J. of Animal And Poultry Production, Vol. 1 (1): 23 - 32, 2010

EFFECT OF PMSG ADMINISTRATION IN RELATION TO FOLLICULAR DIAMETER ON SUPEROVULATORY RESPONSE AND EMBRYO QUALITY IN FRIESIAN COWS.

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ABSTRACT

A total of 20 Friesian cows (450-550 Kg LBW, 3.5-5.5 years old and 1-3 parities) were used as donor cows. Cows were divided into 4 groups (5 animals in each) according to time of PMSG injection. Before treatment, cows in all groups were injected with 2 ml $PGF_2\alpha$ (Estrumate) to start oestrous cycles. Cows in the 1st group (Control, n=5) were i.m. injected with a single dose of 2500 IU PMSG on day 10 of the oestrous cycle. However, in the 2nd, 3rd and 4th groups, cows were injected i.m. with a single dose of 2500 IU PMSG when diameter of the dominant follicle reach 5-7.5 mm (G2, n=5), >7.5-10 mm (G3, n=5) and >10 mm (G4, n=5). After 48 h of PMSG injection in each group, cows in all groups were injected with 2 ml Estrumate and artificially inseminated (AI) when excepted estrous. Flushing was conducted 7 days after AI to determine the ovulatory response**.** Ultrasonography device was used during treatment period to record the number of follicles and CL and diameter of the follicles for PMSG injection. Results showed that day of PMSG treatment averaged 7.2, 9.6 and 8.2 for cows in G2, G3 and G4, respectively. All cows in G1 and G3 (100%) produced CL *vs.* 60 and 80% of cows in G2 and G4, respectively. Response rate of embryo production was only detected in G1 and G3, being 60 and 80%, respectively. No embryos were recovered from cows in G2 and G4. Average number of CLs/cow was higher (P<0.05) in G1 and G3 (8.0 and 7.4) than in G2 and G4 (1.0 and 3.0), respectively. Cows in G1 and G3 showed the highest (P<0.05) total response (CLs and UOF), being 9.4 and 9.8/cow, respectively. Averages number of total and transferable embryos were higher (P<0.05) in G3 by about 42 and 44% than in G1. Recovery rate of total and transferable embryos were higher in G3 than in G1 (73.0 and 70.0% *vs.* 47.5 and 45.0%, respectively). Yield of embryos at morula and blastocyst stages was higher in G3 than in G1. Cows in G3 showed the highest distribution of embryos at morula stage (37.0%) versus the highest distribution of compact morula in G1 (42.1%). Cows in G3 increased yield of excellent embryos/cow (2.2 *vs.* 1.0) and good embryos/cow (3.0 *vs.* 1.8) as compared to those in G1. Excellent and good embryos represented 40.7 and 55.6% in G3, being higher than 26.3 and 47.4% in G1. The differences in production of embryos at different stages or grades were not significant.

In conclusion, the obtained results, indicated that the potentiality of PMSG injection to induce high superovulatory response in cattle is highly related to follicular diameter . Under the experimental conditions of present study, appropriate time for SO in Friesian cows treated with 2500 IU of PMSG was when diameter of the dominant follicle reached a rang from ≥7.5-10 mm to reflect the highest ovulatory response and the best number of transferable embryos of excellent grade.

Keywords: Cows, follicular diameter, PMSG, SO, embryo**.**

INTRODUCTION

Superovulation (SO) is an efficient technique for obtaining progeny from genetically valuable females. The ovarian response of each female depends on the number of gonadotropin-sensitive follicles present at the time that treatment is initiated (Adams, 1994). Identification of the number of such follicles in each female would improve the efficacy of SO, by allocating potential non-responders to groups where other techniques are used for SO. One of the main factors influencing response to SO is stage of the follicular wave when gonadotropin treatments are given (Driancourt, 2001).

Variability in superovulatory response (SOR) after gonadotropin treatments continues to be the greatest problem for commercial embryo transfer (Mapletoft *et al*., 2002 and Barros and Nogueira, 2004). The conventional protocol of initiating ovarian superstimulation during mid-cycle was originally based on anecdotal and experimental information in which a greater SOR was reported when gonadotropin treatments were initiated 8-12 days after oestrus (Bò *et al*., 1995). However, early experiments and field studies concluded that superovulatory treatments initiated 9–10 days after oestrous detection resulted in a greater SOR as compared with those initiated 2–6 or 12–13 days after oestrous detection (Lindsell *et al*., 1986). None of these early studies utilized ultrasonography to evaluate follicular status of the animals when superstimulation treatments were initiated. It is now known that 8-12 days after estrus (Days 7-11 after ovulation) would be the approximate time of emergence of the second follicular wave in cattle. However, the day of emergence of the second follicular wave differs among individuals and is 1 or 2 days later in 2-wave cycles, as compared to 3-wave cycles (Ginther *et al*., 1989).

Several studies have demonstrated the importance of initiating gonadotropin treatments at the time of follicular wave emergence. The absence of a dominant follicle at the beginning of treatment increased the efficacy of the super-stimulatory treatments (Adams, 1994). This approach presents difficulties because it requires oestrus detection prior to initiation of treatment, and because there is a great individual variation in the day of emergence of the 2nd follicular wave of cows (Mapletoft *et al*., 2002). A significant individual variation in the number of follicles >8 mm in diameter at the time of oestrus and in the number of CL at the time of ova/embryo collection were reported by Martins *et al*. (2005).

The aim of this study was to determine the effect of administration of PMSG at mid-luteal stage of the oestrous cycle, before the selection of the dominant follicle, based on follicular diameter on the superovulatory response obtained compared with that of treatment at mid- or late-luteal stages of the oestrous cycle.

MATERIALS AND METHODS

This study was conducted at Animal Production Department, Faculty of Agriculture, Mansoura University in co-operation with Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture. The experimental work was carried out at Animal Production

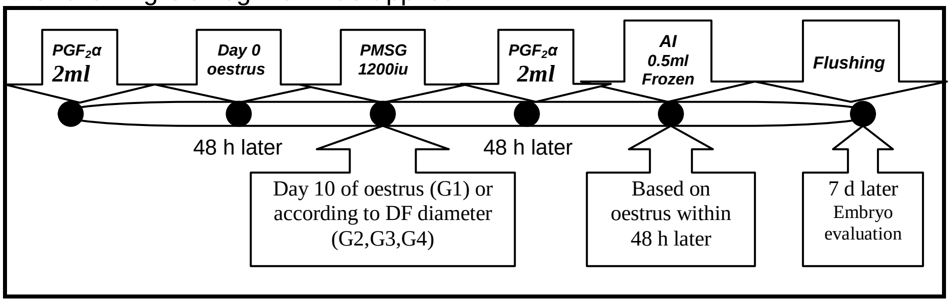
Research Stations, Sakha and International Livestock Management Training Center, Sakha (ILMTC), belonging to APRI and located in the north eastern part of the Nile Delta, Kafer El Sheikh Governorate during the period from January to September 2009.

Animals:

A total of 20 Friesian cows having LBW of 450-550 kg, 3.5-5.5 years old of age and 1-3 parities were used as donor cows after showing at least 1-2 post-partum oestrous cycles prior to SO treatment. Cows were divided into 4 experimental groups (5 animals in each) according to time of PMSG injection. Cows in all experimental groups were subjected to the same managerial and feeding conditions**.**

Superovulation (SO) regimen:

Before treatment, cows in all experimental groups were injected with 2 ml $PGF_2\alpha$ (Estrumate, containing 263 µg of cloprostenol sodium BP, Vet., equivalent to 250 µg of cloprostenol, Friesoythe, Germany) to bring them on heat to start oestrous cycles. Based on the beginning of the oestrous cycle, the following SO regimen was applied:



Based on the beginning of the oestrous cycle, cows in the first group (Control, n=5) were injected intramuscularly with a single dose of 2500 IU PMSG (Folligon, Holland, containing 1000 IU PMSG/ml) on day 10 of the oestrous cycle. However, in the 2^{nd}, 3^{rd} and 4^{th} treatment groups, cows were injected i.m. with a single dose of 2500 IU PMSG when diameter of the dominant follicle reach 5-7.5 mm (G2, n=5), >7.5-10 mm (G3, n=5) and >10 mm (G4, n=5).

Cows in all experimental groups were daily subjected to ultrasonography device during treatment period starting from day 0 of oestrus (heat) after PGF2α (Estrumate) injection to record the diameter of the ovarian follicles at the time of PMSG injection and number of CLs and unovulated follicles on day of flushing.

After 48 hours of PMSG injection in each group, cows in all groups were given an injection of 2 ml Estrumate (500 µg of cloprostenol) to induce luteal regression. Cows were kept under observation for heat detection; thereafter all cows treated with Estrumate were artificially inseminated (AI) 48 h later three times with frozen semen of the same bull at 12 hours intervals following the detection of standing heat.

Flushing was conducted 7 days after AI to determine the ovulatory response to SO by ultrasonography examination of the ovaries in term of counting the number of corpora lutea (CLs) as well as number and diameter of the visual unovulated follicles presented on the ovarian surface.

Embryos were recovered non-surgically 7 days after day of artificial insemination. Technique of non-surgical flushing was followed using the continuous-flow or the closed system. It was done according to the method described for cattle by Newcomb *et al*. (1978a). One percent of fetal or oestrus cow serum was added to the Dulbecco's phosphate buffer saline (PBS) for flushing. Number of recovered embryos in morula and blastocyst stages, and transferable embryos as well as unfertilized ova and degenerated embryos were recorded. Embryos were also evaluated morphologically according to Takeda (1986) into excellent, good, fair and poor on basis of their morphological symmetry, stage of blastomeres and age of embryo in relation to stage of the donor oestrous cycle as well as the presence of vesicles and color of embryo. Number of transferable embryos was calculated as number of embryos at morula, compact morula and blastocyst stages only on excellent and good grades.

Statistical analysis:

The effects of SO treatment on differences among the total number of ova/embryos obtained, number of unfertilized ova, number of degenerate embryos and number of transferable embryos were analyzed by the general linear models procedure of SAS (1989). The significance level of differences among groups was tested using Duncan Multiple Range Test (Duncan 1955) at $P<0.05$.

RESULTS AND DISCUSION

Response rate of superovulated cows:

Results in Table (1) show that day from PMSG treatment to the oestrus was significantly ($P<0.05$) earlier for cows in G2 than those in G1 (day 7.2 *vs.* day 10.0), but did not differ significantly in G3 and G4 (day 9.6 and day 8.2, respectively) from that in G1. The early time of PMSG treatment of cows in G2 as compared to those in G3 and G4 was expected, where cows were injected based on smaller follicular diameter in G2 than in G3 and G4. However, the observed insignificant early time of treatment in G4 than in G3 was associated with wider range of treatment time in G4 than in G3 (day 6-12 *vs.* day 8-10).

The noticeable variability in treatment time based on follicular diameter may be attributed to type of follicular waves in each cow. In both two- and three-wave oestrous cycles, emergence of the 1^{st} follicular wave occurs consistently on the day of ovulation (day 0). Emergence of the 2^{nd} wave occurs on day 9 or 10 for two-wave cycles, and on day 8 or 9 for three-wave cycles. In three-wave cycles, a third wave emerges on day 15 or 16 (Mapletoft *et al.,* 2002).In this respect, starting SO in cattle beginning 8 to 12 days after oestrus (Hahn, 1992) or about 9–12 days after detection of oestrus (Mapletoft and Pierson, 1993).

It is of interest to note that all treated cows exhibited oestrous activity within 48 h of $PGF_2\alpha$ injection. However, variable responses to treatment in term of CL and embryo production were observed among experimental groups. All cows in G1 and G3 (100%) produced CL versus 60 and 80% of cows in G2 and G4, respectively.

Table (1): Response rate (%) of corpus luteum (CL) and embryo production in different experimental groups.

Item	Experimental group			
	G1	G2	G3	G4
Treated cows (n)	5	5	5	5
Day of PMSG injection (mean)	10±0.00	7.2±0.96	9.6±0.67	8.2±0.80
Day of PMSG injection (range)	-	5-10	8-10	6-12
Oestrus rate (%)	100	100	100	100
Cows produced CL (n)	5	3	5	4
Response rate of CL (%)	100	60	100	80
Cows produced embryos (n)	3	0.0	4	0.0
Response rate of embryos (%)	60	0.0	80	0.0

However, response rate of embryo production was only detected in G1 and G3, being 60 and 80%, respectively. However, no embryos were recovered from cows in G2 and G4.

In agreement with the present results, Mahmood *et al*. (1989) found that i.m. injection of cows with 3000 IU PMSG on day 9, 11 or 14 of the oestrous cycle and 500 µg $PGF_2α$ 48 h later showed marked differences in response to treatment although all treated cows were in heat within 36-48 h after PG injection. The superovulatory response was highest in animals treated on day 12 and lowest in these treated on day 14 of the oestrous cycle. No embryos were recovered from cows treated on day 9 or 14 and only one embryo was recovered from each of two animals treated on day 12 of the oestrous cycle.

Superovulatory response and embryo recovery rate:

Table (2) show that superovulatory response in term of average number of CLs was significantly (P<0.05) higher in G1 and G3 (8.0 and 7.4/cow) than in G2 and G4 (1.0 and 3.0/cow), respectively. However, the difference in average number of unovulated follicles (UOF)/cow was significant (P<0.05) only between G1 and G4. Such trend reflected significantly (P<0.05) the highest total response (CLs and UOF) in G1 and G3 (9.4 and 9.8/cow), followed by G4 (6.6/cow) and the lowest in G2 (3.6/cow), respectively

Nearly similar results were obtained by Holy (1987), who found that number of CL averaged 8.8 and 8.0/cow for cows injected with PMSG (2000-3000 IU) or PMSG and anti-gonadotropin, respectively. However, greater number of CLs (11.6/cow) was recorded by Slimane and Ouali (1991) for French Friesian (FF), FF x Holstein or FF x Charolais cows superovulated with 2500 IU PMSG. Zeitoun *et al.* (1991) found that the number of CL tended to be higher at 3000 IU PMSG (23.0) than 1500 IU (14.1) in Hereford cows. Moreover, Saumande and Chupin (1986) reported that the mean number of ovulations and follicles in group of crossbred heifers superovulated by 2500 IU PMSG was 13.3±12.6 and 1.3±1.8, respectively.

On the other hand, Misra *et al*. (1994) found that treatment with PMSG (3000 IU) resulted in lower number of ovulations (3.76/cow). Arora *et al*. (1996) found that mean number of CLs was 6.6±1.2/cow for lactating

Jersey x red Sindhi cows induced with PMSG (2000 IU) on day 11 of the oestrous cycle.

Table (2): Ovarian response and embryos recovered from superovulated cows in different experimental groups. (mean ± SE)

Item	Experimental group			
	G1	G2	G3	G4
Average number of ovarian structure/cow at flushing:				
Number of CLs/cow	8.0±0.49 [a]	1.0±0.27[b]	7.4±0.83[a]	3.0±0.40[b]
Unovulated follicles/cow	1.4±0.42[b]	2.6±0.45[ab]	2.2.4±0.51[a]	3.6±0.51[a]
Total ovulatory response[(1)]	9.4±0.84[a]	3.6±0.35[c]	9.8±0.61[a]	6.6±0.47[b]
Average number of recovered embryos/cow:				
Total embryos[(2)]	3.8±0.59	-	5.4±1.50	-
Transferable embryos	3.6±1.24	-	5.2±2.13	-
Recovery rate (%):				
Total embryos	47.5±7.5	-	73.0±6.3	-
Transferable embryos	45.0±3.7	-	70.0±4.1	-
Transferable/total embryos (%)	95.0±1.5	-	96.3±1.8	-

[a, b and c]: Means denoted within the same row with different superscripts are significantly different at P<0.05.
[(1)]: Total response to SO = Number of CL+NOF.
[(2)]: Number of total embryos (normal and degenerated) and unfertilized ova.

Generally, PMSG treatment of cows with medium follicular diameter from 5 to 7.5 mm on day 9.6 of oestrous cycle in G3 showed the highest total SOR as found in G1 treated with PMSG on day 10 of oestrous cycle. However, PMSG treatment of cows with shorter (G2) or larger diameter (G4) significantly (P<0.05) showed the lowest total SOR (Table 2).

It is worthy noting that only cows superovulated in G1 and G3 produced embryos, however, non embryos were recovered from those in G2 and G4. In comparing embryo production of G1 and G3, results revealed that averages number of total and transferable embryos were greater in G3 by about 42 and 44% than in G1. These findings are associated with higher recovery rate of total and transferable embryos in G3 than in G1 (73.0 and 70.0% *vs.* 47.5 and 45.0%), respectively. However, percentage of transferable embryos relative to total embryos was nearly similar in G1 and G3, being 95.0 and 96.3%, respectively (Table 2).

Primary factors limiting embryo production in cattle are the variability of the ovarian response following induction of SO with commercially available gonadotrophin preparation and the competence of the oocytes ovulated. Recent result obtained by (Ganah *et al*., 2009) clearly indicated that at the dosage administrated, treatment with 2500 IU of PMSG on day 10 of oestrous cycle resulted in significantly (P<0.05) acceptable ovulatory response in term of number of CLs (11.67/cow), unovulated (1.4/cow) and total follicles (13.0/cow).

In cows superovulated by different PMSG levels, number of recovered embryos was 6.0, representing 51.8% as a recovery rate (Silmane and Ouali (1991) and the number of transferable embryos was 1.0/cow. Misra *et al*. (1994) found that number of total embryos recovered (1.6/cow) and number of viable embryos per flush (0.56 embryos/cow). In Holstein cows, Basile *et al*. (1994) found that the number of embryos was 4.65/cow and the percentage of viable embryos was 55.3%. Mohammed and Ismail (1999) recovered 8.6 embryos ova/cow, 7.0 fertilized ova/cow and 6.0 transferable embryos/cow. Kasira *et al*. (2000) using FSH or equal chronionic gonadotropin (eCG) found that total and viable embryo recovered rate were 5.5 and 1.70/cow, respectively.

Embryo production:

Stages of recovered embryos:

Results in Table (3) regarding embryo stage show that cows in G3 yielded more embryos at morula and blastocyst stages in term of total number per group or average number per cow than those in G1. However, yield of embryos at 4-cell and compact morula stages was nearly similar in both groups.

Concerning the frequency distribution of embryos recovered at different stages, cows in G3 showed the highest distribution of embryos at morula stage (37.0%) versus the highest distribution of compact morula in G1 (42.1%). On the other hand, the frequency distribution of embryos at 4-cell and blastocyst stages was nearly similar in both groups (Table 3).

Embryos recovered from the superovulated cow often display a wide range of development cell stage. The highest proportion of morula and compact morula was recovered 5 to 6 days after oestrus. Early blastocyst and blastocyst were prevalent on day 7, as expanded blastocysts were on day 8 and 9 (Shea, 1981 and Lindner and Wrigh, 1983).

Table (3): Number and frequency distribution of embryos recovered from superovulated cows in experimental groups at different stages.

Embryo stage	Experimental group G1	G3	Significance
4-cell:			
Total number/group	1	1	-
Number/cow	0.2±0.2	0.2±0.2	-
Frequency distribution (%)	5.3	3.7	-
Morula:			
Total number/group	6	10	-
Number/cow	1.2±0.96	2.0±1.3	NS
Frequency distribution (%)	31.6	37.0	-
Compact morula:			
Total number/group	8	9	-
Number/cow	1.6±1.02	1.8±1.10	NS
Frequency distribution (%)	42.1	33.3	-
Blastocyst:			
Total number/group	4	7	-
Number/cow	0.8±0.37	1.4±0.60	NS
Frequency distribution (%)	21.0	25.9	-

NS: Not significant.

Grades of recovered embryos:

Results in Table (4) show that cows in G3 produced higher number of excellent embryos (grade I: 2.2 *vs.* 1.0/cow) and good embryos (grad II, 3.0 *vs.* 1.8/cow) as compared to those in G1. Although cows in G3 produced

0.2 embryo/cow in grade III (fair), cows in G1 produced 1.0 embryo/cow in grade III (fair).

With regard to the frequency distribution of embryos recovered at different grades, cows in G3 yielded 40.7 and 55.6% of excellent and good embryos, being higher than 26.3 and 47.4% in G1 (Table 4).

Table (4): Number and frequency distribution of embryos recovered from superovulated cows in experimental groups at different grades.

Embryo grade	Experimental group G1	Experimental group G3	Significance
I (Excellent):			
Total number/group	5	11	-
Number/cow	1.0±0.45	2.2±1.36	NS
Frequency distribution (%)	26.3	40.7	-
II (Good):			
Total number/group	9	15	-
Number/cow	1.8±0.73	3.0±1.22	NS
Frequency distribution (%)	47.4	55.6	-
III (Fair):			
Total number/group	5	1	-
Number/cow	1.0±0.45	0.2±0.0	-
Frequency distribution (%)	26.3	3.70	-

NS: Not significant.

Ravindranatha *et al.* (2001) superovulated Holstein cows using Follitropi on day-10 of estrous cycle, the embryos were collected non-surgically. They found that the mean embryo recovery was 5.14±0.086 and the mean yield of excellent, good, fair, and poor quality embryos recorded were 1.21±0,28, 1.0±0.18, 1.5±1.31, and 1.42±0.37/donor, respectively.

In conclusion, the obtained results, indicated that the potentiality of PMSG injection to induce high superovulatory response in cattle is highly related to follicular diameter . Under the experimental conditions of present study, appropriate time for SO in Friesian cows treated with 2500 IU of PMSG was when diameter of the dominant follicle reached a rang from ≥7.5-10 mm to reflect the highest ovulatory response and the best number of transferable embryos of excellent grade.

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تاثير الحقن بهرمون سيرم الفرس الحامل (PMSG) بناء على قطر الحويصلات على الاستجابة للتبويض المتعدد وجودة الأجنه لأبقار الفريزيان

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اجريت هذه الدراسة على 20 بقرة فريزيان تتراوح اوزانها بين (450-550 كجم) واعمارها بين (3.5-5.5 سنة) وفى الموسم (1-3) . قسمت الابقار الى 4 مجاميع (5 حيوانات فى كل مجموعة) حسب توقيت الحقن بهرمون الـ PMSG. قبل بدء المعامله تم حقن جميع الحيوانات فى كل المجاميع بـ 2سم من هرمون البروستاجلاندين (PGF2α) لبدء دورة الشياع. الابقار فى المجموعة الاولى (الكنترول, 5 حيوانات) تم حقنهم عضليا بجرعة قدرها 2500 وحدة دولية من هرمون الـ PMSG فى اليوم العاشر من دورة الشياع. بينما فى المجاميع الثانية والثالثة والرابعة بنفس الجرعة من الهرمون لكن عندما يصل قطر الحويصلة السائدة الى (5-7.5مم) وذلك بالنسبة للمجموعة الثانية , وعندما يصل قطر الحويصلة السائدة الى (اكبر من 7.5-10مم) بالنسبة للمجموعة الثالثة, اما المجموعة الرابعة يتم الحقن بهرمون الـ PMSG عندما يصل قطر الحويصلة السائدة الى (اكبر من 10مم). فى الاربعة مجموعات بعد 48 ساعة من الحقن بهرمون الـ PMSG يتم حقن جميع الحيوانات بـ 2سم من هرمون البروستاجلاندين PGF2α بعد 48 ساعة يتم التلقيح الصناعى لجميع الحيوانات. يتم جمع الاجنة غير جراحيا بعد 7 ايام من التلقيح لتقييم الاستجابة المبيضية للمعامله. تم استخدام جهاز التشخيص بالموجات فوق الصوتية (السونار) اثناء فترةالمعامله لتسجيل عدد الحويصلات والاجسام الصفراء وقطر الحويصلات وذلك لتحديد توقيت الحقن بـهرمون الـ PMSG. اوضحت النتائج ان توقيت الحقن بالـ PMSG يتراوح فى المتوسط بين الايام 7.2 و 9.6 و8.2 بالنسبة للمجاميع الثانية والثالثة والرابعة على التوالى. كل الابقار فى المجموعتين الاولى والثالثة اظهرت اجسام صفراء على مبايضها بنسبة (100%), بينما اظهرت المجموعتين الثانية والرابعة اجسام صفراء بنسب (60 و80%) على التوالى. تم الحصول على اجنة (معدل الاستجابة) من المجموعتين الاولى والثالثة بنسب 60% للمجموعة الاولى و 80% للمجموعة الثالثة, ولم يتم الحصول على اجنة من اى من المجموعتين الثانية والرابعة. كان متوسط عدد الاجسام الصفراء مرتفع معنويا فى المجموعتين الاولى والثالثة (8.0 و 7.4 على التوالى) مقارنة بالمجموعتين الثانية والرابعة (1 و 3 على التوالى) اظهرت الابقار فى المجموعتين الاولى والثالثة معنوية عالية بالنسبة للاستجابة الكلية (الاجسام الصفراء والحويصلات التى لم يحدث لها تبويض) حيث كانت 9.4 و 9.8 لكل بقرة على التوالى. كان متوسط اعداد الاجنة الكلية و الاجنة القابلة للنقل عالية المعنوية فى المجموعة الثالثة بمقدار 42 و 44% فى المجموعة الاولى. العدد الكلى للاجنة و عدد الاجنة القابلة للنقل المتحصل عليهم مرتفع فى المجموعة الثالثة عنها فى المجموعة الاولى (73 و 70 % مقابل 47.5 و 45% على التوالى). كان الناتج المتحصل عليه من الاجنة فى مرحلتى الموريولا (Morula) و البلاستوسيت (Blastocyst) مرتفعا فى المجموعة الثالثة عن المجموعة الاولى. اظهرت الابقار فى المجموعة الثالثة اكبر توزيع من الاجنة فى مرحلة الموريولا (Morula) (37%), مقابل توزيع عالى من الاجنة فى مرحلة الـ Compact morula فى المجموعة الاولى (42.1%). اعطت ابقار المجموعة الثالثة عددا كبيرا من الاجنة ذات الجودة الممتازة لكل بقرة (Excellent) (2.2مقابل 1) و الاجنة الجيدة لكل بقرة (Good) (3 مقابل 1.8) بالمقارنة بالمجموعة الاولى. كانت نسبة الاجنة الممتازة والجيدة (Excellent and Good) 40.7 و 55.6% من الاجنة فى المجموعة الثالثة وذلك كان أعلى مما فى المجموعة الاولى (26.3 و 47.4%). كانت الاختلافات فى ناتج الاجنة فى مختلف المراحل والرتب غير معنوية.من النتائج المتحصل عليها تبين امكانية معرفة توقيت الحقن بهرمون الـ PMSG للحصول على أعلى استجابة لاحداث التبويض المتعدد فى الماشية. تحت الظروف التجريبية للدراسة الحالية, وجد أن أنسب توقيت لبدء المعاملة بتنشيط التبويض باستخدام 2500 وحدة دولية من هرمون الـ PMSG هو عند وصول قطر الحويصلة السائدة ما بين (≤7.5-10مم) للحصول على أعلى استجابة للتبويض وأعلى عدد من الأجنة القابلة للزرع بجودة ممتازة (Excellent grade).

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