrus.

#### ***Superovulatory treatment was induced in the following regimens:***

##### **Experiment (1) Superovulation using 1000 IU eCG:**

Ten cows and heifers were used in this experiment. After synchronization a single dose of 1000 IU eCG was injected IM on day 10 of the estrus cycle (0 = day of estrus). 48 hrs after the eCG injection (day 12 of estrus cycle), a dose of luteolytic hormone (PGF<sub>2</sub> $\alpha$ ) was injected. At the time of PGF<sub>2</sub> $\alpha$  injection all animals were scanned transrectally with linear-array ultrasonography to determine the degree of ovarian responses by determining the number of growing follicles on each ovary.

All animals were observed 3 times daily for 5 days after luteolytic hormone injection to detect estrus. On the estrus day, the animals were naturally mated or inseminated artificially every 12 hrs for 36 hrs and scanned with transrectal linear-array ultrasonography at the time of the first insemination to determine the number of mature graafian follicles on each ovary. On the day of embryo collection (7 days after insemination) the efficiency of superovulation was determined by estimating the number of ovulations (number of mature CL) on each animal and counting the number of unovulated follicles by transrectal ultrasound scanning of the ovaries of each animal. Based on this scanning, cows were categorized as either non responders ( $\leq$ two CL) or responder ( $>$ two CL).

##### **Experiment (2) Superovulation using 1500 IU eCG:**

This experiment was conducted on 10 animals. The animals received a single dose of 1500 IU eCG (Folligon) on day 10 of the cycle after estrus synchronization and all other steps were carried out as in experiment (1).

##### **Experiment (3) Superovulation using 2000 IU eCG:**

In this trial, the regimen of superovulation was carried out on 10 animals. 10 days after estrus synchronization, all animals were injected with 2000 IU eCG for each animal and all other steps were carried out as in experiment (1).

##### **Experiment (4) Superovulation using 2500 IU ECG:**

Ten animals were used in this experiment. 10 days after estrus synchronization all animals were injected with 2500 IU eCG. All steps of this regimen were carried out as in experiment (1).

##### **Experiment (5) Superovulation using 2500 IU eCG + GnRH:**

This regimen of superovulation was applied on 10 animals. 10 days after estrus synchronization, all animals were injected with 2500 IU eCG. All steps in this regimen were carried out as in experiment (1) plus IM injection of 2.5 mg Receptal (10  $\mu$ g Buserelin) at the time of first mating or insemination.

**2. Embryo recovery:** Recovery of embryos was performed non-surgically on day 7 – 8 after insemination using the method described by **NewComb, Christie and Rowson (1978)**.

**3. Embryo evaluation:** Morphological evaluation of the collected embryos was carried out according to **Elsden et al. (1978)**, **Lindner and Wright (1983)** and **Takeda (1986)** the embryos were classified into four groups (excellent, good, fair and poor) based on morphological symmetry, stage of individual blastomeres and embryos age in relation to stage of the donor estrus cycle.

**4. Non-surgical transfer:** In this experiment only 8 animals were used as recipients, to them only excellent or good embryos were transferred. All the recipients were synchronized with  $PGF_2\alpha$  to be in estrus at the same day or  $\pm 24$  hrs as the donors. The embryo was deposited approximately in the anterior third of the uterine horn ipsilateral to the ovary carrying CL or at the level of the inter-cornual ligament and the gun was withdrawn slowly. After transfer the recipients were observed for estrus signs and success of transfer which was estimated when recipients did not return to estrus up to 25 days of transfer and scanned with ultrasonography at 30 days for pregnancy diagnosis and re-examined with ultrasonography at 60 days to confirm pregnancy.

## Results

### 1. Superovulation:

**Experiment (1)** Ten non pregnant cows and heifers were used in this experiment. Nine of these animals (90%) came into standing estrus following estrus synchronization (using  $PGF_2\alpha$ ).

\* Pie Medical equipment B.V. Philips-Weg1, 6227 AJ. Maastricht, the Netherland.

\*\* Sony Corporation Tokyo Japan. Rengo Co., LTD. Toyohashi Japan.

Only three of the nine superovulated animals (33.33%) responded to the superovulatory treatment (more than 2 CL). The number of ovulations ranged from 1 – 7, while that of unovulated follicles ( $> 10$  mm diameter) ranged from 0 – 3 as shown in ultrasonographic image (1-A). The percentage of ovulation ranged from 33.3% to 100% (mean 66.6%). For the number of recovered, fertilized and transferable embryos see table 2. **Experiment (2)** Ten non pregnant cows and heifers were used in this experiment. All animals came into standing heat following estrus synchronization with  $PGF_2\alpha$ . Ultrasonographic scanning at the time of  $PGF_2\alpha$  injection (48 hrs post eCG injection) showed that all animals carried structures on one or both ovaries (Image 1-B). Ultrasonographic scanning at the time of insemination showed that all animals carried more than 2 follicles on one or both ovaries (Image 2-B). The third ultrasonographic scanning (at the time of embryo recovery) showed that only 6 cows (60%) responded to superovulation (more than 2 CL) as showed in image 3-B. The number of ovulated follicles (percentage of ovulation) ranged from 1 – 5 (20 – 100%), while that of non ovulated follicles ( $> 10$  mm diameter) ranged from 1 – 4. For the number of recovered, fertilized and transferable embryos see table 2. **Experiment (3)** All animals (10 cows and heifers) in this experiment came into standing estrus after estrus synchronization with  $PGF_2\alpha$ . Ultrasonographic scanning at the time of  $PGF_2\alpha$  injection (48 hrs after eCG injection) revealed that all animals were responded with more than two follicles either on one or both ovaries (Image 1-C). Ultrasonographic scanning at the time of insemination showed that all animals were carrying more than two pre-ovulatory follicles

(Image 2-C). Ultrasonographic scanning 7 days post insemination (day of embryo recovery) showed that only 7 cows (70%) responded to superovulation (more than 2 CL) as shown in image (3-C). The number of ovulations ranged from 2 – 8, while that of unovulated follicles (> 10 mm diameter) ranged from 7. The percentage of ovulation was ranged from 22.2% to 70%. For the number of recovered, fertilized and transferable embryos see table 2. **Experiment 4** Ten non-pregnant cows and heifers were used in this experiment. All animals came into standing heat following estrus synchronization with PGF<sub>2</sub>α. Ultrasonographic scanning at the day of PGF<sub>2</sub>α injection showed that all animals responded with more than two follicles either on one or both ovaries (Image 2-D). Ultrasonographic scanning at the day of insemination showed that responded ovaries were carrying more than two pre-ovulatory follicles on or both ovaries (Image 2-D). Ultrasonographic scanning at the day of embryo recovery showed that only 8 animals (80%) responded to superovulation treatment (more than 2 CL). The number of ovulations ranged from 1 – 8, while that of unovulated follicles (> 10 mm diameter) ranged from 1 – 11 unovulated follicles. The percentage of ovulations ranged from 15.38% - 87.5%. For the number of recovered, fertilized and transferable embryos see table 2. **Experiment (5)** Ten non pregnant cows and heifers were used in this experiment. All animals came into standing estrus following estrus synchronization with PGF<sub>2</sub> α. Ultrasonographic scanning at the day of PGF<sub>2</sub> α injection (48 hrs post eCG injection) showed that all animals responded with more than 2 follicles either on one or both ovaries (Image 1-E). Ultrasonographic scanning at the day of insemination showed that the responded animals were carrying more than two pre-ovulatory follicles on one or both ovaries (Image 1-E). Ultrasonographic scanning at the day of embryo recovery showed that all animals have developed multiple cysts on both ovaries (Image 3-E). Ten animals showed estrus behavior for about 50 hrs. The other animals responded to superovulatory treatment with more than two CL. The number of ovulations ranged from 4 – 9, while that of unovulated follicles ranged from 1 – 11 unovulated follicles (> 10 mm diameter). The percentage of ovulations ranged from 53.85% - 100%. For the total number of recovered, fertilized and transferable embryos see table 2.

Table (1):

## Superovulatory response to different doses of eCG

Dose of eCG	Number of treated animals	Number of responded animals	Percentage responded animals
1000 IU	9	3	33.33%
1500 IU	10	6	60%
2000 IU	10	7	70%
2500 IU	10	8	80%
2500 IU +10 µg GnRH	10	7	70%
Total	49	31	62%

Table (2):

Percentage of ovulation and embryo recovery using different doses of eCG

Dose of eCG	Percentage of ovulations	Number of recovered embryos (%)	Number of fertilized ova	Number of transferable embryos
1000 IU	67.5	6 (37.5%)	5	3
1500 IU	69.18	9 (37.5%)	7	6
2000 IU	55.88	18 (47.37%)	12	9
2500 IU	48.29	23 (50%)	18	13
2500 IU +10 µg GnRH	72.32	23 (50%)	20	15
Total	62.63	79 (46.47%)	62	46

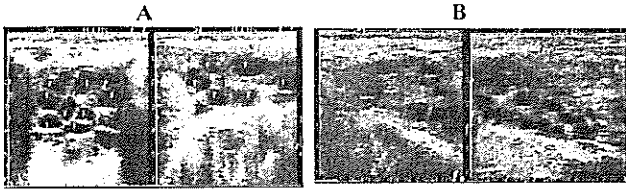
Table (3): Description of the stages of fertilized ova using different doses of eCG

Dose of eCG	Morula	Compact morula	Blastocyst	Hatched blastocyst	Total
1000 IU	2	0	3	0	5
1500 IU	2	2	3	0	7
2000 IU	6	0	5	1	12
2500 IU	6	0	10	2	18
2500 IU +10 µg GnRH	7	1	9	3	20
Total	23(37.1%)	3 (4.48%)	30 (48.39%)	6 (9.68%)	62

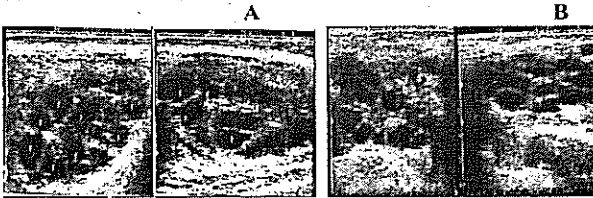
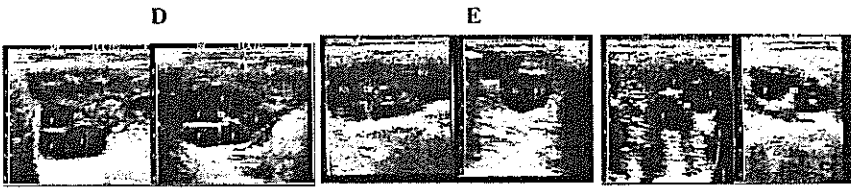
Table (4):

Description of the quality of the embryos using different doses of eCG

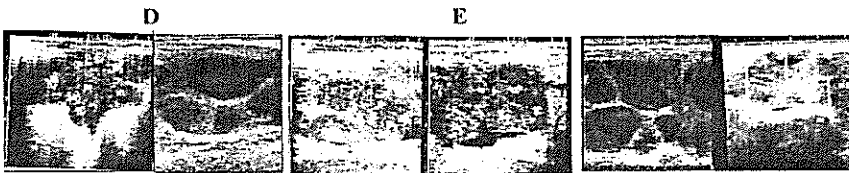
Dose of eCG	Excellent	Good	Fair	Poor
1000 IU	2	1	0	2
1500 IU	3	4	1	0
2000 IU	4	5	2	2
2500 IU	6	5	3	2
2500 IU +10 µg GnRH	6	9	2	3
Total	21 (33.87%)	24 (38.71%)	8 (12.9%)	9 (14.52%)



**Image 1:** Show the ovaries of cows 48 hrs of injection of ECG: A) 1000, B) 1500, C) 2000, D) 2500 or E) 2500 IU + 10 ug GnRH.



**Image 2:** Show the ovaries of some cows at the time of insemination. A) group 1, B) Group 2, C) Group 3, D) Group 4 and E) Group 5

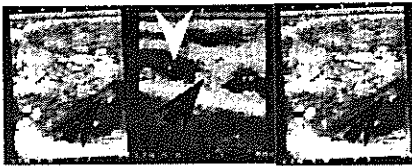


**Image 3:** Shows the ovaries of some cows at the day of embryo recovery. A) Group 1, B) group 2, C) Group 3, D) Group 4 and E) Group 5.

D

E

**3. Transfer of embryos:** A total of 8 recipient cows were synchronized with  $PGF_{2\alpha}$  to be in estrus at the same day or  $\pm 24$  hrs as the donors. Two recipient cows were in estrus about one day before the donors, three recipients were in estrus at the same day as the donors and three recipient cows were in heat one day after the donors. Four recipient cows received single embryo (either morula or blastocyst) and the other four recipient cows received two embryos. All recipient cows were observed for 25 days post-transfer to detect any signs of estrus to determine the non return rate. Only one recipient cow showed signs of estrus (87.5% non return rate). Ultrasonographic scanning 30 days post transfer for early pregnancy diagnosis revealed that only 5 recipient cows (62.5%) became pregnant (Image 4). Ultrasonographic scanning 60 days post transfer revealed that only 4 recipient cows (50%) were pregnant of which 2 recipient cows of that received two embryos and two recipients of that received one



**Image (4):** Shows the uteri of some cows at day 30 after transfer. Notice the amount of fetal fluids (large anechoic areas, white arrow) and the size of the embryo (hypo-echoic areas, black arrows).



**Image (5):** Shows the uteri of some cows at day 60 after transfer. Notice the amount of fetal fluids (large anechoic areas, white arrows) and the size of the embryos (hypo-echoic areas, black arrows).

embryo (Image 5). The pregnant recipient cows were followed by pregnancy diagnosis every 2 months till parturition.

#### **Superovulation and embryo recovery:**

Superovulation is an important step in the embryo transfer procedure. Attainment of the best response, based on ovulation rate, recovery rate and transferable embryos, is the ultimate goal in any embryo transfer procedure. eCG is readily available, cheap and convenient to administer (Zeitoun et al 1991). For these reasons eCG was used in this work although its low superovulatory effect than P-FSH.

#### **Discussion**

In the current work the use of  $PGF_{2\alpha}$  which was given to induce estrus synchronization post eCG injection was given 48 hrs after eCG injection which agrees with what was mentioned by Greve et al. (1979); Boland et al. (1980); Sergeev et al. (1982); Vlachov et al. (1983); Boland et al. (1984); Budevich et al. (1988); Mahmood et al. (1989) and Petr et al. (1992).



In this work the percentage of animals responded (number of animals responded with more than 2 CL / total number of animals) to different doses of eCG (animals with more than two CL) was 33.33%, 60%, 70%, 80% and 70% for 1000 IU, 1500 IU, 2000 IU, 2500 IU and 2500 IU + 10 µg GnRH respectively with a total percentage of responded animals in the five trials of 62.67%. This is similar to 69.6% recorded by Budevich et al. (1988) and lower than 85% for cows and 93.75% for heifers obtained by Sergeev et al. (1982) and 81% obtained by Vlachov et al. (1983). This difference in superovulatory response might be attributed to some variability resides in genetic and physiologic make-up of the animal (individual variability) and breed may also be a factor to be considered. Mapletoft et al. (2002) recorded that Holstein cows required a high proportion of FSH whereas Charolais required a higher proportion of LH for maximal superovulation.

In the current work the percentage of ovulation (total number of CL / total number of ovarian structures) to the different doses of eCG was 67.5%, 69.18%, 55.88%, 48.29% and 72.32% for 1000 IU, 1500 IU, 2000 IU, 2500 IU and 2500 IU + 10 µg GnRH respectively, with a mean percentage of ovulation for the five trials of 62.63%. However, the ovulation rate was 5.33, 4.33, 5.57 and 6.57 with mean ovulation rate of 5.54 for the five trials. This is similar to 6.4 recorded by Vlachov et al. (1983) and Mohamed (1995) but lower than 8.0 recorded by Mahmood et al. (1989); 7.90 recorded by Brown et al. (1989) obtained by Holy (1987) and 13.3 obtained by Saumande and Chupin (1986). Gordon (1975); Elsden et al. (1978) and Newcomb (1980) mention that the major problems with superovulation in cattle include high variability of responses (measured as palpable corpora lutea or number of recovered embryos), treated cows not expressing overt estrus, and cows ovulate over a wide interval of time. In addition, superovulatory treatment was said to be affected because of age, breed, season, nutrition, level of milk production, frequency of breeding, the type and batch of superovulatory compound used.

Increasing the dose of eCG from 1000 to 2500 IU in the present study, seemed to be associated with increase in the number of the dominant pre-ovulatory follicles which may ovulate to increase the ovulation rate or may continue to grow to form follicular cysts depending on the level of LH surge. This is similar to that recorded by Hafez et al. (1963) who reported that higher doses of eCG resulted in the formation of large number of follicles. These follicles were developed as a second follicular wave after ovulation due to the long half-life of eCG and were observed on the ovary at the time of embryo recovery. In the same line, Kummer et al. (1980); Saumande and Chupin (1986) and Dieler et al. (1987) recorded that follicles were observed more with high doses of eCG and their presence with the corpora lutea would create an unfavorable estrone:progesterone ratio which allegedly affected embryo transport and was consequently associated with a low quality of recovered embryos.

It is important to note that embryo recovery in cows was planned to be performed on day 7 which agrees with day 7 – 9 (Mohamed, 1995). This depends on the knowledge cited by Kuzan (1986) that the embryos recovered from superovulated cows often displayed a wide range of developmental stages. The highest proportion of morulae and compact morulae was observed

to 6 days after estrus. Early blastocysts and blastocysts were more prevalent on day 7 and expanded blastocysts were more prevalent on day 8 – 9 of the estrus.

In the current study, out of 170 corpora lutea, 79 eggs (46.47%) were recovered from the five trials with a recovery rate of 37.5% (6/16), 37.5% (9/24), 47.37% (18/38), 50% (23/46) and 50% (23/46) for 1000 IU, 1500 IU, 2000 IU, 2500 IU and 2500 IU + 10 µg GnRH, respectively. Out of the 79 eggs recovered from the flushed cows, 62 (78.48%) were fertilized. Amongst these fertilized eggs, 46 (74.19%) were transferable embryos. Similar values for the percentage of fertilized and transferable embryos 71 – 83% and 70 – 80% respectively, were reported in cattle by Boland et al. (1984); Foote et al. (1989); and Peter et al. (1990) but lower than 85.1% and 85.63% for fertilized and transferable embryos respectively, were recorded by Mohamed (1995). This difference in the percentage of fertilized and transferable embryos might be attributed to many factors as semen quality, time and number of inseminations, age of the donor, experience of the embryo evaluator and even the technique used for embryo evaluation. A high degree of variability was observed in morphological development and embryo quality within and among donors (Lindner and Wright, 1983).

According to the stage of embryonic development, transferable embryos in this study were classified into morula (n = 23; 37.1%), compact morula (n = 3; 4.84%), blastocyst (n = 30; 48.39%) and hatched blastocyst (n = 6; 9.68%). Similar results for morula and hatched blastocyst (35.09% and 10.92%, respectively) were recorded by Issa (2002). According to the quality of the embryo, transferable embryos were classified into excellent embryos (n = 21; 33.87%), good embryos (n = 24; 38.72%), fair embryos (n = 8; 12.9%) and poor embryos (n = 9; 14.52%). Similar results for excellent and poor quality embryos (29.92% and 13.14%, respectively) were recorded by Mohamed (1995).

In the current study, for both groups of superovulation using 2500 IU eCG alone and using 2500 IU eCG + 10 µg GnRH, the rate of ovulation was 48.29% and 72.32%; recovery rate was 50% and 50%; the percentage of fertilized ova was 78.26% and 86.96% and the percentage of transferable embryos was 72.2% and 75%, respectively. These results indicate that there is no difference between the two groups of superovulation except there is slight improvement in the ovulation rate in the group of superovulation using 2500 IU eCG + 10 µg GnRH. Similar results were recorded by Savage et al. (1987). They also, concluded that although treatment with GnRH tended to be associated with an increased ovulation response, there was no significant difference in the number of corpora lutea, recovery rate, fertilization rate and percentage of transferable embryos. Also, Foote et al. (1989) summarized that the use of Buserelin may increase the number of yielded embryos (fertilized ova) slightly, but Wubishet et al. (1986) concluded that administration of GnRH on the day of estrus increases fertilization rate and number of transferable embryos in superovulated cows.

### **3. Transfer of embryos:**

The process of embryo transfer consists of a chain of events; synchronization, superovulation, embryo collection, embryo evaluation and transfer. In Egypt all the treatments were approached in cattle and buffaloes by many researchers

(Hussen, 1981; Hamam, 1987; El-Nahala, 1989; El-Menoufy and Abdou, 1991; Ismail et al., 1991; Ismail et al. 1992; Mohamed, 1995, Issa, 2002 and Kani et al., 2007).

In the present study, 8 recipient cows were received either one embryo or embryos, 7 of them (87.5%) did not return to heat 25 days following embryo transfer. Ultrasonographic scanning on day 30 and 60 post transfer revealed 62.5% (5/8) and 50% (4/8) pregnancy rate, respectively. This means that embryonic loss between day 30 and 60 after embryo transfer was 12.5%. These findings were lower than 19% recorded by Markette et al. (1984). The pregnancy rate at 60 days post transfer (50%) was higher than 47.9% recorded by Mas et al. (1986); 33.3% recorded by Subramaniam and Devarajan (1991); 38% recorded by Totey et al. (1991); 43% recorded by Mishra et al. (1992); 42.4% recorded by Mohamed (1995) and 42.1% recorded by Smith-Grimmer (2005). However, this was lower than 53.8% recorded by Niemar et al. (1986); 54.4 – 60% recorded by Donaldson (1986); 56% recorded by Hoogenkamp (1986); 59.3 – 65.9% recorded by Gaber (1991); 64.2% recorded by Stubbings and Walton (1986) and 68.1 – 77.1% recorded by Hasler (2005). This difference might be attributed to the limited number of recipient cows used in this study, the stage of embryo development, the grade of embryo, recipient synchrony or the technique of transfer itself (surgical or non surgical). Schneider et al. (1980) reported that the pregnancy rate for embryos transferred non surgically was lower (44%) than the pregnancy rate of embryos transferred surgically (66%) during the same period. Josef and Wright (1981) reported pregnancy rates from morulae (44%); advanced morulae (53%), morulae and blastocysts (65%), blastocysts (66%) and advanced blastocysts (66%). Pregnancy rates related to synchronization of estrus between donor and recipients were -36 hrs (before donor) (59%), -24 hrs (61%), -12 hrs (68%), -6 hrs (59%), +12 hrs (after donor) (61%), +24 hrs (58%) and +36 hrs (41%).

From the current experiments of superovulation we concluded that there was an increase in the number of responded cows and the number of recovered embryos associated with the increase in the dose of eCG. Under non-surgical conditions, the most appropriate superovulatory dose of eCG ranged from 2500 – 2500 IU and should be injected mostly around the 10<sup>th</sup> day of the estrus cycle. There was no difference in the superovulatory response between 2500 IU eCG alone and 2500 IU eCG + 10 µg GnRH. However, further studies on this topic are required especially for the time of injection of GnRH. Under non-surgical conditions, transfer of embryos from Holstein cows to native cows can successfully be applied and can produce pregnancy rates corresponding to those reported internationally.

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## الملخص العربي

### دراسات عن نقل و زرع الأجنة في الماشية بمساعدة الموجات فوق صوتية

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أجريت هذه الدراسة لمعرفة فاعلية الأنظمة المختلفة لإكثار الإباضة في إنتاج أجنة ذات جودة عالية وذلك باستخدام الهرمون الحاث للغدة المنسلية (eCG) بمفرده بجرعات مختلفة أو بإضافة الهرمون الحاث لإفراز الهرمونات المنشطة للمبايض (GnRH) وذلك أثناء تلقيح البقر المحلى و الهولشتاين بمساعدة الفحص بجهاز الموجات فوق صوتية في المراحل المختلفة من نشاط المبايض لإكثار الإباضة.

تم استخدام عدد ٣٠ بقرة غير عشار و قسمت إلى ٥ مجموعات و تم حقنها بجرعات مختلفة من الهرمون الحاث للغدة المنسلية (eCG) تراوحت من ١٠٠٠ إلى ٢٥٠٠ وحدة دولية بالنسبة للمجموعات الأربع الأولى أما المجموعة الخامسة فقد تم حقنها بجرعة ٢٥٠٠ وحدة دولية مصحوبة بالهرمون الحاس لإفراز الهرمونات المنشطة للمبايض (GnRH) وتم متابعة ذلك بالفحص المتتابع بجهاز الموجات فوق صوتية عن طريق المستقيم و تم تلقيح هذه الحيوانات بالسائل المنوي المجمد من طلائق منتخبة بثلاث جرعات بتزامن قدره ١٢ ساعة و ذلك بعد مرور ١٢ ساعة على بداية ظهور الشبق على هذه الحيوانات. تم جمع الأجنة وفحصها وتصنيفها كما تم نقل الأجنة المنتخبة إلى عدد ٨ أبقار من البقر المحلى وذلك في اليوم السابع من الدورة التناسلية وتلي ذلك متابعة الفحص بالموجات فوق صوتية لتشخيص الحمل في هذه الحيوانات بعد مرور ٣٠ و ٦٠ يوما وحتى نهاية فترة الحمل. أظهرت الدراسة أن الجرعة المناسبة من الهرمون الحاث للغدة المنسلية اللازم لإكثار الإباضة تراوحت بين ٢٠٠٠ و ٢٥٠٠ وحدة دولية كما أظهرت المتابعة بالفحص بالموجات فوق صوتية أن عدد ٥ بقرات كانت عشار عند ٣٠ يوم وان عدد ٤ بقرات منها هي التي أتمت العشار وولدت أجنة حية وكاملة النمو.