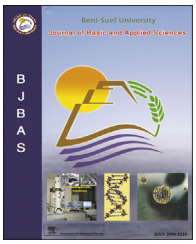
HOSTED BY

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/bjbas



Full Length Article

Preliminary study of factors affecting the superovulatory response of high producing dairy cows superstimulated regardless of the stage of estrous cycle in Egypt

M.M. Hussein [a], R.L. Abdel Aziz [a,*], A. Abdel-Wahab [b], H. El-Said [c]

[a] Department of Theriogenology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt
[b] Department of Physiology, Faculty of Veterinary Medicine, Minia University, El-Minia, Egypt
[c] Veterinary and Embryo Transfer Section, Milkys Farm, Al-buhyrah, Egypt

ARTICLE INFO

Article history:
Received 25 September 2014
Accepted 15 November 2014
Available online 9 December 2014

Keywords:
Superovulation
Dairy cow
Milk yield
Parity
Propylene glycol

ABSTRACT

This work was conducted as a first time commercial production of embryos from lactating Holstein and Brown Swiss cows using multiple ovulation embryo transfer (MOET) technology in Egypt. We studied factors affecting the superovulatory response (SR) in superovulated cows and effects of propylene glycol (PG) on embryo quality. Daily milk production at flushing had significantly negative effects on SR and embryo yields in superovulated cows. In addition, Brown Swiss cows had better SR than Holstein cows. Moreover, cows having more than 3 parities yielded better response, compared to cows in the first three parities. However, factors such as body weight at flushing, body condition score (BCS) at flushing and days in milk (DIM) at flushing did not have any association with SR in cows. In addition, drenching of PG prior to and during the superovulatory treatment improved SR (Right CL number, $P < 0.05$; Left CL number, $P < 0.05$), total embryos per flush ($P < 0.05$), first grade embryos per flush ($P < 0.01$) and tended to improve transferable embryos ($P = 0.13$) and second grade embryos per flush ($P = 0.11$). However, it tended to increase the number of degenerated embryos per flush (0.06). In conclusion, commercial production of embryos from lactating Holstein and Brown Swiss cows regardless of stage of estrous cycle by MOET proved successful under Egyptian conditions. Milk yield was negatively associated with SR and embryo yields in superovulated cows. Moreover, drenching of PG prior to and during the superovulatory treatment improved SR and embryo yields.

* *Corresponding author.*
E-mail addresses: rabi3zahran@yahoo.com, rabea.ramadan@vet.bsu.edu.eg (R.L. Abdel Aziz).
Peer review under the responsibility of Beni-Suef University.
http://dx.doi.org/10.1016/j.bjbas.2014.11.002

1. Introduction

Multiple ovulation embryo transfer (MOET) can circumvent the low fertility, improve genetics in dairy cows and can be used to obtain over 80% of embryos produced for commercial purposes (Lamb, 2005; Hasler, 2012). However, the variable SR (Bo and Mapletoft, 2014) and yield of viable embryos (Greve et al., 1995) still limit the success of MOET in cattle. Donor factors leading to this variable response are not fully understood in spite of extensive research. Superovulation is usually initiated during mid-cycle, 8–12 days after estrus (Bo et al., 1995) which was considered to coincide with the time of emergence of the second follicular wave in cows (Ginther et al., 1989). Hormonal control of the timing of follicular wave emergence can target the superovulatory treatments to be initiated at the beginning of a follicular wave in normally cyclic cows (Bo et al., 2006) without the need to know the time of the base heat which is a difficult task in large dairy herds. Controlled internal drug release (CIDR) was the most commonly used hormonal source to control follicular wave emergence in combination with injections of estradiol -at varying dosages- and progesterone with variable results (Bo et al., 2002;Colazo et al., 2005 and Hasler, 2014). Moreover, estrus detection during the superstimulatory protocol can be eliminated by using protocols which synchronize ovulation (Bo et al., 2006). However, estradiol treatment which is a commonly used approach for synchronization of follicular wave emergence for superstimulation cannot be used in many countries because of side effects and public health significance of estrogens. Recently, a protocol which allows removal of the progesterone device in the PM of day 7 (day zero is the day of device insertion), injection of GnRH in the PM of day 8 and AI 12 and 24 h later yielded more synchronous ovulations and higher numbers of transferable embryos (Bo and Mapletoft, 2014). In addition, an increase in nutrient intake during superovulation and an acute change to a low nutritional intake regime immediately after ovulation was estimated to maximize ovulation rate and enhance embryo quality in cows (Kakar et al., 2005).

Accordingly, the current study was conducted to evaluate factors which affect donors' responses to a superovulatory protocol initiated regardless of the stage of estrous cycle in dairy cows of a large herd under Egyptian conditions as a first attempt for commercial production of embryos using MOET technique. Another aim was to study effects of PG on SR and embryo quality in dairy cows.

2. Material and methods

2.1. Study design

This study was carried out during the period from December, 2011 to March, 2013. In experiment 1, we studied factors affecting SR and embryo yields of 69 high producing dairy cows superstimulated regardless of the stage of estrous cycle and submitted to timed artificial insemination (TAI). In experiment 2, we examined effects of PG on SR and embryo yields of high producing dairy cows. Forty two treated cows of experiment 2 were examined during December, 2012–March, 2013; while 27 cows superovulated during December, 2011–March, 2012 served as control. Table 1 provides data about characters of superovulated cows during the two years.

2.2. Animals, feeding and management

Sixty nine lactating high producing (average daily milk at flush 39.16 Kg) dairy cows (57 Holstein and 12 Brown Swiss) belonging to a private dairy herd in North-western Egypt were included in the study. Animals were housed in a free, partially roofed head-lock yard system. They were fed according to the National Research Council (2001) recommendations on eight occasions to maximize their feed intake. Cows were milked three times per day. The voluntary waiting period of the herd was 45 days then cows were subjected to a pre-synch-ovsynch TAI program which began on day 45 postpartum by injection of 500 μg cloprostenol (2 ml Estrumate®, MSD, USA) and another dose on day 59 postpartum. Twelve days later, animals were subjected to an ovsynch program including injection of 12 μg busrelin (3 ml Receptal®, MSD, USA) on day 70, 500 μg cloprostenol on day 77, 12 μg busrelin on day 79 and timed insemination 16 h later (Pursley et al., 1995). The resynchronization of ovulation began on day 41 post-insemination by intramuscular injection of 12 μg busrelin. On day 48 post-insemination, pregnancy diagnosis was carried out via trans-rectal palpation and open cows were injected with 500 μg Cloprostenol. On day 50 post-insemination, these open cows were injected with another dose, 12 μg busrelin and were timely inseminated 16 h later by experienced farm veterinarians using frozen-thawed semen from bulls of proven fertility (Galvao et al., 2007).

2.3. Superovulation, embryo recovery and evaluation

As shown in Table 2, a controlled internal drug releasing device (CIDR® insert, Pfizer, animal health care, USA) was aseptically inserted into the anterior vagina of cows at 6:00 AM on day zero. At 6:00 AM on day 2, each cow received 9 μg Busrelin, (Receptal®, MSD, USA) intramuscularly. The superovulatory FSH treatment was initiated 4 days after insertion of CIDR with a total dose of 400 mg pFSH (Folltropin-V®, Agtech inc, USA)in twice daily decreasing doses over 4 days. On 18:00 PM of the last day of FSH treatment, 500 μg cloprostenol (Estrumate®, MSD, USA) was given by intramuscular rout together with removal of CIDR. 24 h later, cows received 9 μg busrelin

Table 1 – Characters (Mean ± SE) of superovulated cows during the two experimental periods.

Parameter	Cows superovulated (December, 2012–March, 2013)	Cows superovulated (December, 2013–March, 2014)
Age	66.62 ± 2.43	71.63 ± 2.86
Parity	3.30 ± 0.16	3.76 ± 0.19
Body weight at flushing	674.58 ± 12.12	722.05 ± 8.64
BCS at flushing	3.25 ± 0.10	3.02 ± 0.07
DIM at flushing	338.81 ± 23.00	241.83 ± 31.99
Daily milk at flushing	41.72 ± 2.13	38.26 ± 1.79

Table 2 – Superovulation protocol with timed artificial insemination in dairy cows.

Days of treatment	6:00 (AM)	18:00 (PM)
0	CIDR insertion	
2	Busrelin (9 μg)	
4	FSH (80 mg)	FSH (80 mg)
5	FSH (60 mg)	FSH (60 mg)
6	FSH (40 mg)	FSH (40 mg)
7	FSH (20 mg)	FSH (20 mg) + Cloprostenol sodium (500 μg) + CIDR removal
8		Cloprostenol sodium (500 μg) + Busrelin (9 μg)
9	TAI	TAI
16	Uterine flush	

and 500 μg cloprostenol I/M. Cows were expected to be due in heat 12 h later, thus timed AI was applied 12 and 24 h after the last injection (Bo and Mapletoft, 2014).

Embryos were collected by flushing the uteri of superovulated cows on day 7 (6:00 AM) post-insemination. Each horn was flushed separately using 500 ml of flushing medium (VIGRO® complete flushing medium, Agtech inc, USA). After flushing, each cow received 750 μg cloprostenol (3 ml Estrumate®, MSD, USA) to induce regression of corpora lutea and to initiate a new cycle for normal breeding. Number of corpora lutea on each ovary were recorded for each cow during the flushing process by means of manual per rectal palpation. Embryos were received in an embryo filter which was cleaned into a squared embryo searching dish using 50 ml of the flushing medium by a sterile 50 ml capacity plastic syringe. Embryos were searched using a stereo microscope and were classified for quality. The evaluation process was done by the same experienced person along the course of the study. Grading of embryos, concerning quality and developmental stage, was done according to the International Embryo Transfer Society (IETS, 1998). All stages and grades of embryos were recorded for each cow individually. First grade embryos were symmetrical and spherical with blastomeres uniform in size, color and density with 85% of cellular material appearing as intact, viable embryonic mass, second grade embryos showed moderate irregularities in shape, color and density of embryonic mass with at least 50% of cellular material appearing as intact, viable embryonic mass. Third grade embryos were poor embryos with major irregularities in shape, size, color and density of embryonic mass presenting at least 25% intact viable cellular material. However, degenerated embryos appeared as a non-viable deformed shape arrested at various stages and may be an oocyte. First and second grade embryos were classified as freezable embryos. While first, second and third grade embryos were set as transferable embryos.

For each cow, at flushing, data concerning to breed, age, Parity, body weight, DIM, BCS and daily milk yield were recorded. BCS was estimated for each individual cow on a five point scale system with quarter points according to Edmonson et al. (1989). First grade embryos were transferred frozen, whereas second and third grade embryos were transferred fresh to recipient cows and heifers and achieved reasonable conception rates.

2.4. *Drenching of propylene glycol (PG) prior to and during superstimulation of dairy cows*

This experiment was carried out to investigate the effect of drenching PG (as a glucogenic agent) to dairy cows prior to and during the superovulatory treatment on SR and embryo yields. Dairy cows were divided into two groups. The control group included 27 Cows (superstimulated during December, 2012–March, 2013) which did not receive any treatment. The treated group included 42 cows (superstimulated during December, 2013–March, 2014). Each cow in the treated group was drenched 300 ml of monopropylene glycol (Propylene Glycol, SKC Ltd, Korea), once daily for 20 days prior to the beginning of -and during- the superovulation protocol.

2.5. *Statistical analyses*

Data were processed statistically using the software of SPSS (2007) package. Student's *T* test was used to examine the significance of different factors-nearly below or equal to average and above average of the group- affecting superovulatory response of dairy cows including failure of response (Experiment 1). *T* test was used also to examine the statistical significance between PG-treated cows and control cows (Experiment 2). Statistical significance was declared at $P < 0.05$ and was interpreted as extreme at $P < 0.01$, while $0.05 \leq P$ values ≤ 0.15 was considered a tendency.

3. Results

3.1. *Experiment 1*

3.1.1. *Descriptive statistics*

As shown in Table 3, Average numbers of right corpora lutea and left corpora lutea at flushing were 4.75 ± 0.44 and 3.91 ± 0.40, respectively. The average number of total embryos per flush was 7.48 ± 0.78 representing a recovery rate of 81.16 ± 3.93%. Average numbers of transferable embryos per flush, first, second and third grade and degenerated embryos were 4.86 ± 0.57, 3.68 ± 0.50, 0.90 ± 0.30, 0.25 ± 0.08 and 2.33 ± 0.40, respectively. More embryos were recovered at the Morula stage (2.26 ± 0.30), compared to early blastocyst (0.67 ± 0.15), blastocyst (1.30 ± 0.24) or expanded blastocyst stages (012 ± 0.07).

3.1.2. *Differences between cows with SR and those without SR*

No significant differences were observed between cows with SR and those without SR in the average values of age (71.35 ± 2.27 vs. 65.26 ± 8.18 months, respectively, $P = 0.32$), parity (3.67 ± 0.14 vs. 3.21 ± 0.32, respectively, $P = 0.16$), body

Table 3 – Descriptive statistics of embryo and ovarian characteristics in superovulated cows.

		Minimum	Maximum	Mean	Standard error
Right CL number		0	16	4.75	0.44
Left CL number		0	11	3.91	0.40
Total embryos per flush (n.)		0	22	7.48	0.78
Recovery rate (%)[a]		0	100	81.16	3.93
Transferable embryos per flush	n.	0	20	4.86	0.57
	%	0	100	68.46	4.10
Embryo grades					
1st grade embryos	n.	0	19	3.68	0.50
	% of total	0	100	50.54	4.47
2nd grade embryos	n.	0	17	0.9	0.30
	% of total	0	100	14.31	2.84
3rd grade embryos		0	3	0.25	0.08
Degenerated embryos/unfertilized oocytes	n.	0	12	2.36	0.40
	% of total	0	100	31.36	4.03
Embryo stages (number/flush)					
Morula		0	12	2.26	0.30
Early blastocyst		0	6	0.67	0.15
Blastocyst		0	8	1.30	0.24
Expanded blastocyst		0	4	0.12	0.07

[a] Recovery rate: number of collected embryos divided by number of CL before flushing multiplied by 100. n: number.

weight at flushing (705.96 ± 8.67 vs. 693.57 ± 16.13 kg, respectively, $P = 0.52$) and DIM at flushing (284.50 ± 31.37 vs. 217.80 ± 36.71 days, respectively, $P = 0.31$). However, the average daily milk yield at flushing day was significantly higher in cows without SR, compared to those with SR (46.12 ± 2.80 vs. 37.95 ± 1.52 kg, respectively, $P = 0.017$) as declared in Table 4.

3.1.3. *Donor factors affecting embryo yield and quality*

Table 5 represented animal factors responsible for the variability in response to superstimulation in examined cows. Holstein cows produced significantly higher ($P < 0.05$) number of degenerated embryos per flush, compared to Brown Swiss cows (2.71 ± 0.47 vs. 0.58 ± 0.26 embryos, respectively). Moreover, the response to superovulation was higher in cows having more than three parities, compared to those in the first three parities. Cows having greater than 3 parities had significantly higher ($P < 0.05$) number of total and degenerated embryos per flush (9.42 ± 1.12 and 3.33 ± 0.66 embryos, respectively), compared to cows in the first three parities (5.69 ± 0.99 and 1.42 ± 0.43 embryos, respectively). Total embryos per flush ($P < 0.01$), transferable and first grade embryos per flush ($P < 0.05$) were significantly higher in low producing donors (≤40 Kg milk at flushing day), compared to high producing donors (>40 Kg milk at flushing day). In addition, cows producing >40 kg of milk at flushing yielded lower ($P < 0.01$) numbers of degenerated embryos per flush (1.19 ± 0.37 vs. 3.58 ± 0.68) as compared to cows producing ≤40 kg.

Table 4 – Differences in donor factors between cows with SR (CL number greater than or equal to 3) and those without SR (CL numbers less than 3).

Factors	Cows with SR		Cows without SR		P value
	Mean	S.E.	Mean	S.E.	
Age (month)	71.35	2.27	65.26	8.18	0.32
Parity	3.67	0.14	3.21	0.32	0.16
Body weight at flushing (Kg)	705.96	8.67	693.57	16.13	0.52
BCS at flushing	3.09	0.08	2.89	0.08	0.23
DIM at flushing (day)	284.50	31.37	217.80	36.71	0.31
Daily milk at flushing (Kg)	37.95	1.52	46.12	2.80	**0.017**

Bold significance indicates significantly different means at P < 0.05.

3.2. *Experiment 2*

3.2.1. *Effect of drenching PG to donor cows prior to- and during – superovulation on SR and embryo yields*

As shown in Table 6, both SR and embryo yields were improved in PG-treated cows, compared to control cows. Average numbers of right and left corpora lutea were significantly ($P < 0.05$) higher in the PG-treated group (5.50 ± 0.55 and 4.68 ± 0.69, respectively), in comparison with 3.59 ± 0.69 and 2.74 ± 0.61, respectively, in the control group. Similarly, total embryos per flush were significantly ($P < 0.05$) increased in the PG-treated group (8.88 ± 0.98), compared to the control group (5.30 ± 1.16). Moreover, transferable embryos per flush tended ($P = 0.13$)to be higher in the PG-treated cows, compared to the control cows (5.55 ± 0.72 vs. 3.78 ± 0.93, respectively). Also, first grade embryos per flush were significantly ($P < 0.01$) increased in the PG-treated group (4.73 ± 0.70), compared to 2.08 ± 0.52 in the control group. In spite of that, second grade embryos per flush tended ($P = 0.11$) to be higher in the control group, compared to the PG-treated group (1.44 ± 0.67 vs. 0.54 ± 0.13, respectively). In addition, degenerated embryos per flush tended ($P = 0.06$) to be higher in the PG-treated group (2.95 ± 0.59), compared to the control group (1.37 ± 0.43).

4. Discussion

The present study was conducted as a trial for commercial production of embryos for the first time using MOET in Egypt.

Table 5 – Donor factors affecting embryo yield and quality following superovulation (Mean ± S.E.) in dairy cows.

Factors		Response			
		Total embryos/flush	Transferable embryos/flush	First grade embryos/flush	Degenerated embryos/flush
Breed	Holstein	7.36 ± 0.82	4.65 ± 0.57	3.65 ± 0.49	2.71 ± 0.47
	Brown Swiss	8.00 ± 2.23	5.83 ± 1.91	3.92 ± 1.62	0.58 ± 0.26
	P value	0.76	0.44	0.84	**0.04**
Parity	≤3	5.69 ± 0.99	4.08 ± 0.73	3.28 ± 0.64	1.42 ± 0.43
	>3	9.42 ± 1.12	5.70 ± 0.89	4.15 ± 0.75	3.33 ± 0.66
	P value	**0.02**	0.16	0.38	**0.02**
Body weight at flushing (Kg)	≤700	7.15 ± 1.09	4.42 ± 0.78	3.36 ± 0.60	2.24 ± 0.54
	>700	7.78 ± 1.11	5.25 ± 0.84	4.00 ± 0.76	2.42 ± 0.60
	P value	0.69	0.47	0.52	0.83
BCS[a] at flushing	≤3	8.57 ± 1.37	5.79 ± 0.99	4.18 ± 0.86	2.32 ± 0.65
	>3	6.77 ± 1.49	4.46 ± 1.22	3.85 ± 1.18	2.00 ± 0.w88
	P value	0.43	0.43	0.83	0.78
DIM[b] at flushing (Day)	≤272	7.94 ± 1.25	5.16 ± 30.92	0.71 ± 0.79	2.36 ± 0.62
	>272	8.10 ± 1.32	4.71 ± 0.89	3.86 ± 0.75	3.00 ± 0.87
	P value	0.93	0.74	0.90	0.54
Daily milk at flushing (Kg)	≤40	9.91 ± 1.07	6.09 ± 0.88	4.91 ± 0.79	3.58 ± 0.68
	>40	5.25 ± 0.99	3.72 ± 0.71	2.58 ± 0.54	1.19 ± 0.37
	P value	**<0.01**	**0.04**	**0.02**	**<0.01**

Bold significance indicates significantly different means at $P < 0.05$.
[a] BCS. Body condition score.
[b] DIM. Days in milk.

In this work, superovulation was carried on high producing dairy cows having unknown stages of estrous cycles using TAI.

The average number of total embryos recovered per flush in the present study came in accordance with that reported by Chebel et al. (2008). However, mean number of right and left CL was lower and recovery rate was higher than those previously reported by Garcia Guerra et al. (2012) who used transrectal ultrasonography in the counting process. Also, Carvalho et al. (2013) recovered less than 50% of embryos. It may be possible that the numbers of corpora lutea were underestimated in the present study as they were counted by transrectal palpation of the ovaries without using ultrasonography or hormonal assays (Drillich et al., 2012).

Table 6 – Effect of drenching of PG to donor cows prior to superovulation on CL numbers, embryo yields and quality (Mean ± S.E.).

	Control group	PG-treated group	P value
Right CL number	3.59 ± 0.69	5.50 ± 0.55	**0.03**
Left CL number	2.74 ± 0.61	4.68 ± 0.49	**0.02**
Total embryos per flush	5.30 ± 1.16	8.88 ± 0.98	**0.02**
Transferable embryos per flush	3.78 ± 0.93	5.55 ± 0.72	0.13
1st grade embryos per flush	2.08 ± 0.52	4.73 ± 0.70	**<0.01**
Second grade embryos per flush	1.44 ± 0.67	0.54 ± 0.13	0.11
Degenerated embryos per flush	1.37 ± 0.43	2.95 ± 0.59	0.06

Bold significance indicates significantly different means at $P < 0.05$.

The mean number and percentage of transferable embryos were similar to those reported by Drillich et al. (2012) but were higher than those obtained by Garcia Guerra et al. (2012) and Viera et al. (2014). Differences in the study design, and regime of superovulation may account for these results. In addition, number of transferable embryos obtained per flush may be affected by several factors such as gonadotrophic hormone, donor, nutrition and/or dominant follicle in the donor's ovary (Betteridge, 2006). Results concerning first grade embryos indicated a good superovulatory response according to Kohram and Poorhamdollah (2012) who stated that objective criteria of embryo production used in the embryo transfer industry indicated that on average, 3 to 4. First-grade embryos are collected following superovulation in dairy cows. The mean number of degenerated embryos per flush in the present study was higher than that observed by Peippo et al. (2009) and Viera et al. (2014). The average daily milk of superovulated cows at flushing was 39.61 kg. The higher number and percentage of degenerated embryos may be due to higher incidence of poor fertilization results (Viera et al., 2014), rather than poor embryo quality, which accompany high levels of milk production in lactating dairy cows (Leroy et al., 2005; Peippo et al., 2009) and this is supported by results of transferable embryos per flush since we observed high number of them.

None of the investigated animal factors had a significant effect on SR but for the average daily milk yield at flushing, where cows without SR produced significantly ($P < 0.05$) higher daily milk at flushing, compared to those with SR. Although hormonal analyses were not carried out, it is possible that low progesterone levels in cows without SR might have been the cause of failure of response in these cows (Chagas e Silva et al., 2002). Moreover, the greater dry matter intake of lactating dairy cows increases the metabolism of estradiol leading to follicular

persistence and reduced quality of oocytes (Mann and Lamming, 2000; Sangsritavong et al., 2002 and Wiltbank et al., 2006).

In the present study, Brown Swiss cows yielded 5.83 transferable embryos per flush. This result is greater than previously reported by Bulbul et al. (2010) who used progesterone and estradiol pretreatment to synchronize the estrous cycle before superstimulation. Additionally, Holstein cows produced significantly ($P < 0.05$) higher number of degenerated embryos than Brown Swiss cows. Leroy et al. (2005) reported that lactating Holstein Friesian cows produced significantly more embryos of darker color and inferior quality, compared to embryos from Belgian blue beef cows. Moreover, Correa-Calderon et al. (2005) reported that Brown Swiss cows were better adapted to adverse environmental conditions, compared to Holstein cows. Breed differences in timing of ovulation, fertilization or events leading to cleavage were reported by Krininger III et al. (2003).

Cows of more than three parities produced significantly ($P < 0.05$) higher total embryos per flush and significantly ($P < 0.05$) lower degenerated embryos per flush as compared to those in the first three parities, in the present study. Similarly, Prdhan et al. (2008)reported that >3 parity cows produced higher number of ova/embryos and significantly ($P < 0.05$) higher number of transferable embryos, compared to 2–3 parity cows. It is clear that cows in the first three parities included primiparous and biparous cows which were still growing while producing high amounts of milk. Such stressors are believed to depress early embryonic development and overall fertility of these cows (Walters et al., 2002).

There were no significant effects of body weight at flushing and BCS at flushing on SR and embryo yields in the present study. This is not surprising because the minimal value of BCS recorded for cows in this study was 2.75.

Milk production had significant negative effects on embryo yields in the present study which came in agreement with Chebel et al. (2008). It was reported that high feed intake and increased steroid metabolism were reported to lower progesterone concentrations in blood of high producing dairy cows (Sangsritavong et al., 2002). Rivera et al. (2011) reported that superstimulation of dairy cows in an environment of low progesterone concentration results in reduced ova/embryo quality.

In the present study, drenching PG to lactating donors prior to – and during- superovulation had positive effects on average numbers of right and left corpora lutea, total embryos, transferable embryos and first grade embryos per flush. Gamarra et al. (2014) mentioned that PG supplementation to dairy cows may be effective in improving oocyte production when combined with hormonal treatments to stimulate follicular growth for superovulation or ovum pick up. Plasma concentrations of glucose and insulin are known to increase in response to dietary PG (Miyoshi et al., 2001). An increase in insulin concentrations over a short period of time has been shown to improve the growth of small ovarian follicles prior to superovulatory treatment and exert a beneficial effect on subsequent embryonic development (Freret et al., 2006). An unexpected increase in the average number of degenerated embryos per flush was observed in treated cows. This result in unexplainable and deserves further investigation. One criticism of this experiment may be that treated cows were not in the same year of control cows. We can explain that although treated and control cows were in different years (2014 vs. 2013), both were managed strictly under the same managerial procedures and superovulatory treatments were scheduled to begin on same days of experimental periods (December to March).

5. Conclusion

First time commercial production of embryos from high producing lactating dairy cows using MOET technique regardless of stage of estrous cycle proved successful under Egyptian conditions. Factors other than milk yield and parity did not have significant effects on results of the superovulatory treatment. In addition, drenching of PG to lactating dairy cows prior to and during the superovulatory treatment improved SR and embryo yields. Future research is warranted to explain the beneficial effects of PG on superovulatory responses of lactating donors.

Acknowledgments

The authors thank all employees and workers of Milkys dairy farm for their kind cooperation.

REFERENCES

Betteridge KJ. Farm animal embryo technologies: achievements and perspectives. Theriogenol 2006;65:905–13.

Bo GA, Adams GP, Pierson RA, Mapletoft RJ. Exogenous control of follicular wave emergence in cattle. Theriogenol 1995;43:31–40.

Bo GA, Baruselli PS, Moreno D, Cutaia L, Caccia M, Tribulo R. The control of follicular wave development for self-appointed embryo transfer programs in cattle. Theriogenol 2002;57:53–72.

Bo GA, Baruselli PS, Chesta PM, Martins CM. The timing of ovulation and insemination schedules in superstimulated cattle. Theriogenol 2006;65:89–101.

Bo GA, Mapletoft RJ. Historical perspectives and recent research on superovulation in cattle. Theriogenol 2014;81:38–48.

Bulbul B, Kirbas M, Kose M, Dursun S. Investigation of superovulation response in Brown Swiss cows after synchronization using progesterone and estradiol valerate. Kafkas Univ Vet Fac Derg 2010;16:463–8.

Carvalho PD, Souza AH, Sartori R, Hackbart KS, Dresch AR, Vieira LM. Effects of deep-horn AI on fertilization and embryo production in superovulated cows and heifers. Theriogenol 2013;80:1074–81.

Chagas e Silva J, Lopes da Costa L, Robalo Silva J. Embryo yield and plasma progesterone profiles in superovulated dairy cows and heifers. Anim Reprod Sci 2002;69:1–8.

Chebel RC, Demetrio DGB, Metzger J. Factors affecting success of embryo collection and transfer in large dairy herds. Theriogenol 2008;69:98–106.

Colazo MG, Martinez MF, Small JA, Kastelic JP, Burnley CA, Ward D, et al. Effects of estradiol valerate on ovarian follicular dynamics and superovulatory response in progestin-treated cattle. Theriogenol 2005;63:1454–68.

Correa-Calderon A, Armstrong DV, Ray DE, DeNise SK, Enns RM, Howison CM. Productive and reproductive responses of Holstein and Brown Swiss heat stressed dairy cows to two different cooling systems. J Anim Vet Adv 2005;4:572–8.
Drillich M, Tesfaye D, Rings F, Schellander K, Heuwieser W, Hoelker M. Effects of polymorphonuclear neutrophil infiltration into the endometrial environment on embryonic development in superovulated cows. Theriogenol 2012;77:570–8.
Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G. A body condition scoring chart for Holstein dairy cows. J Dairy Sci 1989;72:68–78.
Freret S, Grimard B, Ponter AA, Joly C, Ponsart C, Humblot P. Reduction of body weight gain enhances in vitro embryo production in overfed superovulated dairy heifers. Reproduction 2006;131:783–94.
Galvao KN, Santos JEP, Cerri RL, Chebel RC, Rutigliano HM, Bruno RG, et al. Evaluation of methods of resynchronization for insemination in cows of unknown pregnancy status. J Dairy Sci 2007;90:4240–52.
Gamarra G, Ponsart C, Lacaze S, Le Guienne B, Deloche MC, Monniaux D, et al. Short term dietary propylene glycol supplementation affects circulating metabolic hormones, progesterone concentrations and follicular growth in dairy heifers. Livest Sci 2014;162:240–51.
Garcia Guerra A, Tribulo A, Yapura J, Singh J, Mapletoft RJ. Lengthening the superstimulatory treatment protocol increases ovarian response and number of transferable embryos in beef cows. Theriogenol 2012;78:353–60.
Ginther OJ, Kopf L, Kastelic JP. Temporal associations among ovarian events in cattle during estrous cycles with two or three follicular waves. J Reprod Fertil 1989;87:223–30.
Greve T, Callessen H, Hyttel R, Assey R. The effects of exogenous gonadotropins. Theriogenol 1995;43:41–50.
Hasler JF. Bovine embryo transfer: are efficiencies improving?. In: Proceedings, applied reproductive strategies in beef cattle 2012; December 3–4, Sioux Falls, SD; 2012.
Hasler JF. Forty years of embryo transfer in cattle: 299 a review focusing on the journal Theriogenology, the growth of the industry in North America and personal reminisces. Theriogenol 2014;81:152–69.
IETS. Manual of the international embryo transfer society. 3rd ed. 1998.
Kakar MA, Maddocks S, Lorimer MF, Kleemann DO, Rudiger SR, Hartwich KM, et al. The effect of peri-conception nutrition on embryo quality in the superovulated ewe. Theriogenol 2005;64:1090–103.
Kohram H, Poorhamdollah M. Relationships between the ovarian status and superovulatory responses in dairy cattle. Anim Reprod Sci 2012;131:123–8.
Krininger III CE, Block J, Al-Katanani YM, Rivera RM, Chase Jr CC, Hansen PJ. Differences between Brahman and Holstein cows in response to estrus synchronization, superovulation and resistance of embryos to heat shock. Anim Reprod Sci 2003;78:13–24.
Lamb C. Factors affecting an embryo transfer program. In: Proceedings, applied reproductive strategies in beef cattle 2005; October 27–28, Reno, Nevada; 2005.
Leroy JL, Opsomer G, De Vliegher S, Vanholder T, Goossens L, Geldhof A, et al. Comparison of embryo quality in high-yielding dairy cows, in dairy heifers and in beef cows. Theriogenol 2005;64:2022–36.
Mann GE, Lamming GE. The role of suboptimal preovulatory oestradiol secretion in the etiology of premature luteolysis during the short estrous cycle in the cow. Anim Reprod Sci 2000;64:171–80.
Miyoshi S, Pate JL, Palmquist DL. Effects of propylene glycol drenching on energy balance, plasma glucose, plasma insulin, ovarian function and conception in dairy cows. Anim Reprod Sci 2001;68:29–43.
National Research Council. Nutrient requirements of dairy cattle. 7th revised ed. Washington DC: National Academic Science; 2001
Peippo J, Vartia K, Kananen-Anttila K, Räty M, Korhonen K, Hurme T, et al. Embryo production from superovulated Holstein-Friesian dairy heifers and cows after insemination with frozen-thawed sex sorted X spermatozoa or unsorted semen. Anim Reprod Sci 2009;111:80–92.
Prdhan R, Oshima K, Ochiai Y, Kojima T, Yamamoto N, Ghanem M, et al. Influence of season and parity on embryo recovery and subsequent reproductive performance in early postpartum suckling Japanese black cows. Livest Res Rur Dev 2008;20:2 (Abstract).
Pursley JR, Mee M, Wiltbank MC. Synchronization of ovulation in dairy cows using prostaglandin F2 alpha and GnRH. Theriogenol 1995;44:915–23.
Rivera FA, Mendonca LGD, Lopes Jr G, Santos JEP, Perez RV, Amstalden M, et al. Reduced progesterone concentration during growth of the first follicular wave affects embryo quality but has no effect on embryo survival post transfer in lactating dairy cows. Reprod 2011;141:333–42.
Sangsritavong S, Combs DK, Sartori R, Armentano LE, Wiltbank MC. High feed intake increases liver blood flow and metabolism of progesterone and estradiol 17-beta in dairy cattle. J Dairy Sci 2002;85:2831–42.
SPSS. User's guide for statistical analysis. 2007. Version 15, Free Software Internet Download.
Vieira LM, Rodrigues CA, Castro Netto A, Guerreiro BM, Silveira CRA, Moreira RJC, et al. Superstimulation prior to the ovum pick-up to improve *in vitro* embryo production in lactating and non-lactating Holstein cows. Theriogenol 2014;82(2):318–24.
Walters AH, Bailey TL, Pearson RE. Parity-related changes in bovine follicle and oocyte populations, oocyte quality, and hormones to 90 days postpartum. J Dairy Sci 2002;85:824–32.
Wiltbank MC, Lopez H, Sartori R, Sangsritavong S, Gumen A. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenol 2006;65:17–29.