Impact of Single or Multiple Doses of Pregnant Mare Serum Gonadotropin (PMSG) on Superovulatory Response of Post-Partum Friesian Cows

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ABSTRACT

This study aimed to compare the effect of using PMSG at a level of 2500 IU in term of one dose, two doses (1250 IU for each at 12 h interval) or 4 doses (650 IU for each at 12 h interval) on superovulatory response (SOVR), and yield and quality of embryos. A total of 21 Friesian cows (450-550 kg LBW, 3.5-5.5 y old and 1-3 parities) were used as embryo donors. Cows were fed and managed under the same conditions. All cows were i.m. injected with 3 ml PGF2α/cow (Estrumate) for estrous synchronization. Cows in the 1st protocol (P1) were i.m. injected with one dose of PMSG (2500 IU/cow) on day 10 of estrous cycle (control), while those in the 2nd and 3rd protocols were i.m. injected with two doses of PMSG (1250 IU/dose/cow) at 12 h interval on day 10 of estrous cycle (P2) and with four doses of PMSG (625 IU/dose/cow) at 12 h interval on day 10 and 11 of estrous cycle (P3), respectively. After 48 hours of the last PMSG dose in all protocols, all cows were i.m. injected with 2 ml PGF2α/cow. Both AI and GnRH (5 ml Receptal/animal) injections were done on day 14 in P1 and P2 and day 15 in P3. Embryos were un-surgically flushed 7 days post-AI. Number of total follicles (TFN), large follicles (LFN) and CLs (CLN), and diameter of follicles/cow on day of AI and flushing were determined. Ovulation rate (OR), embryo recovery rate (ERR) and quality of non-surgically recovered 7 days post-AI were determined. Results showed that on day of AI, FN, LFN and follicular diameter tended to be the highest in P3, moderate in P2 and the lowest in P1. On day of flushing, total and large follicles were the greatest and the widest in P2, followed by P1, and the least and the narrowest in P3. Ovulatory sites (CLN) was greater in P1 and P3 (2.00 and 2.07/ovary) than in P2 (1.57/ovary). The effect of protocol, ovarian side and their interaction on follicular number and diameter, and CLs number was not significant on AI and flushing days. The response to CLs formation on day of flushing on the right, left or both ovaries was 100% in each protocol. Showing CLs was higher on the right than on the left ovaries in P1 (85.7 vs. 71.4%), being the opposite (71.4 vs. 85.7%) in P2 and similar in P3 (85.7 on each side). OR was higher (P<0.05) in P1 than in P2 (69.9 vs. 52.3%), but did not differ in P3 (59.1%) from that in P1 and P2. Percentage of cows produced embryos was highest in P3 (71.4%), moderate in P1 (57.1%) and the lowest in P2 (14.2%) with average number of 1.71, 1.86 and 0.57 embryos/cow, respectively. ERR was higher in P1 and P3 (46.5 and 41.3%) than in P2 (18.1%). About 25% of cows in P1 produced 5 embryos, and 40% in P3 produced 2 embryos versus one cow in P2 (14.3%) produced 4 embryos. Distribution of embryos at morula stage was higher (50%) in P2 than in P1 (30.8%) and P3 (33.4%). Distribution of embryos at compact morula stage was the highest in P1 and P3 (53.8 and 41.7%) than in P2 (25%) versus 15.4 and 16.6% at blastocyst stage in P1 and P3. Distribution of transferable embryos was 95.3, 50.0 and 83.3% in P1, P2 and P3, respectively. Superovulation of Friesian cows with PMSG (2500 IU/cow) on day 10 of the estrous cycle, in term of 4 doses (625 IU) at 12-h interval, showed the best follicular response (number and diameter) on day of AI and the higher ovulatory response, in term of number of un-ovulated follicles and CLs as well as percentage of cows responded to produce embryos (71.4%) on day of flushing. The same PMSG as a single dose showed the highest response, in term of number of transferable embryos/cow (1.71/cow).

Keywords: Cattle, superovulation, PMSG, dose interval, ovulatory response, embryos.

INTRODUCTION

The main purpose of embryo transfer (ET) in domestic ruminants is to spread the genetic quality of livestock production for desirable traits. Although the basic procedures employed in ET are now well established, there is considerable scope for improvement of ET technology in various areas. In ET, the production of a sufficient number of viable embryos is essential for maximum utilization of genetically superior donors. Superovulation (SO) is a critical step in ET success, and several gonadotropins have been utilized for SO in different species, but PMSG is most often used to induce multiple ovulations from the ovary for increased production of oocytes/embryos (Marte Mucci *et al.*, 1988; McKiernan and Bavister, 1998). The SO is still widely used to produce valuable bovine embryos for breeding around the world, despite the fact that variability of response remains a major limiting factor in its use (Hahn, 1992 and Adams, 1994). Variability in superovulatory response (SOVR) after gonadotropin treatments continues to be the greatest problem for commercial ET (Mapletoft *et al.*, 2002 and Barros and Nogueira, 2004).

The gonadotropins commonly used for SO are Pregnant Mare Serum Gonadotropin (PMSG) or Follicles Stimulating Hormone (FSH) of porcine or ovine origin (Elsden *et al.*, 1978). The PMSG is a glycoprotein containing more than 10% of sialic acid and has a molecular weight of 70000, and these characteristics allow SO induction by a single injection of PMSG (Schams *et al.*, 1978). The SOV protocols using either PMSG or FSH have been established in cattle by many authors (Lindsell *et al.*, 1986 and Goulding *et al.*, 1990). However, PMSG had disadvantages of poor quality embryos production for several reasons (Schams *et al.*, 1978 and Ziecik *et al.*, 2005), and also SO with PMSG increases the incidence of embryonic mortality and abortion (Kiewisz *et al.*, 2011), but PMSG at different doses may vary in response in terms of estrus behavior, fertility and productivity of farm animals (Barrett *et al.*, 2004).

The conventional protocol of initiating ovarian super-stimulation during mid-estrous 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 estrus (Bò *et al.*, 1995; Abdel-Khalek *et al.*, 2010 and 2016) in cows and buffaloes. Traditionally, a single dose of 1500 to 3000 IU of PMSG during the mid-luteal phase of the estrous cycle has been used to SO of cows. The advantage of using PMSG for SO is its availability in large quantities for a low cost. PMSG can be also administered, as a single dose compared with the multiple injections normally required when using pituitary preparations (Alfuraiji *et al.*, 1993).

The response of individual donors mainly depends on the number of gonadotropin-sensitive follicles present at the time of treatment initiation (Monniaux *et al*., 1983 and Cushman *et al*., 1999). Lower SOR with PMSG may be associated with its relatively long half-life in blood circulation (Moor *et al*., 1985 and Murphy and Martinuk 1991), resulting in excessive follicular development and failure of ovulation, with follicular growth continued through to embryo collection. Existing evidence indicates that PMSG antibodies can be used to neutralize the PMSG in the circulation and thus reduce post-estrus ovarian follicular stimulation. Treatment with PMSG antibodies have been reported to improve ovulation rates (Dhondt, *et al*., 1978 and Dieleman and Bevers 1987) and embryo quality (Kummer, *et al*., 1980 and Saumande, *et al*., 1984). These results suggest that blood PMSG levels decreased after antiserum injection.

The aim of this study was to compare the effect of using PMSG at a level of 2500 IU in term of one dose, two doses (1250 IU for each at 12 h interval) or 4 doses (625 IU for each at 12 h intervals) on follicular dynamics, superovulatory response, and yield and quality of Friesian embryos.

MATERIALS AND METHODS

This study was conducted at Animal Production Research Stations in El-Karada and Sakha and International Livestock Management Training Center (ILMTC), Sakha, Kafrelshiekh governorate, belonging to Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Ministry of Agriculture in co-operation with Animal Production Department, Faculty of Agriculture, Mansoura University during the period from January 2017 to March 2018.

Animals:

A total of 21 Friesian cows having live body weight (LBW) of 450-550 kg, 3.5-5.5 years of age and 1-3 parities were used as embryo donors at 1-2 post-partum estrous cycles prior to SOV protocols. Cows were taken from El-Karada and Sakha herds and divided into three experimental groups (7 animals in each). Donor cows were subjected to clinical examination of the ovaries and reproductive tract before SOV protocols. Each animal was subjected to rectal palpation to exclude any abnormalities of the reproductive organs before starting the experiment. Furthermore, the size of the cervical canal of the donors was tested for the suitability of passing the folly catheter through it. Generally, all donor cows were cyclic, fit and free of diseases.

Feeding and management systems:

Experimental cows were fed and managed under the same conditions applied in Animal Production Research Institute Station. Animals were housed in a semi-open shaded yard and fed based on the recommendation of the Ministry of Agriculture (APRI). During winter and spring months, animals were fed on berseem (*Trifolium alexandrinum*), concentrate feed mixture (CFM) and rice straw (RS), while during summer and autumn months, animals were fed the same CFM, berseem hay (BH), corn silage and RS to cover their nutritional requirements according to their LBW and milk production.

Superovulation protocols:

The experimental cows were divided into three treatment groups (7 per each) for three SOV protocols as illustrated in the following diagram:
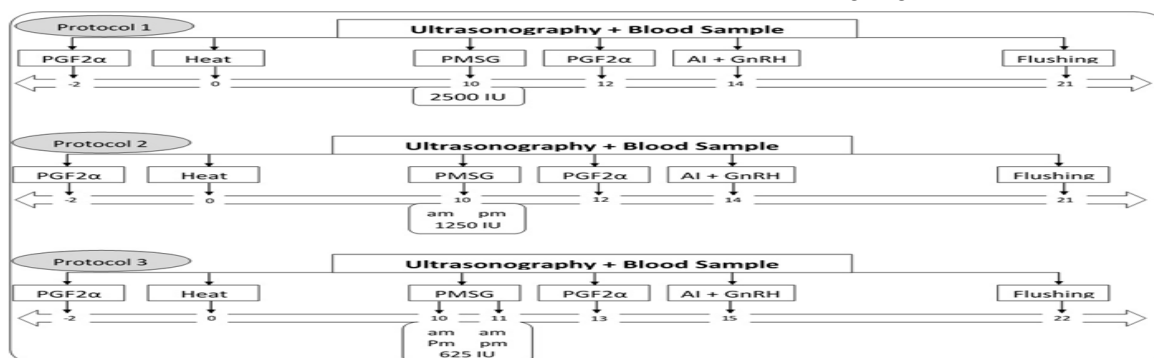
According to the previous diagram, all cows were intramuscularly (i.m.) injected with 3 ml PGF2α/cow (Estrumate, containing 263 µg Cloprostenol Sodium BP (Vet) equivalent to 250 µg Cloprostenol; Friesoythe, Germany) to bring them on heat (start of estrous cycles). Cows in the 1st protocol (P1) were i.m. injected with one dose of Pregnant Mare Serum Gonadotrophin (PMSG, Folligon, Intervet International B.V., Boxmeer, Netherlands) at a level of 2500 IU/cow on day 10 of the synchronized estrous cycle. Cows received this protocol were considered as control protocol. Cows in the 2nd protocol (P2) were i.m. injected with two doses of PMSG (1250 IU/dose/cow) at 12 h interval on day 10 of the synchronized estrous cycle. However, cows in the 3rd protocol (P3) were i.m. injected with four doses of PMSG (625 IU/dose/cow) at 12 h intervals on day 10 and 11 of the synchronized estrous cycle.

After 48 hours of the single dose of PMSG in P1, the 2nd dose of PMSG in P2, and the 4th PMSG dose in P3, all animals were i.m. injected with 2 ml PGF2α/cow to induce corpus luteum (CL) regression. All treated animals were artificially inseminated (AI) and injected with GnRH (5 ml Receptal/animal) at insemination on day 14 of animals in P1 and P2 and on day 15 of animals in P3. All treated animals were un-surgically flushed 7 days post-AI, on day 21 of in P1 and P2 and on day 22 in P3.

Ultrasonography examination:

Cows in all protocols were subjected to ultrasonography device (ESAOTE Pie Medical Aquila Pro Vet + Probe 6.0/8.0 Mhz LA Rectal Veterinary Transducer) during protocol days for counting the number

of follicles and corpora lutea, and estimating the diameter of follicles on the ovarian surface on day of estrus, PMSG, PGF2α, AI and flushing. Ovulation rate (OR%) was calculated as the following:

OR (%) = (Number of CLs on day of flushing/number of follicles on day of AI) x 100.

Heat detection and artificial insemination:

For estrous synchronization at the beginning of each protocols, cows treated with PGF2α were kept under observation for heat detection for two times/day at 8.00 a.m. and 16.00 p.m. Cows in heat were considered on day 0 of each protocol. At fixed AI time for each protocol, cows were artificially inseminated two times (12 hours interval) with frozen proven semen of the same bull and by the same inseminator.

Uterine flushing and embryo recovery:

Embryos were recovered non-surgically 7 days post-insemination. Epidural anaesthesia was performed; the tail head was clipped, then scrubbed with iodine soap and swabbed with 70 percent alcohol to prevent infection of the spinal column then donor injected with 5 ml of a sterile 2 percent solution of procaine in water using a new 18-gauge needle each time. Epidural anaesthesia monitored by flaccidity of the tail.

Technique of non-surgical flushing was followed using the closed system. It was done according to the method described for cattle by Newcomb *et al.* (1978). Embryos were collected in Modified Dulbecco's phosphate buffer saline (PBS). The pH value of the medium was adjusted to be in the range between 7.25-7.50, while osmolarity level ranged between 260-310 mOsm/kg. One percent of fetal or estrus cow serum was added to Modified Dulbecco's PBS for flushing. Composition of Modified Dulbecco's PBS (GIBCO Laboratories USA) is shown in the following table:

Content of PBS	g/ liter
Calcium chloride ($CaCl_2.2H_2O$)	0.133
Magnesium Sulphate ($MgSO_4\ 7H_2O$)	0.120
Sodium chloride (NaCl)	8.000
Potassium chloride (KCl)	0.200
Sodium phosphate ($NaHPO_4$)	2.170
Potassium phosphate (KH_2PO_4)	1.000
Glucose	1.000
Sodium pyrovate	0.036
Streptomycin sulphate	0.050
Sodium penicillin	100,000 (IU)

Embryo searching and evaluation:

Dish of the collected embryos with PBS for each donor was searched under a stereomicroscope (x 20-80). When embryo was identified, it was picked up by a 0.5 ml glass pack syringe connected to a capillary plastic hose and then transferred into a small dish (holding capacity of 35 x 10 mm) containing filtered culture media. After searching, number of recovered embryos at different stages (morula, compact morula, early blastocyst and blastocyst) was recorded and evaluated. Also, degenerated and abnormal embryos were recorded for each responded cow. Then embryo recovery rate (ERR) was calculated as the following:

ERR (%) = (Total number of recovered embryos/number of CLs on day of flushing) x 100.

Collected embryos were evaluated morphologically according to Takeda (1986). Embryos were classified into different grades basis on their morphological symmetry, stage of blastomeres and age of embryo in relation to stage of the donor estrous cycle as well as the presence of vesicles and colour of embryo. Embryos were classified into *excellent embryos* (Score I): at normal stage of development at time of examination, symmetrical blastomeres are polygonal in shape forming a tight mass at morula stage, *good embryos* (Score II): as excellent but embryos are asymmetrical, contain blastomers extruded from the main morula mass, *fair embryos* (Score III): spherical rather polygonal blastomers, contained blastomers of different sizes, had signs of degeneration such large vesicles in the cells and/or darker or lighter than normal, and *poor embryos* (Score IV): having several faults than fair.

Number of transferable embryos was expressed as a summation of excellent and good embryos at morula, compact morula, early blastocyst and blastocyst stages.

Statistical analysis:

Data in each experiment were subjected to factorial design (3 protocols x 2 ovarian side) according to Snedecor and Cochran (1982) using program of SAS (2004) to study the effect of SOV protocol, ovarian side and their interaction on number of follicles and CLs and follicular diameter on day of AI and flushing. The differences among means were set at P<0.05 using Multiple Range Test (Duncan 1955).

RESULTS AND DISCUSSION

Ovarian structures on day of AI:

Effect of SOV protocol on ovarian structure on day of AI was not significant, although number and diameter of total and large follicles on the ovarian surface tended to be the highest in P3, moderate in P2 and the lowest in P1. These results may reveal that PMSG injection (2500 IU/cow) on day 10 of the estrous cycle, in term of 4 doses at 12-h interval had pronounced impact on increasing number and size of total and large ovarian follicles on day of AI as compared to PMSG in term of 2 doses at 12-h interval or as a single dose. In this respect, effect of ovarian side on ovarian structure was not significant with different trend on the right and left ovary, regardless type of protocol. It is of interest to note that no CLs were found on the ovarian surface on day of AI (Table 1).

Table 1. Number and diameter of follicles on the surface of the right and left ovaries of cows in different superovulation protocols on days of AI.

Item	Total ovarian follicles		Large follicles*		Corpora lutea number
	Number	Diameter (cm)	Number	Diameter (cm)	
Effect of protocol:					
P1	4.57	0.97	2.50	1.34	-
P2	4.93	1.06	2.86	1.39	-
P3	5.21	1.10	3.29	1.49	-
±SEM	0.516	0.053	0.425	0.100	-
Effect of ovarian side:					
Right	4.95	1.07	3.14	1.44	-
Left	4.86	1.01	2.62	1.37	-
±SEM	0.422	0.044	0.347	0.082	-

* Large follicles of 0.95 cm.

The small ovarian follicles require FSH to develop, and evidence suggests that follicles as small as 1 mm in diameter will commence growth under the influence of FSH (Adams *et al.*, 2008). It is unknown if the high variation in number of follicles in ovarian reserves among individuals causes a highly variable number of un-ovulated follicles to grow during estrous cycles (Burns *et al.* 2005). The observed trend of differences in number of all and large follicles on day of AI may be related to number of follicles during the 2nd follicular wave in cows. In superovulted cattle, a range of 20-30 follicles/cow emerge during each follicular wave and most of them have the ability to develop to pre-ovulatory stages (Adams, 1994), and about 45 small follicles less than 5 mm in diameter were recorded during the 1st follicular wave in Zebu heifers (Buratini *et al.*, 2000). In this respect, Monniaux *et al.* (1983) suggested that the follicular number on the ovarian surface at the time of gonadotropin treatments affects number of follicles in superovulated cattle. Therefore, the recorded tendency of increase in follicular of all and large follicles on day of AI in P3 (the same PMSG dose at 4 intervals) may suggest more follicular development after the last dose of PMSG, because during a normal follicular wave, subordinate follicles regress as a result of decreasing FSH concentrations in blood circulation by the secretion of estradiol and inhibin of the cohort and especially of the dominant follicle (Adams *et al.*, 1992).

Superovulatory response on day of flushing:

Effect of SOV protocol on ovarian structure on day of flushing was not significant, although total follicles or un-ovulated follicles were the greatest and the widest in P2, followed by P1, and the least and the narrowest in P3. It is worth noting that number of CLs, as ovulatory sites, showed adverse situation in different protocols, being the greatest in P3 and the lowest in P2. These results indicated negative relationship between CLs number and un-ovulated follicles number on day of flushing. Also, number and size of ovarian structure was not affected significantly by ovarian side, showing different trend on the right and left ovary. However, number of CLs was greater on right than on the left ovary, regardless type of protocols, indicating higher ovarian activity on right than on left ovaries (Table 1).

The obtained results indicated low SOVR in all protocols in comparing with the results of Ganah *et al.* (2009), who reported greater number of CLs (11.67/cow), un-ovulated follicles (1.4/cow) and total follicles (13.0/cow) in Friesian cows superovulated with 2500 IU of PMSG on day 10 of the estrous cycle. Also, Mohammed and Ismail (1999) found that number of CLs and follicles was 12.8±1.06 and 2.6±0.40 in non-lactating Friesian cows injected with 2500 IU PMSG. Nearly similar number of CLs (11.6/cow) was recorded by Slimane and Ouali (1991) in French Friesian cows superovulated with 2500 IU PMSG. In Hereford cows, CLs number was 23 and 14.1/cow for animals treated with 3000 and 1500 IU PMSG, respectively (Zeitoun *et al.*, 1991). Mean number of follicles and ovulatory sites of crossbred heifers superovulated by 2500 IU PMSG was 13.3 and 1.3/cow, respectively (Saumande and Chupin, 1986).

Although PMSG has been used for SOV, the response in terms of the number of CLs per flush is highly variable and very low (Karaivanov, 1986; Misra, 1993; Madan *et al.*, 1996). Lower SOVR with PMSG may be attributed to its relatively long circulating half-life (Moor *et al.*, 1985; Murphy and Martinuk, 1991), resulting in excessive follicular development and failure of ovulation, with follicular growth continued through to embryo collection. Other authors postulated that the main cause for variation may originate from deviations occurring during the follicular period (Dieleman *et al.*, 1993).

The effect of interaction between protocol type and ovarian side was not significant on number of ovarian follicles on days of AI and flushing, reflecting the greatest number of total follicles and large follicles of cows in P3 on the left and right ovaries on AI day, respectively. Cows in P2 showed the greatest number of total or large follicles on the left ovaries on day of flushing. On the other hand, diameter of total or large follicles was the highest on the right ovaries on AI and flushing days (Figs. 1-4).

In comparing ovarian follicles between AI and flushing days, number of total or large follicles was higher on AI than on flushing day for all protocols (Figs. 1 and 2), while follicular diameter showed an opposite trend (Figs. 3 and 4). This finding may indicate higher ovulation rate of right than of left ovaries.
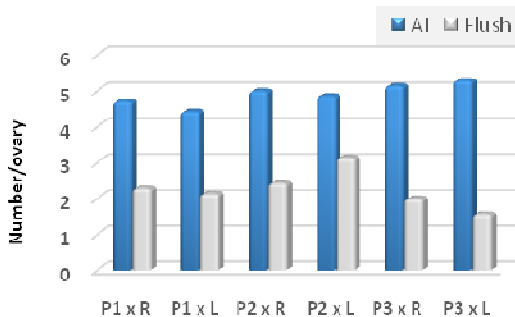

Fig. 1. Total number of follicles/ovary on days of AI and flushing in different protocols.
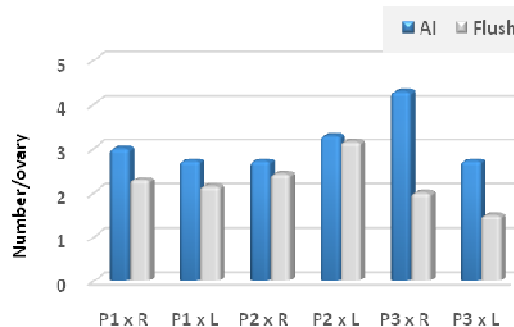
Fig. 2. Large follicular number/ovary on days of AI and flushing in different protocols.
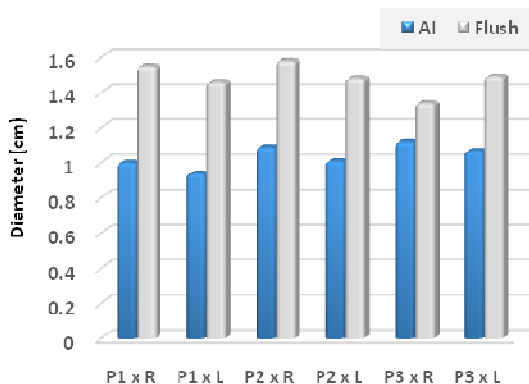
Fig. 3. Total follicular diameter (cm) on days of AI and flushing in different protocols.

Fig. 4. Large follicular diameter (cm) on days of AI and flushing in different protocols.

In agreement with the present effect of ovarian side, Testart (1972) found similar number of large follicles on the right and left ovaries in the cow after PMSG injection. Also, Saumande and Chupin (1982) reported similar ovarian activity in the two ovaries of cow after PMSG injection.

Ovulatory sites:

The SOVR, in term of CLs formation on day of flushing, all cows (100%) in each protocol showed CLs on the right, left or both ovaries. Percentage of responded cows showing CLs on the right ovaries was higher than those showing CLs on the left ovaries in P1 (85.7 vs. 71.4%), being the opposite in P2 (71.4 vs. 85.7%) and similar in P3 (85.7 on each side, Fig. 5). Such finding suggested that ovarian side had no effect on CLs response in each SOV protocol.

Based on number of large follicles on day of AI and number of CLs on day of flushing, ovulation rate was significantly (P<0.05) higher in P1 than in P2 (75.68 vs. 55.0%), but did not differ significantly in P3 (63.04%) from that in P1 and P2 (Fig. 6). Although number of the large follicles was higher in P3 than in P1 on day of AI, CLs number was nearly similarity in P1 and P3. Decreasing ovulation rate of cows in P3 than in P1 was associated with higher number of large follicles on day of flushing in P3 than in P1 (Table 2).
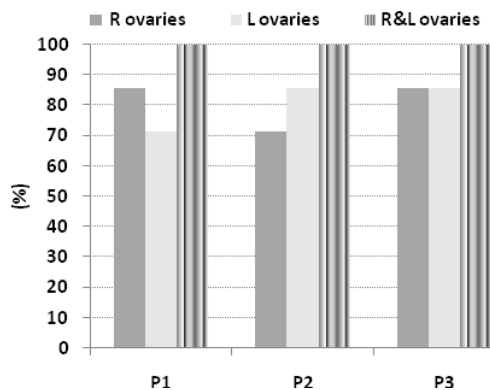
Table 2. Number and diameter of follicles and corpora lutea on the surface of the right and left ovaries of cows in different superovulation protocols on days of flushing.

Item	Total ovarian follicles		Un-ovulated follicles*		Corpora lutea Number (CLs)
	Number	Diameter (cm)	Number	Diameter (cm)	
Effect of protocol:					
P1	2.21±0.524	1.51±0.091	2.14±0.499	1.75±0.150	2.00±0.400
P2	2.79±0.524	1.53±0.091	2.71±0.499	1.80±0.150	1.57±0.400
P3	1.79±0.524	1.42±0.095	1.57±0.499	1.67±0.157	2.07±0.400
Effect of ovarian side:					
Right	2.24±0.428	1.49±0.078	2.14±0.408	1.76±0.128	2.05±0.323
Left	2.29±0.428	1.48±0.074	2.14±0.408	1.73±0.121	1.71±0.323

* Large follicles of 0.95 cm.

In comparable with our study, the present CLs number on day of flushing was 4.0, 3.14 and 4.14/cow in P1, P2 and P3, respectively. Similarly, Misra *et al.* (1994) found lower number of ovulations (3.76/cow) in cows treated with PMSG at a level of 3000 IU. However, Arora *et al.* (1996) found that mean number of CLs was 6.6/cow for lactating Jersey x red Sindhi cows induced by 2000 IU PMSG on day 11 of the estrous cycle. Holy (1987) found that CLs number averaged 8.8 and 8.0/cow for cows injected with PMSG at levels of 2000 and 3000 IU and anti-gonadotropin, respectively.

Fig. 5. Percentage of responded cows showing CLs on the right (R), left (L) or both (R&L) ovaries in different SOV protocols.

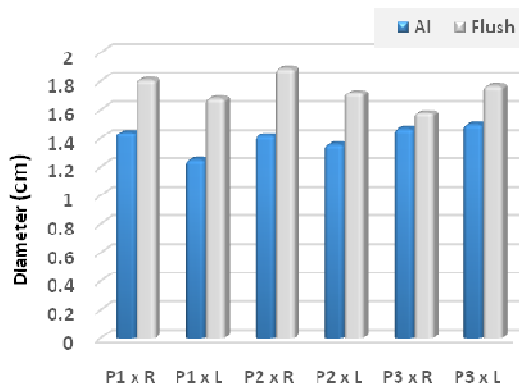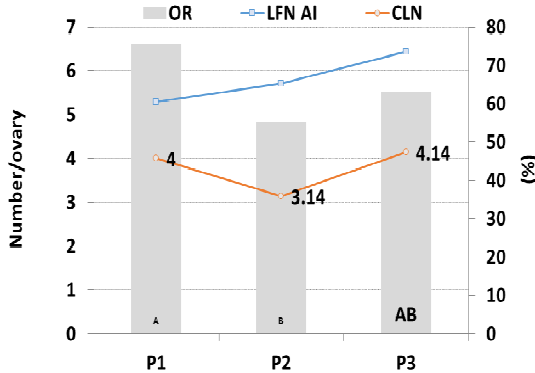**Fig. 6. Number of large follicles on day of AI (LFN AI) and CLs (CLN) on day of flushing per cow, and ovulation rate in different SOV protocols. (A and B: significant differences at P<0.05)**

The PMSG is used to induce more ovulation rate (Sommer *et al.*, 2007) by increasing multiple ovulation or multiple births (Mehaisen *et al.*, 2005 and Talebkhan Garoussi *et al.*, 2012). Therefore, PMSG treatment is considered to enhance ovarian follicular growth and fertility at the end of estrus synchronization program in dairy cows (Souza *et al.*, 2009; Rostami *et al.*, 2011). Initiating treatment of gonadotropins at the time of follicular wave emergence will optimize the number of follicles capable of responding and should result in improved SOVR (Adams, 1994 and Bergfelt *et al.*, 1997). The presence of a dominant follicle at the time of initiation of SOV treatment was suggested to be associated with a reduction of 40- 50% in the ovulation rate (Guilbault *et al.*, 1991).

Variability in ovulation rate by PMSG may be attributed to secondary follicles production after ovulation, due to long half life of PMSG (Callesen *et al.*, 1986). In similarity with the obtained results, Guilbault *et al.* (1991) observed large variation, ranging from 0 to 14 ovulations in heifers super-stimulated by PMSG.

Embryos yield:

In response to embryo production on day of flushing, percentage of cows produced embryos was the highest in P3 (4 out of 7 cows, 71.4%), moderate in P1 (1 out of 7 cows, 57.1%) and the lowest in P3 (5 out of 7 cows, 14.2%) with average number of 1.71, 1.86 and 0.57 embryos/cow, respectively (Fig. 7).
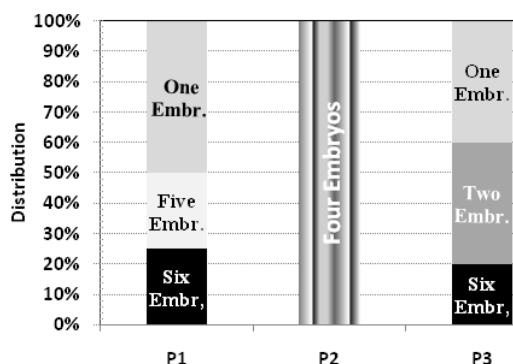
It is of interest to note that all non-responded cows to embryo production in all protocols yielded CLs. In P1, out of 3 non-responded cows, one cow give CLs on right and left ovaries, while other two cows give one and two CLs on the right ovary, respectively. In P2, all non-responded cows (n=6), cows give CLs on the right, left and both ovaries. In P3, out of 2 non-responded cows, one cow give CLs on the right and left ovaries and another give one CL on the left ovary. Therefore, embryo recovery differed in each protocol, being higher in P1 and P3 (46.5 and 41.3%) than in P2 (18.1%), which may suggest effect of protocol type on embryo recovery rate (Fig. 7).

The response to embryo production was evaluated for each protocol according to number of embryos/cow within each protocol. Frequency distribution of cows produced one embryo was 50% (2/4) in P1 versus 40% (2/5) in P3. Corresponding distribution was 25% (1/4) and 20% (1/5) for cows produced 6 embryos, respectively. On the other hand, about 25% (1/4) of cows in P1 produced 5 embryos, and 40% (2/5) of cows in P3 produced 2 embryos versus only one cow out of 7 cows in P2 (14.3%) produced 4 embryos (Fig. 8).

These results indicated that about 28.6% of cows (2/7) in P1 showed the highest embryo production (5-6 embryos) versus 14.3% of cows (1/7) in P3 (6 embryos) and only 14.3% of cows (1/7) in P2 responded and produced 4 embryos.
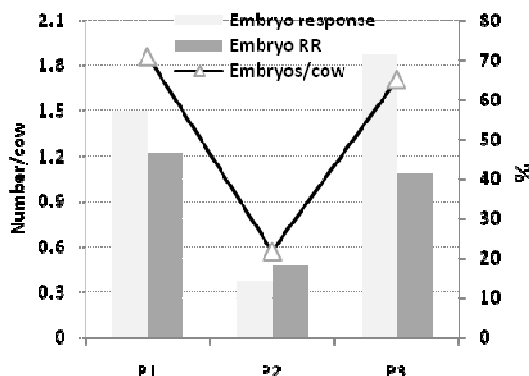

Fig. 7. Frequency distribution of cows producing from 1 to 6 embryos in different SOV protocols.

Fig. 8. Number of embryos per cow, embryo response and recovery rate in different SOV protocols.

Wide SOVR and embryo yield have been detailed in several reviews of commercial embryo transfer records. In a report of 2048 beef donor collections, Looney (1986) recorded a mean number of 11.5 ova/embryos per cow. This is higher than that obtained in our study. In general, the overall number of embryos recovered per donor flushed in the practical multiple ovulation-embryo transfer (MOET) schemes ranges from 4 to 7 (Lange, 1995; McGuirk, 1995). Number of recovered embryos was 6.0 in cows superovulated by different PMSG levels (Slimane and Ouali (1991), 1.6/cow (Misra *et al.,* 1994), or 4.65/cow (Basile *et al.*, 1994).

From normally ovulating lactating cows by deep flushing technique, Sartori *et al.* (2002) found that embryo/ova recovery rate was 30.9% from ovulated follicles. Non-surgical recovery rate of embryos/ova varied widely from a range of 20–25% (Fricke *et al.*, 1994 and

Mitchell *et al.*, 1998) to 60–80% (Kelly *et al.*, 1997 and Kim *et al.*, 2001). The observed reduction in embryo recovery rate in P2 than in other protocols was associated with appropriate ovulation rate (52.3%) and the highest number of un-ovulated follicles. In this line, Gonzalez *et al.* (1994) observed that higher number of un-ovulated follicles were associated with a detectable increase in uterine tone, which in turn, made embryo collection difficult.

In Danish cattle, the recovery rate was 67%, but was not correlated to the estimated number of CLs (Callesen *et al.*, 1992), which was observed in all protocols in the present study. Generally, recovery rate of embryos was 51.8% in cows superovulated by different levels of PMSG (Slimane and Ouali, 1991).

Embryonic stage and quality:

Frequency distribution of embryonic stages was affected by type of protocol. Cows in P2 showed higher distribution of embryos at morula stage (2/4, 50%) than P1 (4/13, 30.8%) and P3 (4/12, 33.4%). Meanwhile, cows in P1 and P3 showed higher distribution of embryos at compact morula stage (53.8 and 15.4%) than in P2 (25%) versus appropriate distribution of those at blastocyst stage (15.4 and 16.6%) in P1 and P3. It is of interest to note that cows in P3 produced embryos at 4-cell stage, while no embryos at blastocyst stage were produced by cows in P2. This means that number of embryos at morula and blastocyst stages was 1.86, 0.57 and 1.57 per cow in P1, P2 and P3, respectively (Table 3).

Regarding embryo quality, frequency distribution of transferable embryos (excellent and good) was 95.3, 50.0

and 83.3% for P1, P2 and P3, representing 1.71, 0.29 and 1.42 transferable embryos per cow, respectively (Table 3).

It is worth noting that results of embryonic stage and quality are in consistent with ovulatory response and embryonic yield in each protocol. On the other hand, cows in P3 showed the highest number of total and large follicles on day of AI, being with an opposite trend with the highest number of CLs on day of flushing as compared to those in P1.

Embryos recovered from the superovulated cows often display a wide range of development to different embryonic stages. In accordance with the present embryonic stages, embryos at early blastocyst and blastocyst stages were prevalent on day 7 (flushing day) as reported by some authors (Shea, 1981; Lindner and Wright, 1983).

Table 3. Stage and quality of embryos recovered from cows in different superovulation protocols on day of flushing

Item	Superovulation protocol								
	P1			P2			P3		
	N/cow	N/group	%*	N/cow	N/group	%*	N/cow	N/group	%*
Stage of fresh embryos:									
4-Cell	-	-	-	-	-	-	0.14	1	8.3
Morula	0.57	4	30.8	0.29	2	50.0	0.57	4	33.4
Compact morula	1.00	7	53.8	0.14	1	25.0	0.71	5	41.7
Early blastocyst & blastocyst	0.29	2	15.4	0.14	1	25.0	0.29	2	16.6
Total	1.86	13	100	0.575	4	100	1.71	12	100
Quality of fresh embryos:									
Excellent	1.00	7	53.8	-	-	-	0.42	3	25.0
Good	0.71	5	38.5	0.29	2	50.0	1.00	7	58.3
Transferable	1.71	12	92.3	0.29	2	50.0	1.42	10	83.3
Fair	0.14	1	7.7	0.29	2	50.0	0.29	2	16.7
Total	1.86	13	100	0.575	4	100	1.71	12	100

* Frequency distribution.

Embryos were collected from most donors on day 7 post-mating, thus the majority of embryos collected were at late or compact morula and blastocyst stages (Stringfellow and Seidel, 1998). Since embryo age corresponds rather closely to stage of development, it can be stated rather conclusively, based on a large number of fresh *in vivo* derived embryo transfers, that embryonic stages ranging from late morula to expanded blastocyst stage (Hasler *et al.*, 1987).

Regarding the quality of recovered embryos, Ravindranatha *et al.* (2001) recorded that mean number of collected non-surgically embryos was 5.14/cow, being 1.21, 1.0, 1.5 and 1.42/donor as excellent, good, fair, and poor quality embryos, respectively, in superovulated Holstein cows using Folltropin on day-10 of estrous cycle. However, number of viable embryos per flush was 0.56/cow (Misra *et al.,* 1994) and the percentage of viable embryos was 55.3% in Holstein cows (Basile *et al.*, 1994). The observed great variability in embryo quality was observed by Lerner *et al.* (1986). They found that about 24% of the collections produce un-viable embryos, while 64% produced fewer than average numbers of transferable embryos and 30% yielded 70% of the embryos. In beef donor collections, Looney (1986) reported that mean number of transferable embryos was of 6.2/cow. In cows, total number of transferable embryos was 6.0/cow (Mohammed and Ismail, 1999) and 2.0/cow (Arora *et al.*, 1996), being higher than that obtained

in this study. However, Slimane and Ouali (1991) found that mean number of transferable embryos was 1.0/cow, being lower than that obtained in P1 and P3 in our study. The noted reduction in embryo quality in P2 was associated with the highest number of un-ovulated follicles on day of flushing. In this way, Saumande *et al.* (1984) mentioned that the un-ovulated follicles on day of flushing produce abnormally estradiol and progesterone at high levels, leading to adverse effects on development of embryos.

Generally, PMSG had disadvantages of poor quality embryos production for several reasons. In this respect, Schams *et al.* (1978) stated that PMSG still remains at a certain level in the blood circulation during LH phase in superovulated animals. Also, Ziecik *et al.* (2005) observed that using the high PMSG dose may result in excessive development of the ovarian follicles leading to ovulatory failure. The remains of these follicles without ovulation may secrete abnormally high levels of E2, which may have adverse effects on embryo development. It has also been reported that SO with PMSG increases the incidence of embryonic mortality and abortion (Kiewisz *et al.*, 2011).

CONCLUSION

Superovulation of Friesian cows with PMSG (2500 IU/cow) on day 10 of the estrous cycle, in term of 4 doses (625 IU) at 12-h intervals, showed the best follicular response (number and diameter) on day of AI and the

higher ovulatory response, in term of number of un-ovulated follicles and CLs as well as percentage of cows responded to produce embryos (71.5%) on day of flushing. The same PMSG as a single dose showed the highest response, in term of number of transferable embryos/cow (1.71/cow). However, the same PMSG level as 2 doses (1250 IU) at 12-h interval showed the lowest results. Further studies are required to study the problem of decreasing ovulation rate of cows superovulated by 4 dose of PMSG (625 IU) by increasing level of GnRH or using LH on day of AI.

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تأثير إستخدام جرعة واحده أو جرعات متعدده من هرمون الفرس الحامل (PMSG) على الإستجابة للتبويض الفائق فى أبقار الفريزيان بعد الولاده

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تهدف هذه الدراسة إلى مقارنة تأثير استخدام جرعة واحدة من هرمون (PMSG) (٢٥٠٠ وحدة دولية) ، وجرعتين (١٢٥٠ وحدة دولية لكل جرعة بينهم ١٢ ساعة) و ٤ جرعات (٦٢٥ وحدة دولية لكل جرعة بينهم ١٢ ساعة) على الاستجابة للتبويض الفائق وعدد وجودة الأجنة. أجريت هذه الدراسة على ٢١ بقرة فريزيان تتراوح أوزانها بين ٤٥٠-٥٥٠ كجم وأعمارها بين ٣.٥ـ٥.٥ سنة وفى الموسم ١-٣، وتم استخدامهم كأبقار مانحة. تغذية الأبقار وإدارتها تمت تحت نفس الظروف. تم حقن جميع الأبقار بـ ٣ مل من هرمون البروستاجلاندين (PGF2α) لتنظيم الشياع. الأبقار فى المجموعة الأولى تم حقها بجرعة واحدة من هرمون (PMSG) (٢٥٠٠ وحدة دولية) فى اليوم العاشر من دورة الشياع (الكنترول). تم حقن أبقار المجموعة الثانية بجرعتين من هرمون (PMSG) (١٢٥٠ وحدة دولية لكل جرعة بينهم ١٢ ساعة) فى اليوم العاشر من دورة الشياع، بينما تم حقن أبقار المجموعة الثالثه بـ ٤ جرعات من هرمون (PMSG) (٦٢٥ وحدة دولية لكل جرعة بينهم ١٢ ساعة) فى اليوم العاشر والحادى عشر من دورة الشياع. بعد ٤٨ ساعة من أخر جرعة من هرمون (PMSG) تم حقن جميع الأبقار بـ ٢ مل من هرمون البروستاجلاندين. تم تجميع الأجنه غير جراحيا بعد ٧ أيام من التلقيح. تم تحديد عدد الحويصلات الكلى وعدد الحويصلات الناضجة وعدد الأجسام الصفراء وقطر الحويصلات فى يوم التلقيح ويوم تجميع الأجنة. تم حساب معدل التبويض، ونسبة وجودة الأجنة المجمعة غير جراحيا بعد ٧ أيام من التلقيح. أظهرت النتائج أنه فى يوم التلقيح كان العدد الكلى للحويصلات، وعدد الحويصلات الكبيرة وقطر الحويصلات كان أعلى فى المجموعة الثالثه، ومتوسط فى المجموعة الثانيه، وأقل فى المجموعة الأولى. فى يوم التجميع كان العدد الكلى للحويصلات، وعدد الحويصلات الكبيرة والأكبر قطرا فى المجموعة الثانية، ويليها المجموعة الأولى، وكان الأقل عددا وقطرا فى المجموعة الثالثة. عدد الأجسام الصفراء كان الأعلى فى المجموعة الأولى والثالثة (٢ ، ٢.٠٧ / مبيض) عن المجموعة الثانية (١.٥٧ / مبيض). تأثير المجموعة وجانب المبيض والتداخل بينهم كان غير معنوى فى يوم التلقيح ويوم التجميع. الاستجابة لتكوين الأجسام الصفراء فى يوم التجميع على المبيض الأيمن والأيسر أو كلاهما كان ١٠٠% فى كل المجاميع. أعداد الأجسام الصفراء كان أعلى على المبيض الأيمن منه على المبيض الأيسر فى المجموعة الأولى (٨٥.٧% مقابل ٧١.٤%)، وعكس ذلك فى المجموعة الثانية (٧١.٤% مقابل ٨٥.٧%)، ومتساويين فى المجموعة الثالثة (٨٥.٧%% لكل مبيض). معدل التبويض كان أعلى فى المجموعة الأولى عنه فى المجموعة الثانية (٦٩.٩% مقابل ٥٢.٣%)، ولكنه لم يختلف فى المجموعة الثالثة (٥٩.١%) عن المجموعة الأولى والثانية. نسبة الأبقار التى أنتجت أجنة كانت أعلى فى المجموعة الثالثة (٧١.٤%) ومتوسطة فى المجموعة الأولى (٥٧.١%)، وأقل فى المجموعة الثانية (٤٢.١%) بمتوسط عدد أجنه(١.٧١ - ١.٨٦ - ٠.٥٧ / جنين لكل بقرة على التوالى. نسبة الأجنة كانت أعلى فى المجموعة الأولى والثالثة (٤٦.٥% ـ ٤١.٣%) عن المجموعة الثانية (١٨.١%). حوالى ٢٥% من الأبقار فى المجموعة الأولى أنتجت ٥ أجنة و ٤٠% من الأبقار فى المجموعة الثالثة أنتجت جنينان، مقابل بقرة واحدة فى المجموعة الثانية أنتجت أربع أجنة. تصنيف الأجنه فى مرحلة Morula كان أعلى فى المجموعة الثانية (٥٠%) عن المجموعة الأولى (٣٠.٨%) والمجموعة الثالثة (٣٣.٤%). مرحلة Compact morula كانت أعلى فى المجموعة الأولى والثالثة (٥٣.٨% - ٤١.٧%) عن المجموعة الثانية (٢٥%)، مقابل ١٥.٤% - ١٦.٦% لمرحلة Blastocyst فى المجموعة الأولى والثالثة. الأجنه القابلة للنقل كانت ٩٥.٣% ـ ٥٠% ـ ٨٣.٣% للمجموعة الأولى والثانية والثالثة على التوالى. التبويض الفائق للأبقار الفريزيان بواسطة هرمون PMSG (٢٥٠٠ وحدة دولية لكل بقرة) فى اليوم العاشر من دورة الشياع مقسمة على أربع جرعات (٦٢٥ وحدة دولية لكل جرعة بينهم ١٢ ساعة) أظهر أفضل إستجابة حويصلية من حيث العدد والقطر فى يوم التلقيح، وأظهرت أعلى استجابة مبيضية من حيث عدد الحويصلات التى لم يحدث لها تبويض، وعدد الأجسام الصفراء، ونسبة الأبقار التى استجابت لإنتاج الأجنه (٧١.٤%) فى يوم التجميع. بينما الجرعة الواحده من هرمون PMSG أظهرت أعلى استجابة من حيث عدد الأجنه القابله للنقل لكل بقره (١.٧١ جنين لكل بقرة).