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Superovulatory Response of Egyptian Buffaloes Treated with A Single Dose of Pmsg as Affected by Diameter of The Dominant Follicle

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ABSTRACT

The present work was conducted to evaluate the efficacy of two superovulatory regimens to determine the diameter of follicle using ultasonography to monitoring the ovarian activity for superovulation in 21 buffalo cows, giving PMSG and PGF2α. Superovulation regime was done by injection PMSG (3000 IU) when follicular diameter of the dominant follicles reach 5-7 mm (G1, n=7), >7-9 mm (G2, n=7,) and >9 mm (G3, n=7). Buffaloes in all groups were injected with 2 ml $PGF_2\alpha$ and naturally inseminated depend on detection of estrous within 48-72 h of PGF_2 injection. Flushing was conducted 7 days after insemination 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 the duration from Day 0 (estrus) up to PMSG injection was earlier ($P<0.05$) in G1 than in G2 and G3 (day 7.04 vs. days 9.2 and 9.8, respectively), but did not differ significantly in G2 and G3. The observed insignificant early time of treatment in G3 than in G2 was associated with wider range of treatment time in G3 than in G2 (day 8-10 vs. day 8-12). All buffalo cows in all groups came in estrus showing an estrus rate of 100% in each group. All buffaloes in G2 (100%) produced CL vs. 71.4 and 85.7% of buffaloes in G1 and G3, respectively. No embryos were produced from animals in G1, although 71.4% of animals in this group produced ovulation sites. Response rate of embryo production doubled in G2 as compared to G3 (57.1 vs. 28.6%). Buffalo cows in G2 showed insignificantly the highest total ovulatory response as compared to G1 and G3 (2.71 vs. 2.14 and 2.29, respectively). Number of unovulated follicles was insignificantly lower, while number of CLs per animal (total or responded animals) was higher ($P<0.05$) in G2 than in G1 and G3.Animals in G2 showed the highest ($P<0.05$) average number of CLs per responded animal, while number of embryos per responded animals or animals produced embryos was insignificantly the highest in G2 as compared to other groups. Buffalo cows in G2 produced embryos at morula (2 embryos/group) and blastocyst (2 embryos/group) stages, while those in G3 yielded embryos only at compact morula (2 embryos /group) stage. In comparing embryo production of G2 and G3, results revealed that averages number of total and transferable embryos were greater in G2 by about 50% than in G3. These findings are associated with higher recovery rate of total embryos in G2 than in G3, respectively. The obtained results indicated that the potentiality of PMSG injection to induce high superovulatory response in buffaloes is highly related to follicular diameter. Under the experimental conditions of the present study, appropriate time for superovulation in buffalo cows treated with 3000 IU of PMSG was when diameter of the dominant follicle reached a rang between ≤7 and 9 mm to reflect the highest ovulatory response.

Keywords: *Buffaloes, PMSG, superovulation, follicular diameter, embryo*.

INTRODUCTION

Buffalo is an essential cattle aid in numerous nations of South Asia and the Mediterranean regions. The sector population of buffalo is estimated to be 195 million (FAO, 2013). Application of various biotechnology tools like artificial insemination (AI), estrus synchronization for timed AI, more than one ovulation (multiple- or super-ovulation) and embryo transfer and current breeding strategies can be of wonderful use for faster multiplication and propagation of animal species in close to destiny (Mondal *et al*., 2014).

The first successful embryo transfer in buffalo was executed inside the USA (Drost *et al*., 1983). This method contains a series of cautiously included sequential steps such as donor choice, donor remedy, recipient choice, insemination of the donor, embryo recovery, embryo handling and assessment, embryo switch, and recipient care (Warriach *et al*., 2015). In buffaloes, the utility of multiple ovulation and embryo transfer (MOET) is constrained due to variable superovulatory reaction and low yield of more than one transferable embryo (Drost, 2007; Baruselli *et al*., 2013).

Embryo transfer technique has been widely used worldwide, whereas it can increase the number of offspring that can be obtained from supergenetic females (Kandil, *et al*. 2012). Conventional superstimulatory protocols have been used in buffaloes involving the initiation of superstimulatory treatment during mid-cycle (8-12th day) (Narinder Singh, *et al*., 2015). Some studies used protocols for synchronization based on progesterone and gonadotropin releasing hormone (GnRH) administration together with prostaglandin to induce luteolysis during season have yielded quite promising conception rates ranging from 30 to 50% (Warriach *et al*., 2015).

In early reports, buffalo donors generally have a low number of recovered embryos in comparing with bovines as described by several authors (Karainov, 1986; Madan, 1990; Drost, 1996; Zicarelli, 1997). Several authors reported recovery rate of buffalo embryos at a range from 20 to 40%, being lower than in bovine (63 to 80%) as reported by Boland *et al*. (1991), Adams (1994), Vos *et al*. (1994) and Shaw *et al*. (1995). In buffaloes after superovulation treatment, low ovulatory response was recorded in terms of 15 follicles (>8 mm), 60% ovulation rate, 9 CL yield on the time of flushing and 34.8% recovery rate of embryos (Baruselli *et al*., 2000). In bovine, Abdel-Khalek *et al*. (2010) indicated that the potentiality of PMSG injection to induce high superovulatory response in cattle is highly related to follicular diameter. The highest ovulatory response and the best number of transferable and excellent embryos was when Friesian cows were treated with PMSG at a level of 2500 IU/h in presence of dominant follicle of ≤7.5-10 mm. A wide individual variation in the number of follicles >5 mm in diameter on the time of oestrus and in CL number at flushing had reported by Martins *et al*. (2005). Therefore the present

study aimed to evaluate the ovulatory response and embryo production of Egyptian buffaloes treated with single dose of 3000 IU PMSG/h when diameter of the dominant follicle of the estrous cycle was 5-7, >7-9 or >9 mm in diameter.

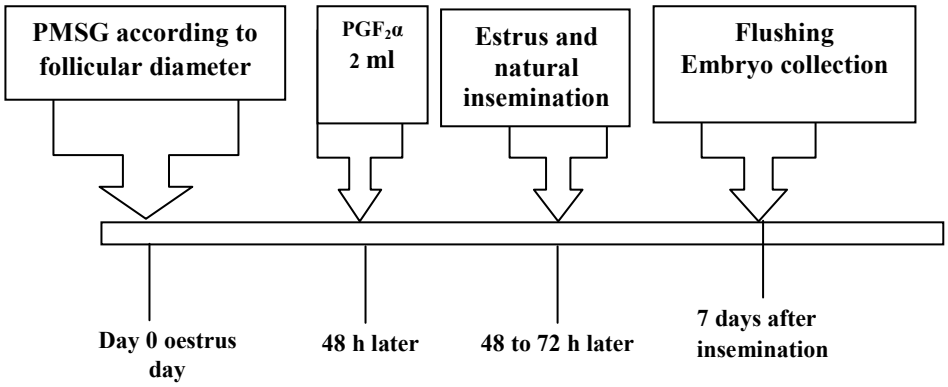
MATERIALS AND METHODS

This study was conducted at buffalo herd selected from Mehalt Mousa Experimental Station, belonging to Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt, during the period from August 2013 to October 2014.

A total of 21 buffalo cows having 450-600 kg live body weight and 5-9 years of age within 90-110 days postpartum were used in this study. Feeding system, watering and managerial conditions were similar for animals in all groups.

At the beginning of the experiment (Day of estrus), animals in all experimental groups were daily subjected to ultrasonography device during treatment period starting from day 0 of estrus (heat) to record number and diameter of the ovarian follicles (dominant follicle). Then, three experimental groups (7 buffaloes in each group) were divided according to diameter of the dominant follicles during the estrous cycle and treated with a single dose of 3 ml PMSG (Folligon, Holland) containing 1000 IU PMSG/ml. Transrectal ultrasonography was carried out using ultrasound scanner supplied with 7 MHZ array transducer (Model DP-30 Vet 50/60HZ SHENZHEN MINDRAY BIO-MEDICAL ELECTRONICS). After 48 h of PMSG treatment in each experimental group, all animals were i.m. injected with 2 ml PGF2α (Estrumate, containing 263 µg of cloprostenol sodium BP, Vet., equivalent to 250 µg of cloprostenol, Friesoythe, Germany) to induce estrus within 48-72 h post- PGF2α. Thereafter, animals in heat were naturally inseminated with fertile buffalo bull and embryos were collected by flushing 7 days later.

Based on the beginning of the estrous, buffalo cows in each group were injected i.m. with a single dose of 3000 IU PMSG when diameter of the dominant follicle reach 5-7 mm in the 1st group (G1, n=7), >7-9 mm in the 2nd group (G2, n=7) and >9 mm in the 3rd group (G3, n=7) as illustrated in the following diagram:



Buffalo cows in all experimental groups were daily subjected to ultrasonography device during treatment period starting from day 0 of estrus (heat) after $PGF_2\alpha$ (Estrumate) injection to record number and diameter of the ovarian follicles at the time of PMSG injection. After 48 hours of PMSG injection buffalo cows in all groups were given an injection of 2.5 ml Estrumate to induce luteal regression. Buffaloes were kept under observation for heat detection till came in heat within 48-72 h of Estrumate injection. All buffalo cows in heat were naturally mated more than once time by a fertile buffalo bull.

Flushing was conducted 7 days after insemination to determine the ovulatory response to each superovulation protocol 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 to demonstrate the response to superovulatory response according to Baruselli *et al*. (1998). Embryo recovery was non-surgically performed on day 7 Newcomb *et al*., (1978). It was conducted by using sterile two-way Foley catheter (size 22). The flushing media was phosphate buffer saline (PBS) containing 1% Bovine serum albumin (BSA). Number and stage (morula, compact morula and blastocyst stages) of recovered embryos were recorded.

Data were statistically analyzes by the General Linear Model procedure of SAS (2004). Differences among group means were set at $P<0.05$ using Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSION

Response rate of superovulated buffalo cows:

According to diameter of the dominant follicles at the beginning of the treatment (Day 0), results in Table (1) revealed that the duration from Day 0 (estrus) up to PMSG injection was significantly ($P<0.05$) earlier for buffalo cows in G1 than those in G2 and G3 (day 7.04 vs. days 9.2 and 9.8, respectively), but did not differ significantly in G2 and G3. The early time of PMSG treatment of buffalo cows in G1 as compared to those in G2 and G3 was expected, where buffalo cows were injected based on smaller follicular diameter in G1 than in G2 and G3. However, the observed insignificant early time of treatment in G3 than in G2 was associated with wider range of treatment time in G3 than in G2 (day 8-10 vs. day 8-12).

The noticeable variability in treatment time based on follicular diameter may be attributed to type of follicular waves in each buffalo cow. There is no difference between second and third wave cycles with regard to the day of emergence of the first wave in buffalo. Since the day of onset of the second wave ranged widely between buffaloes, it is difficult to set up a superovulation schedule (Baruselli *et al*., 1997). Emergence of the 2nd 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).

Table (1): Day of PMSG treatment and rate (%) of animals responded to estrus and corpus luteum (CL) formation on day of flushing in different experimental groups.

Item	Experimental group G1 (DF of 5-7 mm)	G2 (DF of > 7-9 mm)	G3 (DF of > 9 mm)
Number of treated buffaloes (n)	7	7	7
Day of PMSG injection (mean)	7.04±0.68[b]	9.2±0.23[a]	9.8±0.92[a]
Day of PMSG injection (range)	6-9	8-10	8-12
Buffaloes in estrus post-$PGF_2\alpha$ (n)	7	7	7
Estrus rate post-$PGF_2\alpha$ (%)	100	100	100
Buffaloes produced CLs at flushing (n)	5	7	6
Response rate of CLs (%)	71.4	100	85.7
Buffaloes produced embryos (n)	0	4	2
Response rate of embryos (%)	0	57.1	28.6

[a and b] **Means denoted within the same row with different superscripts are significantly different at P<0.05.**

Results in Table (1) also showed that all buffalo cows in all groups came in estrus showing an estrus rate of 100% in each group. Such findings indicated higher response by complete regression of CLs post- PGF2α injection in all animals. However, variable responses to treatment in term of number of unovulated follicles and CLs were observed among experimental groups. All buffaloes in G2 (100%) produced CL vs. 71.4 and 85.7% of buffaloes in G1 and G3, respectively. It is of interest to note that no embryos were produced from animals in G1, although 71.4% of animals in this group produced ovulation sites. On the other hand, response rate of embryo production doubled in G2 as compared to G3 (57.1 vs. 28.6%). These trends indicated that response of superovulated buffalo cows in term of CLs and embryo production was the highest in G2, followed by G3, but those in G1 showed low CLs response. This may be due to failing of embryo recovery in G1 or losing embryos during flushing.

Superovulatory response and embryo recovery rate:

Results presented in Table (2) showed the superovulatory response of buffalo cows in terms of average number of CLs and unovulated follicles/animal. Buffalo cows in G2 showed the highest total ovulatory response as compared to G1 and G2, but the difference was not significant. This trend in G2 was associated with insignificantly lower number of unovulated follicles and significantly (P<0.05) higher number of CLs per animal (total or responded animals). It is worthy noting that animals in G2 showed significantly (P<0.05) the highest average number of CLs per responded animal, while number of embryos per responded animals or animals produced embryos was insignificantly the highest in G2 as compared to other groups.

Generally, Baruselli *et al*. (2000) mentioned that buffaloes have shown follicular responses after superovulation treatment (mean of 15 follicles > 8 mm), moderate ovulation rate (60%) and CL yield at the time of flushing (approximately 9 CL) and low embryo recovery rates (34.8%). In accordance with the present results, many authors observed declined embryo recovery rate in buffaloes (20 to 40%), being lower than 63-80% in bovine (Boland *et al*., 1991; Adams, 1994; Vos *et al*., 1994; Shaw *et al*., 1995).

Table (2): Ovulatory response of buffalo cows on day of flushing in different experimental groups.

Item	Experimental group G1 (DF of 5-7 mm)	G2 (DF of >7-9 mm)	G3 (DF of >9 mm)
Ovulatory response:			
Total number of unovulated follicles	10	8	10
Total number of CLs	5	11	6
Number of unovulated follicles/animal	1.43±0.65	1.14±0.37	1.43±0.65
Number of CLs/ animal	0.71±0.13[b]	1.57±0.15[a]	0.86±0.14[b]
Total ovulatory response/buffalo	2.14±0.78	2.71±0.52	2.29±0.79
Number of CLs/responded animal (n)	1.0±0.00[b]	1.57±0.11[a]	1.0±0.00a
Embryo recovery:			
Total number of embryos (n)	0	4	2
Number of embryo/animal	0	0.57	0.29
Number of embryos/responded animal*	0	0.57	0.33
Number of embryos/animal produce embryo	0	1.0	1.0
Embryo recovery rate (%)*	0	36.4	33.3

[A and b]**Means denoted within the same row with different superscripts are significantly different at P<0.05.**

*** Responded animals with CLs.**

In bovine, Mahmood *et al*. (1989) found marked differences in response of cows i.m. injected with 3000 IU PMSG on day 9, 11 or 14 of the oestrous cycle and 500 µg PGF2α 48 h later. The superovulatory response was highest in animals treated on day 12 and lowest in these treated on day 14 of the estrous 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 estrous cycle. In comparison with buffaloes in the present study, Holy (1987) found that average number of CLs was 8.8 and 8.0/animal for cows treated with 2000-3000 IU PMSG or PMSG and anti-gonadotropin, respectively. In Friesian or crossbred cows, Slimane and Ouali (1991) recorded greater CLs number (11.6/cow) in cows treated with 2500 IU PMSG. In Hereford cows, Zeitoun *et al*. (1991) showed

that CL number was higher (23.0/animal) with 3000 IU than with 1500 IU PMSG (14.1/animal). 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. These finding indicated lower superovulatory response of buffaloes than bovines.

Embryonic stage of recovered embryos:

Based on embryo production only in buffaloes superovulated in G2 and G3, results shown in Table (3) cleared that buffalo cows in G2 produced embryos at morula and blastocyst stages, while those in G3 yielded embryos only at compact morula stage. In comparing embryo production of G2 and G3, results revealed that averages number of total and transferable embryos were greater in G2 by about 50% than in G3. These findings are associated with higher recovery rate of total embryos in G2 than in G3, respectively.

Table (3): Average number and percentage of embryos at different stages of superovulated buffaloes in the 2nd and 3rd groups.

Item	Responded group G2 (DF of > 7-9 mm)	G3 (DF of > 9 mm)
Embryos at morula stage:		
Total number/group	2	-
Number/animal produce embryos	1.0	-
Frequency distribution (%)	50	-
Embryos at compact morula stage:		
Total number/group	-	2
Number/animal produce embryos	-	1.0
Frequency distribution (%)	-	100
Embryos at blastocyst stage:		
Total number/group	2	-
Number/animal produce embryos	1.0	-
Frequency distribution (%)	50	-

Generally, the reason for the low embryo recovery rate in superovulated buffaloes remains unknown, compromising the efficiency and the application of embryo transfer technology in this species. Further studies are needed to enable the use of MOET in buffalo, to allow this technique to be widely used by farmers and to accelerate genetic gain and productivity of buffalo herds.

CONCLUSION

The obtained results indicated that the potentiality of PMSG injection to induce high superovulatory response in buffaloes is highly related to follicular diameter. Under the experimental conditions of the present study, appropriate time for superovulation in buffalo cows treated with 3000 IU of PMSG was when diameter of the dominant follicle reached a rang from ≤7 -9 mm to reflect the highest ovulatory response. According to the present study was 8-10 days of the estrous cycle.

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تأثير الحقن بهرمون سيرم الفرس الحامل (PMSG) بناءاً علي قطر الحويصلات علي الإستجابة للتبويض المتعدد وجودة الأجنة للجاموس المصري

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أجريت هذه الدراسة علي 21 من أناث الجاموس المصري تتراوح أوزانها بين (٤٥٠-٦٠٠كجم) وأعمارها بين (٥-٩ سنوات) وفي الموسم (٣-٥) وفي الفترة من (٩٠-١٢٠) يوم بعد الولادة. قسمت الأبقار إلي ثلاث مجاميع (7 جاموس لكل مجموعة) حسب توقيت الحقن لكل هرمون وبناءاً علي قياس قطرالحويصلات المبيضية المطلوب الوصول إليها. قبل بدء المعاملة أعطيت لكل الحيوانات بالمجاميع حقن بواقع 2 سم من هرمون البروستاجلاندين ($PGF_2\alpha$) بواقع جرعتين بينهما ١١ يوم لتنظيم الشياع والبدء في الموعد المحدد لبدء المعاملة. تم حقن الأبقار في المجاميع الثلاثة بهرمون (PMSG) تركيز ٣٠٠٠ وحدة دولية حقن عضلي عند وصول كل من المجاميع إلي قطر الحويصلة المبيضية السائدة إلي (٥-٧مم)بالنسبة للمجموعة الأولي, وعندما يصل قطر الحويصلة المبيضية السائدة (أكبر من ٧-٩مم) بالنسبة للمجموعة الثانية, وعندما يصل قطر الحويصلة المبيضية السائدة (أكبر من ٩ مم) بالنسبة للمجموعة الثالثة. في الثلاث مجاميع وبعد ٤٨ ساعة من الحقن بهرمون PMSG تم حقن جميع الحيوانات بواقع ٢ سم من هرمون البروستاجلاندين ($PGF_2\alpha$) وبعد ٤٨-٧٢ تم تلقيح الحيوانات بناءاً علي مشاهدة الشياع تلقيح طبيعي ولأكثر من مرتين. تم جمع الأجنة غير جراحياً بعد سبعة أيام من التلقيح لتقييم الإستجابة المبيضية للمعاملة . كما تم إستخدام جهاز الموجات فوق الصوتيه SONAR أثناء فترة المعاملة لتسجيل عدد الحويصلات وقطرها والأجسام الصفراء لتحديد مدي الإستجابة للمعاملات الهرمونية أيضاً لتحديد توقيت الحقن بهرمون PMSG. أوضحت النتائج أن توقيت الحقن بالـ PMSG يتراوح في المتوسط بين الأيام ٧.٠٤و ٩.٢و ٩.٨ بالنسبة للمجاميع ١, ٢ و ٣ علي التوالي. كل الأبقار في المجموعة الثانية أظهرت أجسام صفراء علي المبيض بنسبة ١٠٠% بينما أظهرت المجموعة الثالثة نسبة ٨٥.٧% وتلتها المجموعة الأولي بنسبة ٧١.٤% . تم الحصول علي أجنة وهو ما يظهر معدل الإستجابة من المجموعتين الثانية والثالثة بنسب ٧٥.١% و ٢٨.٦% علي التوالي ولم نتحصل علي أجنة من المجموعة الأولي . كان متوسط عدد الأجسام الصفراء مرتفع معنوياً في المجموعة الثانية ٢.٧١ / حيوان مقارنة بالمجموعة الثالثة ٢.٢٩/ حيوان وتلتها المجموعة الأولي / حيوان ٢.١٤. أظهرت الحيوانات بالمجموعة الثانية والثالثة معنوية عالية بالنسبة للإستجابة (الأجسام الصفراء والحويصلات التي لم يحدث لها تبويض. كان متوسط أعداد الأجنة عالية المعنوية في المجموعة الثانية بمقدار زيادة ٥٠% عن المجموعة الثالثة . كانت النتائج المتحصل عليها من الأجنة في مرحلة الموريولا (Morula) ١٠٠% في المجموعة الثانية. وكانت نسبة الأجنة ذات التقدير ممتاز تحصل عليها من المجموعة الثانية فقط. من النتائج المتحصل عليها تبين إمكانية معرفة التوقيت المناسب للحقن بهرمون الـ PMSG للحصول علي أعلي معدل إستجابة لإحداث عملية التبويض في الجاموس المصري. تحت الظروف التجريبية للدراسة الحالية, وجد أن أنسب توقيت لبدء معاملة تنشيط تعدد التبويض في الجاموس المصري بإستخدام PMSG بتركيز ٣٠٠٠ وحدة دولية هو عند وصول قطر الحويصلات المبيضية السائدة ما بين (≤٧-٩ مم) للحصول علي أعلي إستجابة للتبويض وأعلي عدد أجنة ممكنة قابلة للزرع, خلال الفترة من ٨-١٠ يوم من بداية حدوث الشياع.