

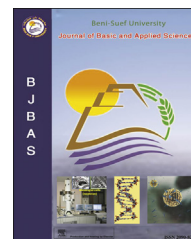
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## Full Length Article

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

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## 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.

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## 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

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