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SUPEROVULATION WITH PMSG (eCG) AND EMBRYO TRANSFER OF EGYPTIAN BUFFALOES

B. Heleil[1], El-S.M. Fattouh[1], B.H. Serur[1], S.A. Darwish[2], Y.M. El-Deeb[1]

[1]Department of Theriogenology, Faculty of Veterinary Medicine, Kafrelsheikh University

[2]Animal Production Research Institute, Mehalet Mousa Buffalo Research Farm

ABSTRACT

The present study was conducted to assess the effects of different superovulatory regimens using pregnant mare serum gonadotropin (PMSG) (eCG equine chorionic gonadotrophin) and influence of administration of GnRH or rbST to recommended superovulatory regimens as well as determination of pregnancy rate after embryo transfer of different superovulatory regimens.

A total of 106 buffaloes were used in the present study. They were clinically healthy and with good body conditions. The Estrous synchronized by administration of prostaglandin (double intramuscular (i.m) injections 11 day apart).A total of 76 buffaloes (3 experiments) were used to evaluate the efficacy of superovulation by eCG. Experiment 1 (43 animals), the animals were divided into 5 groups after synchronization using $PGF_{2\alpha}$ and injected with different doses of eCG (GI: 1500 IU, GII: 2000 IU, GIII: 2500 IU, GIV: 3000 IU, GV: 3500 IU) at mid luteal phase of estrous cycle. Exper. 2: was designed to evaluate the effect of GnRH on the superovulatory response. A total of 8 buffaloes injected with 3000 I.U eCG (control) as recommended dose from 1st exp. and 9 buffaloes were injected with 3000 IU eCG and 20 µg GnRH at the time of insemination. Exper. 3: was conducted to evaluate the effect of rbST on

the superovulatory response. Eight buffaloes received 3000 I.U eCG (control) and other 8 buffaloes received 3000 eCG + 500 mg rbST. Exper. 4: was carried out to determine the pregnancy rate of recovered transferable embryos of previous experiment, a total of 30 fresh buffalo embryos were transferred to prepared recipient in 5-6 days of estrous and pregnancy diagnoses was conducted after 30 days after transfer.

The results of the present study indicated that the using of eCG for superovulation in buffalo characterized by low ovarian response and low embryo recovery in all treated groups with the lowest ovarian response and embryo yields in group I (3.12 $\pm$ 0.22 follicles, 0.0 embryo) and the highest response and embryo recovery in group 4 (7.42 $\pm$ 42 follicles and 1.14 $\pm$ 2.6 embryo). Moreover, the results revealed that administration of 20 µg GnRH or 500 mg rbST do not improve superovulatory response in buffaloes. The pregnancy rate after embryo transfer did not show significant difference between GIII and GIV (2500 IU eCG vs 3000 IU). However, administration of 20 µg GnRH and 500 mg rbst improved the pregnancy rate by 33% and 50%, respectively..

Keyword: buffalo, superovulation, embryo transfer ,PMSG, GnRH, rbST, pregnancy rate.

INTRODUCTION

Embryo transfer technique plays an important role in the genetic improvement of cattle and buffaloes. Successful embryo transfer depends on the coordination of many separate procedures, such as detection and synchronization of estrus, superovulation and embryo collection ***(Thibier, 2001; Sartori et al., 2003)***. Superovulation offers a convenient

procedure for increasing the number of offspring from genetically superior female during short life. The greatest problem with superovulation is the large degree of variation in the superovulation response between individuals of the same species. This variation in the response may be due to age, breed, season, nutrition, nature and dose of superovulation agent and the stage of estrous cycle, ***(Lopes et al., 2001 and Drost, 2007)***.

Embryo transfer in buffaloes is of limited benefit due to lower superovulatory response in buffaloes compared to that in cattle, mainly due to the small number of the primordial follicles reserve, small percentage of easily mobilizable primordial follicles among the serve and high rate of atresia ***(Aboul-Ela, 2000; Manik et al., 2002)***. However, the reason of these phenomena is still not yet known whether it is the species itself or the regimens of superovulation used which have being originally developed to be applied in cattle. Therefore, the current study were designed to compare between the efficacy of several superovulatory regimens as well as study the effect of GnRH and rbst administration on the superovulatory response and pregnancy rate after embryo transfer in Egyptian buffaloes.

MATERIAL AND METHODS

Animals:

This study was carried out on a total of 106 buffaloes aged 6-10 years (76 donor and 30 recipient) belonging to Mahalt Mousa Farm, Animal Production Research Institute, at Kafr El-Sheikh Province. The

number of births for these buffaloes ranged between 2-5. Interval between calving and superovulation ranged between 90-180 days. Body weight of these buffaloes ranged between 450-600 kg. Daily milk production ranged between 9-11 kg. Days open ranged between 70-90 days. The animals included in the present work of a good healthy condition and proved to be free from tuberculosis, brucellosis and leptospirosis as proved by the local veterinary authority. They were fed on hay, silage and concentrate during summer and autumn, while during winter and spring the animal were fed ad-libitum on berseem, concentrate mixture and rice straw. All animals were be examined by ultrasonography before the beginning of the experiments to exclude pregnancy and any abnormalities in their reproductive tract. Moreover, they were observed for at least 2 regular cycles before entering in our experimental programs.

Drugs used in the present study:

1. Hormones:

- **Estrumate** (Schering-Plough Animal Health Co, Germany), synthetic prostaglandin. Each ml contain 263 μg of cloprostenol sodium, equivalent to 250 μg cloprostenol.
- **Pregnant Mare Serum Gonadotrophin** (Folligon/PMSG), (Intervet Co., B.V. Holland). It is presented as a white freeze-dried crystaline plug containing 1000 I.U. PMSG per vial.
- **Gonadotrophin releasing hormone** analogue (Receptal, Intervet Co., B.V. Holland). Each ml contains 0.0042 mg buserelin acetate equivalent to 0.004 mg buserelin.

Recombinant bovine somatotropin (rbST):

rbST (SOMATCH, Elanco Animal Health, Austria).

It is available in syringe. Each 1.4 ml syringe contains 500 mg of rbST in an oil formulation. somatech is equipped with single use needle.

2. Anaesthesia:

- Sedative → zylaject (Adwia Co., Egypt).
- Epidural anesthesia → lidocaine HCl (El-Nasr Co., Egypt).

Chemicals:

1. Flushing and holding media:

Dulebecco's phosphate-buffered saline (DPBS) was used for flushing.

- One percent heat treated bovine serum was added to the prepared flushing medium.
- Holding medium consisted of the same ingredients of the flushing medium plus 10% heat treated bovine serum instead of 1%.

Ultrasonography:

Transrectal ultrasonography was carried out using ultrasound scanner supplied with a 5 MHz linear array transducer (Ultra Scan 900 alliance, Quebec, Canada). All animals would be examined by ultrasonography before the beginning of the experiments to exclude pregnancy and any abnormalities in their reproductive tract and then daily through ultrasound to investigate and demonstrate the response to superovulatory treatment ***(Baruselli et al., 1998)***.

Synchronization of estrous and heat detection:

Estrous cycle of the selected animals (donors and recipients) was synchronized by double doses of $PGF_{2\alpha}$ 11 days apart. After Luteolytic hormone injection, the animals were carefully observed visually twice daily (30-60 min.) by well trained herd man for estrus signs. However special attention was paid for acceptance of buffalo cows to buffalo bulls as standing of buffalo-cows to be mounted by the bull (teaser) which is the most reliable signs of estrus in buffalo ***(Vale et al., 1990)***. Also, ultrasonographic examination of the ovaries aid in estrus detection.

Superovulatory treatments and experimental procedure:

Experiment (1): Effect of different doses of eCG on superovulatory response:

Forty three buffaloes were treated with two I.M. injection of 500 μg of Cloprostenol and assigned to treatment groups of 1500 (n=10 GI), 2000 (n=9GII), 2500 (n=8 GIII), 3000 (n=8 GIV) and 3500 (n=8 GV) IU eCG. Forty eight hours later, all animals in the five groups were given an injection of 2 ml estrumate (500 μg cloprostenol) to induce luteal regression. Animals were under observation of heat detection. They were inseminated artificially 2 times with frozen semen at 12 hr. intervals following detection of standing heat and scanned with ultrasonography at the time of the first insemination to determine the number of mature graffian follicles on each ovary. Embryo recovery was performed non surgically on day 5-6 using the method described by ***New Comb et al. (1978)*** by using a sterile two way foley catheter (size 8-22). Efficiacy of superovulation was determined by estimating number of corpora lutea and counting the unovulated follicles $\geq$10 mm by ultrasonography.

Experiment (2): Effect of GnRH administration on superovulatory response:

Nine buffalo cows were used in this experiment based on the results of previous exp. 1. They were injected intramuscularly by the optimal dose of exp. 1(3000 IU PMSG) on day 10 of the estrous cycle followed

by injection of 2 ml estrumate (500 ug of cloprostenol) 48 hours later. Buffaloes, which exhibited heat sings, were injected with 5 ml receptal (20 ug GnRH) ***(Techakumphu et al., 2001)*** on the day of insemination. All animals were inseminated 2 times at 12 hrs intervals using frozen semen. They were scanned with ultrasonography at the time of the first insemination to determine the number of mature Graffian follicles on each ovary. On the day of embryo collection, efficacy of superovulation was determined as in exp. (1). Eight buffaloes used as control by injection of 3000 I.U eCG only.

Experiment (3): Effect of rbST administration on superovulatory response:

Eight buffalo cows were used in this experiment. The animals were synchronized by 2 ml estrumate, on day 7 of the synchronized estrous cycle, all animals were injected with 500 mg rbST ***(Gong et al. , 1993)*** subcutaneous. On day 10 of estrous cycle, buffaloes were injected with the optimal dose of PMSG of exp. 1 (3000 IU PMSG) followed by injection of 2 ml estrumate 48 hrs. after PMSG treatment to induce luteal regression. Animals on the day of estrous were inseminated 2 times with 12 hrs. intervals using frozen semen and scanned with ultrasonography at the time of the first insemination to determine the number of mature Graffian follicles on each ovary. On the day of embryo collection efficacy of superovulation was determined as in exp. (1). Eight buffaloes were used as control by injection of 3000 I.U eCG only.

Experiment (4): Determination of pregnancy rate of recovered transferable embryos.

To evaluate pregnancy rate of recovered embryo of different doses of eCG or with GnRH and rbST. A total of thirty buffalo cows, clinically healthy. Estrous was synchronized using double injection of PG $F_2\alpha$ 11 days apart. Animals were carefully observed after 48 hr. from the second dose of $PGF_2\alpha$. Embryo transfer was carried out on 5 or 6 days of estrus

using the technique described in buffalo by ***Drost et al. (1983).*** Ultrasonography was conducted for animals at time of transfer to detect the size and maturity of C.L and after 30 days from transfer to detect pregnancy.

Embryo recovery and evaluation:

Recovery of embryos was performed non surgically using the method described by ***New Comb et al. (1978)***. The animal was fastened 24 hrs prior to collection. Epidural anesthesia was undertaken by 5-10 ml of 2% lidocaine. The vulva and perenial region were washed using an antiseptic. A sterile two way foleycatheter (size 18-24 with 30 ml inflatable balloon) was inserted through the cervix into the base of the uterine horn. The balloon was inflated with 10-20 cc-air. After the catheter was fixed in its position, tubing system was connected to the catheter. Each uterine horn was separately irrigated with 500 ml of M.D PBS, containing 1%. BSA. The flushing solution was channeled directly from the uterus through an embryo filter (75 um pore size). Embryo were allowed to settle on the bottom of dishes for 10 minute and identified under a steromicroscope.

Embryos were collected on day 5 to 6 after superovulatory estrus, ***(Drost and Elsden, 1985 and Misra et al., 1998)***. Buffalo embryo are isolated and evaluated at 75 magnification and viable embryos were classified and graded in their morphological appearance ***(Mapletoft 1986).*** Excellent and good grade embryos were selected for transfer to synchronized recipient. All viable embryos are given 10 serial washings in sterilized holding media supplemented with 0.4% BSA, following guidelines of internal embryo transfer society.

Embryo-transfer (ET):

E.T was successful application of Cassou A.I gun. The gun with special sheath with metal tip and two side outlet. In carrying out the transfer made throughout cervix into uterine horn, synchronized recipient

are checked for presence of an active *Corpus luteum* (epidural anaesthesia is induced to minimize straining). The embryos aspirated into a 0.25 ml French straw in a central column of 20 mm holding media between two air pockets. The straw loaded into ET gun, and sheath with a metal tip is fitted over the top. One sanitary sheath is then rolled on top to avoid any contamination from vaginal microflora. The ET gun is passed through the vagina to reach external os uteri. The sanitary sheath is then perforated, and the gun is gently guided through cervix and uterine body to reach upper one third of uterine horn, ispilateral to ovary being bearing CL. The piston of the gun is then pushed gently to deposit embryo into uterine horn and gun gently withdraw.

Statistical analysis:

Data are represented as mean ± SEM. Analysis of variance (ANOVA) was used for comparison of mean values of the various treatments at a significant level of $P<0.05$. All data were statistically analyzed using T-test and F-test according to ***Snedecor and Cochran (1980).***

RESULTS

Experiment (I): The effect of different doses of eCG on superovulatory response:

Ovarian responses and embryo yields in buffalo of the present study are summarized in Table 1. Six buffaloes in this experiment (one from each group except 2 from group I) did not show signs of estrous. The number of mature graffian follicles were significantly increased with the increase of eCG doses, but there were no significant differences between G IV and G V (7.42 ± 42, 7.57 ± 0.42, respectively). The highest number of *Corpora lutea* found in G IV (4.14 ± 0.34). On the other hand, a minimum number of unovulated follicles found in G I (0.62 ± 0.18) and the maximum number found in G V (4.0 ± 0.30) ($P < 0.05$).

There was no embryo recorded in G I & G II and asignificant increase ($P < 0.05$) in recovered embryo was recorded in G IV compared to G III and G V (1.14 ± 0.26, 0.57 ± 0.2 and 0.57 ± 0.20, respectively) and maximum number of transferable embryo of excellent and good grade was found in G IV (0.85 ± 0.18).

Table (1): Ovarian response and embryo recovery by using different doses of eCG (Mean±SE).

Parameter	Groups				
	G I (n = 10)	G II (n = 9)	G III (n = 8)	G IV (n = 8)	G V (n = 8)
Animals in estrus (estrus response)	8/10	8/9	7/8	7/8	7/8
Interval between PG and first signs of oestrus (hr)	33.75±1.97 a	31.37±2.92 a	28.42±2.26 a	30.42±2.24 a	25.71±1.71 a
Number of mature follicles/per buffalo	3.12±0.22 c	4.0±0.46 c	5.57±0.42 b	7.42±0.42 a	7.57±0.42 a
Number of *Corpora lutea*/per buffalo	2.37±0.26 c	2.62±0.32 c	3.71±0.18 ab	4.14±0.34 a	3.14±0.26 bc
Number of unovulated follicles/per buffalo	0.62±0.18 d	1.0±0.26 d	1.57±0.20 c	2.57±0.29 b	4.0±0.30 a
Ovulation percentage	81.22±5.63 a	76.5±6.04 a	70.42±2.97ab	61.61±3.73 b	44.01±2.95 c
Number of recovered embryos /per buffalo	0	0	0.57±0.20 b	1.14±0.26 a	0.57±0.20 b
Number of unfertilized ova/per buffalo	0	0	0.28±0.18 bc	0.57±0.20 ab	0.71±0.18 a
Number of transferable embryos/per buffalo	0	0	0.42±0.20 b	0.85±0.14 a	0.28±0.18 bc
Number of morula	0	0	0.42±0.20 a	0.71±0.18 a	0.28±0.18 a
Number of blastocysts	0	0	0.14±0.14 a	0.42±0.20 a	0.28±0.18 a

Means within same row having different letters are significantly different at $P < 0.05$.

Experiment (2): Effect of GnRH administration on superovulatory response.

The results given in Table (2) revealed that the differences between PMSG alone and PMSG with GnRH (20 µg) were not significant. Three buffaloes in this experiment (one from group I and two from group II) did not show estrus signs. The mean intervals from PG $F_2\alpha$ treatment to estrus were 31.48± 2.24 and 37.57±3.14 hrs for groups I and II,

respectively, It was insignificantly higher in GnRH group than control one. There were no significant differences between groups I and II in the mean number of mature follicles and *Corpora lutea*. Supplementation with GnRH, insignificantly reduced the number of anovulatory follicles to 2.14±0.26 in the GnRH group compared to 2.57±0.29 in the control one. Also, the percentage of ovulations between the two groups did not differ significantly, being 60.61±3.75 and 67.27±3.66 for the two groups respectively. The mean numbers of recovered embryos were 1.19±0.26 and 1.28±0.28 for groups I and II, respectively. The difference between the two groups was insignificant in transferable embryos (0.89±0.15 and 0.85±0.34, respectively). Non significant differences were found between the two groups in the mean number of morula (0.73±0.20 and 0.57±0.20, respectively) and blastocyst (0.45±0.30 and 0.71±0.18, respectively).

Table (2): T-test for differentiation between the effect of PMSG alone (group I) and PMSG with GnRH (group II) (Mean±SE) on the ovarian response and embryo recovery of buffalo cows.

Parameter	Groups		T. value
	I (n = 8)	II (n = 9)	
Animals in estrus	**7/8**	**7/9**	-
Interval between PG and first signs of oestrus (hr)	31.48±2.21	37.57±3.14	1.85 n.s
Number of mature follicles	7.82±0.41	7.14±0.51	0.43 n.s
Number of Corpora lutea	4.25±0.35	4.42±0.36	0.57 n.s
Number of unovulated follicles	2.89±0.29	2.14±0.26	1.08 n.s
Ovulation percentage	60.61±3.75	67.27±3.66	1.08 n.s
Number of recovered embryos	1.19±0.26	1.28±0.28	0.37 n.s
Number of unfertilized ova	0.67±0.30	0.57±0.20	0.0 n.s
Number of transferable embryos	0.89±0.14	0.85±0.34	0.0 n.s
Number of morula	0.73±0.20	0.57±0.20	0.52 n.s
Number of blastocyst	0.45±0.21	0.71±0.18	1.04 n.s

Experiment (3): Effect of rbST administration on superovulatory response:

The results given in Table (3) cleared that the differences between PMSG alone and PMSG with rbST (500 mg) were non significant. Two buffaloes in this experiment (one from each group) did not show estrus signs. There were no significant differences between the two groups in the mean number of mature follicles (7.52±0.41 and 7.71±0.99, respectively) and *Corpora lutea* (4.34±0.34 and 3.85±0.59, respectively). Also, the percentage of ovulations between the two groups did not differ significantly, being 62.61±3.73 and 55.58±4.55 for the two groups, respectively. The mean number of recovered embryos were 1.14±0.26 and 1.0±0.21 for the two groups, respectively. There were non significant differences between the two groups in the mean number of transferable embryos (0.85±0.14 and 0.71±0.18, respectively). Non significant differences were found between the two groups in the mean number of morula (0.71±0.18 and 0.57±0.20, respectively) and blastocyst (0.42±0.2 and 0.42±0.20, respectively).

Table (3): T-test for differentiation between the effect of PMSG alone (group I) and PMSG with rbST (group II) (Mean±SE) on the ovarian response and embryo recovery of buffalo cows.

Parameter	Groups		T. value
	I (n = 8)	II (n = 8)	
Animals in estrus	7/8	7/8	-
Interval between PG and first signs of oestrus (hr)	33.41±2.24	34.28±1.71	1.37 n.s
Number of mature follicles	7.52±0.41	7.71±0.99	0.26 n.s
Number of *Corpora lutea*	4.24±0.34	3.85±0.59	0.42 n.s
Number of unovulated follicles	2.87±0.30	3.0±0.37	0.89 n.s
Ovulation percentage	62.61±3.71	55.58±4.55	1.02 n.s
Number of recovered embryos	1.23±0.26	1.0±0.21	0.42 n.s
Number of unfertilized ova	0.53±0.21	0.71±0.18	0.52 n.s
Number of transferable embryos	0.83±0.15	0.71±0.18	0.61 n.s
Number of morula	0.73±0.19	0.57±0.20	0.52 n.s
Number of blastocyst	0.40±0.21	0.42±0.20	0.0 n.s

n.s. non-significant (P > 0.05)

Experiment (4): Determination of pregnancy rate of recovered transferable embryo:

There were no difference in the pregnancy rate between 2500 and 3000 IU eCG doses. On the other hand, the pregnancy rate decreased to 16% by using a dose of 3500 IU eCG. In spite of injection of GnRH and rbST does not improve superovulatory response, it was clear that injection of rBST improve pregnancy rate to 50% (Table 4).

Table (4): Pregnancy rate after embryo transfer from embryo recovered by different doses of eCG ,GnRH plus eCG and rbST plus eCG.

Parameter	2500 I.U eCG	3000 I.U eCG	3500 I.U eCG	3000 I.U +GnRH	3000 I.U + rbST
No of Recipient	6	6	6	6	6
Pregnant animals	2	2	1	2	3
Pregnancy rate %	33	33	16	33	50

DISCUSSION

Despite considerable researchs during last two decades, few significant changes have occurred in the way of superovulation is induced in buffalo ***(Misra, 1993)*** and the objective of superovulation and embryo transfer is to obtain the greatest number of embryo eliciting high pregnancy potential ***(Bo et al., 1996)***.

After superovulatory treatment buffalo showed good and significant increase in follicular response in G III & IV and G V (5.57 $\pm$ 0.42, 7.42 $\pm$ 0.42and7.57 $\pm$ 0.4, respectively) compared to low follicular response in G I and G II (3.12 $\pm$ 0.46, 3.42 $\pm$ 0.22). Statistical difference in our study ($P < 0.05$) were found between groups of good and low follicular

response. However, the number of follicles > 0.8 cm in estrus day was inferior than results found in bovines ***(Boland et al., 1991, Shaw et al., 1995 and Bo et al., 1995)***. The lower number of follicles in superovulated buffalo may be related to the lower number of follicles recruited per follicular growth wave and as a consequence, the lower number of follicles in the ovaries ***(Danell, 1987, Le Van Ty et al., 1989 and Baruselli et al., 1997)***. It was observed that the average ovulation rate in most treated groups ranged from 60 to 70%. Which similar to the result in bovine by some authors ***(Desaulniers et al., 1995, Shaw et al., 1995 and Stock et al., 1996)***. This result suggests that superovulated buffalo present ovulation rate similar to bovines and confirms that low efficiency of transfer is probably not related to follicular response and to ovulation during superovulatory treatment.

Across all treated buffalo in this study buffaloes of G IV which received 3000 I.U PMSG at the mid-cycle possess highest number of *Corpora lutea* CL (4.14 $\pm$ 0.34), while buffaloes of G I which were treated with 1500 IU PMS developed lowest number of CL (2.37 $\pm$ 0.269). However, the overall mean number of CL in his experiment fails in the range 3-4 as obtained by ***Drost et al. (1988)*** and higher that recorded by ***Karaivanov (1986)***. The results of the present study revealed that there was no recorded embryo in group I and II. However, the number of recovered embryos among buffaloes which was noted in the present study was in accordance with the range of 0.5 to 1 embryo which was reported by ***Mahmoud et al. (1989)***. This was higher than 0.05 embryos which previously reported by ***Ismail et al. (1992)***, and lower than 2 embryos obtained by ***Schallenberger et al. (1990)***. The results of

the present study explained that increasing the superovulatory dose of PMSG from 1500 IU to 2000 IU, 2500 IU and 3000 IU PMSG was associated with a significant increase ($P < 0.01$) in the number of ovulations, number of recovered embryo and the number of transferable embryo. On the other hand, increasing the PMSG dose from 3000 IU to 3500 IU had adverse effect on these criteria. Similarly ***Saher (1989)*** noticed a decline in the ovulation rate of buffaloes from 4.5 CL on application of 3000 IU PMSG to 0.6 CL when the dose was increased to 4800 IU. In contrast to our findings, ***Hamam (1987)*** reported an increase in the superovulatory response with increase in PMSG dose form 3000 to 4800 IU.

In spite of the response of animals to superovulation, there was a very low embryo recovery rate in relation the number of ovulation. These rates are lower than that described in the literature for bovines ***(Boland et al., 1991 and Shaw et al., 1995).*** The conflicting data is due to reproductive tract is smaller ***(Vale et al ., 1990)***, high estrogen concentration in superovulated buffalo ***(Misra et al., 1998)*** and poor recovery of the flushing fluid due to adhering of the uterine wall to the tip of catheter which prevented the back flow of the fluid ***(Sharifuddin and Jainudeen, 1984).*** Moreover the recovery of transferable embryo per flush is highly variable and very low ***(Karaivanov, 1986 ,Misra et al., 1990, Madan et al., 1996)***. The reason for high variability in the number of transferable embryos, which has also been observed in cattle are not fully understood. Some authors have attributed this variability to the production of secondary follicles after ovulation of the first, due to long half life of eCG. These follicle secrete estradiol 17-β that for

exceeds preovulatory concentrations resulting in an abnormal progesterone: estradiol 17-β ratio in the follicular fluid ***(Callesen et al., 1990 and Dieleman et al., 1989)***.

Our results obtained from superovulation using PMSG with combination of GnRH injection on the day of insemination had no significant effect on the number of *Corpora lutea*, unovulated follicles, recovered embryos, unfertilized ova or transferable embryo. Our results are in agreement with the results of ***Ismail et al. (1993), Beg et al. (1996), and Singh and Madan (1999)*** who could not record any change in ovulation rate and embryo recovery when GnRH was administrated at estrus. The same results were obtained by ***Techakumphu et al. (2001)***. The present findings were contradictory with those of ***Misra et al. (1990) and Techakumphu et al. (2000)*** who reported a higher rate of ovarian response in superovulated buffaloes treated with GnRH at estrus than those which were not treated. In the present study timing of GnRH injection might be a reason for the non ovarian response to this hormone. The non responded animals might have been treated with GnRH during or just after the spontaneous LH surge. It has been demonstrated that the LH surge occurred at 36 h after treatment with PGF2α in buffaloes superovulated either with FSH or PMSG ***(Schallenberger et al., 1990)***. Based on these findings and the fact that GnRH was administered at 48 h after PGF2α injection in our experiment it would be expected that the LH surge might have occurred 12 h earlier than the time of GnRH was administred in animals of group II..

In concerning the results of the current work, administration of rbST in a dose of 500 mg did not significantly affect superovulatory response when given in combination with the dose of PMSG (3000 IU)

recommended from exp. 1. The present findings were in accordance with the findings of ***Songsasen et al. (1999)*** who found that there was no significant effect of rbST on the superovulation response in terms of number of *Corpora lutea*, follicles or total embryo recovered, although there was significant difference between rbST treated and untreated cows for the number of transferable embryos per collection (75 vs. 33%). On the other hand, ***Zicarelli et al. (1994)*** recorded that the treatment of Mediterranean buffaloes with rbST increased the mean number of recovered and transferable embryos. It has been shown that rbST treatment enhances the recruitment of small ovarian follicles rather than increasing the growth rate or reducing the rate of follicular atresia and/or insulin plasmatic level ***(Gong et al., 1993, Buratini et al., 2000 and Gong, 2002)***.

In the presented work, it was found that pregnancy rate after transfer of fresh good quality embryo to prepared recipient on day 5 or 6 transfered from embryo superovulated by eCG was 33% in G.III and G.V (2500-3000 IU eCG) and this rate is higher than that recorded by ***Kurup, (1988).*** On the other hand, this pregnancy rate is much lower than rate of 50 to 82% reported following non-surgical embryo transfer in cattle ***(Haselr,. 1992, Newcomb and Rawson, 1975 and Wright, 1981)***. The poor conception rate in buffalo has been attributed to the lack of overt signs of estrus, seasonality in breeding, difficulty in per rectum palpation of C.L, subclinical metritis and generally low fertility of the species due to the lack of selection of breeding stock ***(Jainudeen, 1989, Drost, 1991)*** or may be due to the embryo after transfer on 5.5 day probably perished or was degenerating by day 15 or 16 and was unable to send the signals for materials recognition of pregnancy..

Concerning injection of GnRH and rbST at time of insemination following superovulation by 3000 I.U eCG, our results revealed that GnRH has no influence on pregnancy rate, this may be due effect of GnRH mainly in follicular growth and maturation. On the other hand, rbST increase pregnancy rate to 50%, this explained that rbST was effective at insemination in lactating dairy cow ***(Moreria et al., 2001)***. Moreover, rbST affect both early embryonic development and recipient components to increase pregnancy rate following embryo transfer ***(Moreira et al., 2002)***.

In conclusion, the present study indicated that 3000 I.U eCG was the most suitable dose for superovulation in egyptian buffalo. In addition the using of 3000 I. eCG + 20 µg GnRH and 500 mg rbST does not improve superovulatory response. However, rbST improve pregnancy after embryo transfer. Further research is required to eliciting causes for low embryo yields and low transferable embryo in buffalo and low pregnancy rate after transfer.

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التبويض المتعدد باستخدام هرمون الجونادوتروبين ونقل الاجنة فى الجاموس

بسيونى هليل[1] ، السيد محمد فتوح[1] ، باهى حسين سرور[1] ،
سامى درويش[2] ، ياسر مصطفى الديب[1]

[1] كلية الطب البيطرى ــ جامعة كفرالشيخ

[2] محطة بحوث الانتاج الحيوانى بمحلة موسى ــ كفرالشيخ

أجريت هذه الدراسة لتقييم تأثير الجرعات المختلفة من هرمون الجونادوتروبين (المستخلص من مصل الافراس العشار) لاحداث التبويض المتعدد وتأثير حقن الهرومون الحاس للغدة المنسلية (GNRH) وايضا هرمون النمو البقرى المخلق (rbST) ودراسة معدل الحمل بعد نقل الاجنة بعد حقن الجرعات المختلفة من هرومون الجونادوتروبين فى الجاموس.

أجريت هذه الدراسة على 106 جاموسة (76 جاموسة لتجميع الاجنة و 30 جاموسة لاستقبال الاجنة). **التجربة الاولى: لدراسة تأثير الجرعات المختلفة من هرمون الجونادوتروبين** تم تقسيم 32 جاموسة الى 5 مجموعات بعد تزامن الشياع بحقن جرعتين من هرمون بروستاجلاندين ، كل المجموعات م حقنها بجرعات مختلفة من هرمون الجونادوتروبين ، I.U 3500, I.U. 3000, I.U. 2500, I.U. 2000, I.U. 1500 ، تجميع الاجنة فى اليوم الخامس او السادس. **التجربة الثانية: لدراسة تأثير حقن هرمون (GnRH)** تم حقن 8 جاموسات بـــ I.U. 3000 من هرمون الجونادوتروبين بمفرده وتم حقن 9 جاموسات بـــ I.U. 3000 من هرمون الجونادوتروبين بالاضافة الى 20 µg من هرمون (GnRH). **التجربة الثالثة: لدراسة تأثير حقن هرمون (rbST)** تم حقن 8 جاموسات بـــ I.U. 3000 من هرمون الجونادوتروبين بمفرده وتم حقن 8 جاموسات اخرى بـــ I.U. 3000 من هرمون الجونادوتروبين بالاضافة الى 500 مجم من هرمون (rbST). **التجربة الرابعة: لتحديد معدل الحمل بعد نقل الاجنة فى التجارب المختلفة:** نتائج هذه الدراسة اظهرت ان اقل استجابة للمبايض وتجميع الاجنة كان فى المجموعة المحقونة بـــ I.U 1500 من هرمون الجونادوتروبين وانا اقصى استجابة للمبايض وتجميع الاجنة كان فى المجموعة المحقونة بـــ I.U. 3000 من نفس الهرمون. الدراسة اوضحت انه لا يوجد فرق بين استجابة الجاموس لهرمون الجونادوتروبين بمفرده او استخدامه بالاضافى الى حقن 20 ميكروجرام من هرمون (GnRH) او 500 مجم من هرمون (rbST). ايضا الدراسة اوضحت انه لا يوجد فرق فى معدل الحمل للمجموعات المحقونة بـــ 2500 ، I.U 3000 من هرمون الجونادوتروبين وحقن هرمون (GnRH) (33%) ولكن معدل الحمل ازداد بنسبة 50% بعد حقن هرمون (rbST).