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USE OF PROGESTERONE IN SUPEROVULATION PROTOCOLS FOR EMBRYO PRODUCTION IN FRIESIAN COWS

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ABSTRACT

The current study was designed to determine the effect of different superovulation regimens on ovarian follicular dynamics, superovulatory response and embryo production in Friesian cows. A total of 24 Friesian cows (450-550 Kg LBW, 3.5-5.5 years old and 1-3 parities) were used as donor cows. Cows were divided into 3 groups (8 animals in each group). Cows in the 1st group (Control) before treatment, donor cows were injected i.m. with 2 ml PGF2α to bring them on heat to start oestrous cycles. All cows were injected i.m. with a single dose of 3000 IU PMSG on day 10 of the estrous cycle then injected with 3 ml PGF2α 48 h later. However, in the 2nd group, at random stages of the oestrus cycle cows received Syncro-Mate-B (SMB) implant together with one i.m. injection of 5 mg from estradiol benzoate and 100 mg progesterone, then cows were injected with PMSG (3000 IU) and PGF2α (3 ml) on Day 7 of treatment and implant was removed 2 d later. In the 3rd group, donor cows received Controlled Internal Drug Release (CIDR) together with one i.m. injection of 4 mg estradiol benzoate at random stages of the oestrus cycle, then cows were injected with a single i.m. of PMSG (3000 IU) and PGF2α (3 ml) at the time of CIDR removal on day 7. Cows in all groups artificially inseminated when they exhibited estrus, and flushing was conducted 7 days after AI. Ovulatory response was determined in terms of number of CLs as well as number and diameter of the follicles by ultrasonography device. Results showed that estrus rate was 100% in all groups. Estrus incidence after PGF injection was later ($P<0.05$) in G2 and G3 (3.25 and 5.75 d) than in G1 (2.25 d), being ($P<0.05$) the latest in G3 (5.75 d), moderate in G2 (5.25 d) and the earliest in G1 (2.25 d) after the end of treatment. Percentage of cows produced embryos was higher ($P<0.05$) in G3 (62.5%) than in G1 and G2 (37.5%). On day of flushing, average number of CLs/cow was greater ($P<0.05$) in G3 (7.50) than in G1 and G2 (3.50 and 4.62), respectively. Number of un-ovulated follicles showed an opposite trend ($P\geq0.05$). Ovulation rate was higher ($P<0.05$) in G3 (70.28%) than in G1 and G2 (40.00 and 56.89%), respectively**.** Embryo recovery rate (63.3%) and yield of total embryos (4.75/cow) at early morula, compact morula stages (1.6 and 2.5/cow) were higher ($P<0.05$) in G3 than in G2 (28.4%, 1.28, 0.5 and 0.4/cow) and G1 (43.2%, 1.78, 0.5 and 0.5/cow), respectively. The differences in number of embryos at early blastocyst and blastocyst stages were not significant. Number of embryos at both morula and blastocyst stages was higher ($P<0.05$) in G3 (4.75/cow) than in G2 and G1 (1.28 and 1.65/cow), respectively. Cows in G3 produced the highest ($P<0.05$) number of transferable embryos (1.0 excellent and 2.25 good embryos/cow) as compared to other groups.

In conclusion, using CIDR device as progesterone source in superovulation protocol gives high ovulation rate and acceptable number of transferable embryos of excellent and good grades, which may consider as a useful tool for embryo transfer.

Keywords: Cows, follicle, superovulation, embryo production, embryonic quality.

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.

Superovulation 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; Adams, 1994). 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; Cushman et al., 1999).

Progesterone alters ovarian function in cattle by suppressing estrus and preventing ovulation (Christian and Casida, 1948). Progesterone also suppresses LH release (Savio et al., 1993), which in turn suppresses growth of the dominant follicle in a dose dependent fashion (Adams et al., 1992a). It is noteworthy that progesterone does not suppress FSH secretion (Adams et al., 1992a); therefore, follicular waves continue to emerge in the presence of a functional CL. Although progestins given for intervals exceeding the lifespan of a CL (i.e., >14 days) result in synchronous estrus upon withdrawal, fertility at the ensuing estrus is low. The types and doses of progestins used to control the estrous cycle in cattle are generally less efficacious than endogenous progesterone (from a CL) for suppressing LH; they result in high LH pulse frequency, development of “persistent” follicles (Savio et al., 1993) which contain aged oocytes, and poor fertility (Revah and Butler, 1996).

A combination of steroid hormones may be used to synchronize the emergence of follicular waves in embryo transfer programs of Nelore donors for superovulation. These treatment protocols also minimizes costs of embryo transfer by permitting the use of a large number of donors in a short period of time, making it a valuable tool for animal production programs that use this technology (Andrade et al., 2002**)**.

The aim of this study was to determine the effect of different superovulation regimens on ovarian follicular dynamics, superovulatory response and embryo production in Friesian cows.

MATERIALS AND METHODS

This study was conducted at Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture in co-operation with Animal Production Department, Faculty of Agriculture, Mansoura University. The experimental work was carried out at Karada Animal Production Research Station and International Livestock Management Training Center - Sakha (ILMTC), belonging to APRI and located in the north eastern part of the Nile Delta, Kafrelsheikh Governorate during the period from January to October 2013.

Animals:

A total of 24 Friesian cows having live body weight (LBW) of 450-550 kg, 3.5-5.5 years old of age and 1-3 parities were used as donor cows after showing at least 1-2 post-partum estrous cycles prior to superovulation treatments. Cows were divided into 3 experimental groups (8 animals in each). Cows in all experimental groups were subjected to the same managerial and feeding conditions**.**

Superovulatory regimens:

In Treatment group (1) (Control), before treatment, donor cows were injected with 2 ml $PGF_2\alpha$ (Estrumate, containing 263µg Cloprostenol Sodium BP (Vet) equivalent to 250µg Cloprostenol; Friesoythe, Germany) to bring them on heat to start oestrous cycles. All cows received 3000 I.U. PMSG i.m. (Folligon, Intervet International B.V., Boxmeer, The Netherlands) on day 10 of the oestrus and after 48 hours of PMSG cows were injected with 3 ml PGF2α to induce luteal regression (Fig.1).

In treatment group (2), at random stages of the oestrus cycle donor cows received Syncro-Mate-B (SMB; Intervet, Angers, France, each implant contains 6 mg Norgestomet) implant together with one i.m. injection of 5 mg estradiol benzoate and 100 mg of progesterone. A single i.m. injection of PMSG (3,000 IU) and one of PGF2α (3 ml) were given on Day 7 of treatment and implants were removed 2 days later (Fig.1).

In treatment group (3), at random stages of the oestrus cycle donor cows received Controlled Internal Drug Release (CIDR) (Eazi-Breed CIDR Cattle Insert; Pfizer Animal Health; New Zealand Ltd, each insert contains 1.38 g progesterone) with one i.m. injection of 4 mg estradiol benzoate. A single i.m. injection of PMSG (3,000 IU) and one of PGF2α (3 ml) were given at the time of CIDR removal on Day 7 (Fig.1).

Cows were kept under observation for heat detection; thereafter all cows treated with PGF2α and progestagen removal were artificially inseminated two times with frozen semen of the same bull at 12 hours intervals following the detection of standing heat.

Flushing was conducted 7 days after AI to determine the ovulatory response to superovulation 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 non-ovulated follicles presented on the ovarian surface. 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). One percent of fetal or estrus cow serum was added to the Dulbecco's phosphate buffer saline (PBS) for flushing.

Number of recovered embryos in morula and blastocyst stages, and transferable embryos as well as unfertilized ova and degenerated embryos were recorded. Embryos were also evaluated morphologically according to Takeda (1986) into excellent, good, fair and poor on basis of their morphological symmetry, stage of blastomeres and age of embryo in relation to stage of the donor oestrous cycle as well as the presence of vesicles and color of embryo. Number of transferable embryos was calculated as number

of embryos at morula, compact morula and blastocyst stages only on excellent and good grades.

Ultrasound scanning

Cows in all experimental groups were daily subjected to ultrasonography device (ESAOTE Pie Medical Aquila Pro Vet + Probe 6.0/8.0 Mhz LA Rectal Veterinary Transducer) during treatment period to make examination of the ovaries in term of counting the number of corpora lutea (CLs) as well as number and diameter of follicles presented on the ovarian surface.

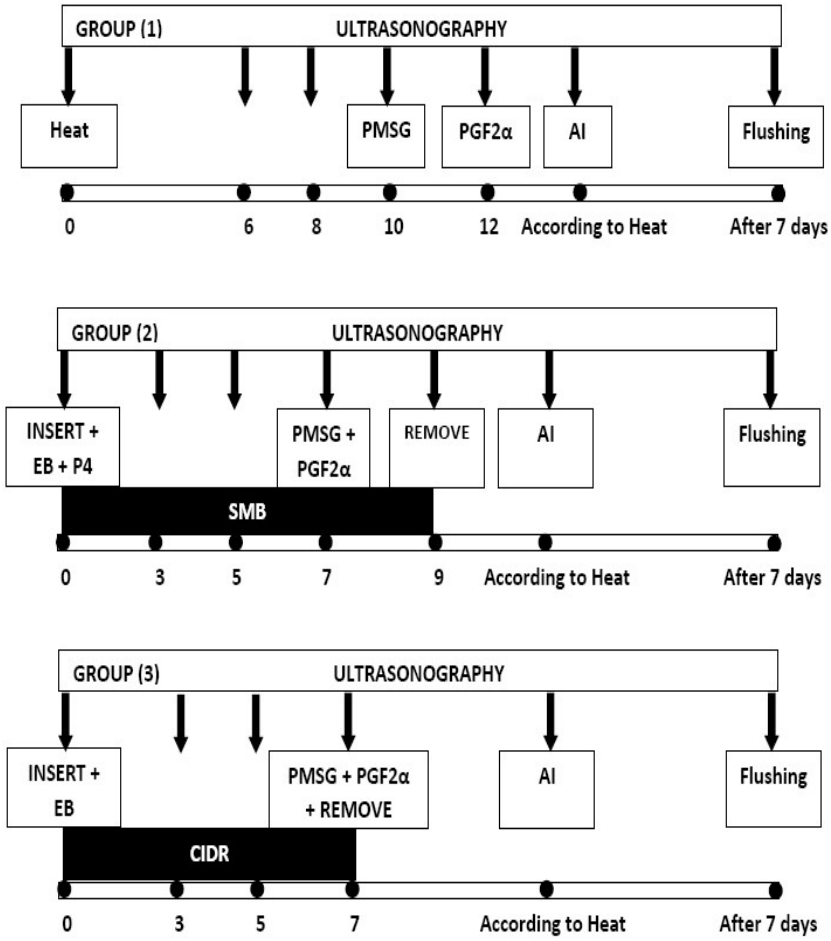

Fig.1: Superovulatory regimens of the experimental group (1) (control), group (2) and group (3).

Statistical analyses

Data in each experiment were subjected to factorial design according to Snedecor and Cochran (1982) using program of SAS (1998). Differences among group means were set at P<0.05 using Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSION

Estrus response and percentage cows produced embryos:

Data in Table 1 revealed incidence of estrus in 100% of cows in all treatment groups after PGF injection, being significantly (P<0.05) later in G2 and G3 (3.25 and 5.75 d) than in G1 (2.25 d) moderate in G2 (3.25 d) and the earliest in G1 (2.25 d). However, after the end of treatment, estrus incidence was significantly (P<0.05) the earlier in G1 (2.25 d), moderate in G2 (5.25 d) and the latest in G3 (5.75 d), respectively. Such difference may be associated with SMB removal on day 9 in G2 versus CIDR removal on day 7 in G3. It is of interest to note that percentage of cows produced embryos was significantly (P<0.05) higher for cows in G3 (62.5%) than in G1 and G2 (37.5% in each).

Regarding estrus response against PMSG administration, Fu et al. (2013) found that the estrus response was more than 70% in different doses of PMSG, although PMSG administration imparted a non-significant effect on the interval from PMSG administration to estrus, ranging between 74.3±6.9 and 98.3±7.6 h. The observed longer incidence of estrus/ovulation in G2 and G3 as compared to G1 may be attributed to that treatment with EB resulted in a longer and more variable interval to follicular wave emergence than treatment with estradiol-17β, which affected preovulatory dominant follicle size following progestin removal, and may have also affected superstimulatory response in Holstein cows (Colazo et al., 2005).

In seasonal dairy herds in New Zealand, cows were synchronized with PGF 13 d apart and supplemented or not with a CIDR for 5 d before the second PGF to increase circulating P4 before AI (Xu et al., 1997). The authors found an increase in the proportion of pregnancy per AI (P/AI) in cows inseminated after detected estrus (65.1 vs. 59.7%) in response to P4 supplementation. CIDR improved fertility in cows in earlier and mid-cycle. They suggested that increasing P4 in cows with lower P4 prior to PGF synchronization improves fertility at the subsequent AI. According to (Patterson et al., 1989), synchronization programs used in this study for G2 based on prolonged treatments with progestins (SMB) caused ovulation of larger follicles and may increase circulating E2 concentrations before breeding, which may reduce fertility. Collectively, E2+P4-based protocols are expected to induce a synchronous follicular wave emergence in 70 to 90% of the cows; most failures in synchronization are related to lack of dominant follicle regression and late wave emergence (Souza et al., 2009; Diskin et al., 2002). One of the key benefits of synchronizing the follicular wave by inhibiting follicle growth rather than by ovulating the dominant follicle is that a new CL is not present during the synchronization protocol, making incomplete

luteal regression less likely in an E2+P4 protocol compared with Ovsynch like protocols (Kim et al., 2007).

Table 1. Estrus response of superovulated cows after the end of PGF injection and end of treatment.

Item	Experimental group		
	G1	G2	G3
Number of treated cows	8	8	8
Treatment period (d)	12	9	7
Number of cows exhibiting estrus	8	8	8
Estrus (%)	100	100	100
Onset of estrus after PGF injection (d)	2.25^{b}	3.25^{ab}	5.75^{a}
Onset of estrus at end of treatment* (d)	2.25^{b}	5.25^{a}	5.75^{a}
cows produced embryos (%)	37.5^{b}	37.5^{b}	62.5^{a}

a and b: Means denoted within the same row with different superscripts are significantly different at P<0.05. * SMB or CIDR removal. * After the end of treatment on day 12, 9 and 7 in G1, G2 and G3.

Generally, increasing the circulating E2 after EB treatment at the beginning of CIDR insertion (G3), in the presence of high P4, causes regression of follicles that are present on the ovaries; approximately 3 to 5 d later, there is initiation of a new follicular wave **(Bo et al., 1995)**. Cows were treated with PGF to regress any CL that are present and 1 d later, and the CIDR is removed. In the presence of low circulating P4 after CIDR removal and PMSG injection at the same time produces a synchronized estrus and ovulation (Souza *et al.,* 2009). Souza *et al.* (2009) reported that combining a CIDR® insert with 2 mg of estradiol benzoate was effective in synchronizing follicular wave emergence 1–5 d after treatment.

Superovulatory response of cows:

Results in Table 2 show that superovulatory response of cows in term of average follicular number and mean diameter of largest follicle per cow at artificial insemination was not affected significantly by superovulation protocol. On day of flushing, average number of CLs/cow was affected significantly (P<0.05) by superovulation protocol, being greater in G3 (7.50/cow) than in G1 and G2 (4.12 and 4.50/cow), respectively. However, number of un-ovulated follicles showed an opposite trend, but the differences were not significant. Such results were reflected in significantly (P<0.05) higher ovulation rate in G3 (71.43%) than in G1 and G2 (47.03 and 55.42%), respectively.

These results may indicate that CIDR insertion for 7 days and injection of PMSG and PGF on day of CIDR withdrawal (G3) had positive effect on number of ovulated follicles as compared to SMB for 9 days and PMSG and PGF injection on day 7 of SMB insertion (G2). In this respect, Bo *et al.* (1991) suggested that the combination of Norgestomet and E2 injection when SMB was inserted in the early stages of the estrous cycle resulted in the inhibition of endogenous progesterone secretion and early CL regression. In the present study, the use of SMB with PMSG resulted in poorer response in G2 than CIDR + PMSG in G3. Such results are in agreement with Almeida (1987), who found that the use of PMSG+SMB significantly decreased the

number of CLs present at the time of embryo collection 7 days after insemination, as compared to other treatment regimens.

Table (2): Superovulatory response of cows on day of artificial insemination and at flushing.

Item	Experimental group		
	G1	G2	G3
At artificial insemination:			
Number of follicles/cow	8.76±0.31	8.12±0.50	10.50±0.61
Diameter of largest follicle (cm)	1.38±0.09	1.24±0.04	1.39±0.05
At flushing:			
Number of non-ovulated follicles/cow	5.26±0.40	3.5±0.30	3.12±0.36
Diameter of largest follicle (cm)	1.62±0.11	1.57±0.08	1.81±0.12
Number of corpora lutea/cow	3.50±0.39^{b}	4.62±0.43^{b}	7.38±0.62^{a}
Ovulation rate (%)	40.00±8.05^{b}	56.89±7.30^{b}	70.28±6.50^{a}

$^{a \text{ and } b}$**: Means denoted within the same row with different superscripts are significantly different at P<0.05.**

Also, Misra *et al.* (1994) found that treatment with PMSG (3000 IU) resulted in lower number of ovulations (3.76/cow), being lower than that obtained in cows of G2 (4.5/cow) or G3 (7.5/cow) recorded in the present study. However, Son *et al.* (2007) found that superovulatory treatments that follow administration of either EB or GnRH (at any stage of the estrous cycle) resulted in similar superovulatory response. They suggested that at any stage of the estrous cycle, artificial control of luteal phase, follicular growth and synchrony of ovulation can be effectively achieved by administration of either EB or GnRH prior to superovulatory treatments in CIDR-treated cows (Son et al, 2007).

Arora *et al.* (1996) found that mean number of CLs was 6.6/cow for lactating Jersey x red Sindhi cows induced with PMSG (2000 IU) on day 11 of the estrous cycle. However, higher number of CLs was reported by several authors, being 17.4 CL by rectal palpation on the day of embryo recovery following superovulatory treatments 4 days after 5 mg E-17β injection in progestogen-treated cows (Colazo´s, 2005), 24.3 CL in progestogen-treated cows slaughtered 72–96 h after estrus following superovulatory treatments 4 days after 5mg E-17β injection (Bo *et al.,* 1996), 10.1 CL in CIDR-treated cows by slaughtered 6 days after insemination following superovulatory treatments 2 days after 100 µg GnRH injection (Deyo et al., 2001) and 20.3 CL at 7 days after insemination following superovulatory treatments 2 days after 100 µg GnRH injection in CIDR-treated cows (Mitchell *et al.,* 1998).

In protocol of superovulation using intravaginal progesterone devices (Vos *et al*., 1994) or norgestomet ear implants (Van de Leemput, 1998; Vos *et al.,* 1995), to delay endogenous LH surge after PGF2a injection, a higher ovulation rate (29.6±3.8 vs. 20.5±2.3) was observed in the group with delayed LH surge compared to control group. This finding is in agreement with the present study using SMB (G2) or CIDR (G3) with higher ovulation rate of 56.9 and 70.3%, respectively. Treatment of dairy cows at random

stages of the estrous cycle with GnRH results in ovulation in only 50 to 70% of the cows (Pursley et al., 1995; Galvao and Santos, 2010).

The use of SMB combined with PMSG in G2 resulted in poorer responses than when CIDR with PMSG was given. This fact was particularly evident in the comparison between PMSG+SMB (G2) and PMSG+PGF regimen (G1) in the present study. This contrasted with the results reported by Saumande et al. (1980), which presented an improvement in super-ovulatory responses when the SMB+gonadotrophin treatment was used. Also, Hill et al. (1986) showed no difference related to the superovulatory response in cows treated with FSH, either alone or combined with SMB. Also, the results presented in this work are poorer than that reported by other authors (Monniaux et al., 1983; Elsden *et al.,* 1978), who reported better superovulatory responses after FSH treatment compared with PMSG.

Embryo production:

Recovery rate and embryonic stage:

Results presented in Table 3 revealed that cows in G3 showed significantly (P<0.05) the higher recovery rate (63.3%) and yield of total embryos (4.75/cow) at early morula, compact morula, early blastocyst and blastocyst stages (1.6 and 2.5/cow) than those in G2 (28.4%, 1.28, 0.5 and 0.4/cow) and G1 (43.2%, 1.78, 0.5 and 0.5/cow), respectively. However, the differences in number of embryos at early blastocyst and blastocyst stages among the experimental groups were not significant. Such results were reflected in significantly (P<0.05) more embryos at both morula and blastocyst stages in G3 (4.75/cow) than in G2 and G1 (1.28 and 1.65/cow), respectively.

Table 3. Recovery and yield of embryos at different stages per superovulated cow in treatment groups.

Item	Experimental group		
	G1	G2	G3
Number of CLs/cow	4.12±0.39^{b}	4.50±0.43^{b}	7.50±0.62^{a}
Number of embryos/cow	1.78	1.28	4.75
Embryo recovery rate (%)	43.2±4.43^{b}	28.4±3.01^{b}	63.3±3.94^{a}
Embryonic stage, n/cow (%)*:			
2-cell	0.13 (7.10)	-	-
Early morula	0.50^{b} (28.6)	0.50^{b} (40.0)	1.60^{a} (34.2)
Compact morula	0.50^{b} (28.6)	0.40^{b} (30.0)	2.50^{a} (52.6)
Early blastocyst	0.25 (14.3)	0.25 (20.0)	0.25 (5.26)
Blastocyst	0.40 (21.4)	0.13 (10.0)	0.40 (7.90)
Total, n/cow (%):			
Morulae	1.00^{b} (57.2)	0.90^{b} (70.0)	4.10^{a} (86.8)
Blastocysts	0.65 (35.7)	0.38 (30.0)	0.65 (13.2)
Morulae + blastocysts	1.65^{b} (92.9)	1.28^{b} (100)	4.75^{a} (100)

$^{a \text{ and } b}$**: Means denoted within the same row with different superscripts are significantly different at P<0.05. * Frequency distribution.**

It is worthy noting that cows in both treatment groups (G2 and G3) did not yield embryos at 2-cell stage versus 0.13/cow in G1(control). Also, cows in G3 showed the highest frequency distribution of embryos at all stages, particularly those at morula stage (Table 3).

The present results are in agreement with Almeida (1987), who found a significantly lower number of ova of cows superovulated with PMSG + SMB. However, Son et al. (2007) found that superovulatory treatments that follow administration of either EB or GnRH (at any stage of the estrous cycle) resulted in similar embryo yield comparable to conventional superovulation protocols. Olivera-Angel et al. (1984) investigated the embryo recovery rate in beef heifers and suckled cows following superovulation induced by 2000 IU PMSG combined with different methods of estrus cycle synchronization (Norgestomet, Prid, Dinolytic, Norgestomet combined with Dinolytic). They showed that embryo recovery rate was 39.5%, being lower than those obtained in the present study. Of the 149 embryos recovered, 48.9% had developed to the expected stage and 67.1% of these appeared normal.

Grades of recovered embryos:

Results in Table 4 show that cows in G3 produced significantly ($P<0.05$) the highest number of transferable embryos (1.0 excellent and 2.25 good embryos/cow) and even the highest number of fair and poor embryos (0.75/cow for each) as compared to other groups. It is worthy noting that, cows in G1 and G3 yielded the highest frequency distribution of good embryos (50.0 and 47.3%, respectively), while those in G2 showed the highest frequency distribution of excellent embryos (70.0%).

The present results suggested that the decrease in the number of transferable embryos in G2 may be associated with the sub-luteal concentration of progestagen released by the SMB which was removed 48 h after PMSG and PGF2a administration. Also, **Kohram et al. (1999)** observed that delayed the LH surge for approximately 24 h (i.e., until 70 h after PGF2a injection) using an intravaginal progesterone device and induced ovulation with GnRH, resulted in a decrease in the number of transferable embryos (3.6±1.2) when compared to control (5.8±1.2).

Table 4. Yield of embryos at different grades per superovulated cow in treatment groups.

Embryo grade n/cow (%)*	Experimental group		
	G1	G2	G3
Excellent	0.50^{b} (28.6)	0.88^{ab} (70.0)	1.00^{a} (21.1)
Good	0.88^{b} (50.0)	0.13^{b} (10.0)	2.25^{a} (47.3)
Fair	0.13^{b} (7.1)	0.13^{b} (10.0)	0.75^{a} (15.8)
Poor	0.25 (14.3)	0.13 (10.0)	0.75 (15.8)
Transferable embryos	1.38^{b} (78.6)	1.03^{b} (80.0)	3.25^{a} (68.4)

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

Andrade et al. (2002) evaluated the effectiveness of synchronization of follicular wave emergence using steroid hormone treatments in Nelore cows.

Donors were between days 9 and 12 of their cycle (TI), whilst those that were in any other stages of their estrus cycle constituted groups TII and TIII. TI donors were submitted to a standard protocol of superovulation, however, TII and TIII donors were treated with SMB or CIDR programs, respectively. They found that total ova and viable (transferable) embryos were 15.8 and 8.3 (TI), 15.6 and 8.9 (TII) and 17.3 and 9.9 (TIII), respectively ($P \geq 0.05$).

A decrease in transferable embryos (2.3 vs 3.4) was observed in the group with delayed LH surge compared to control group using intravaginal progesterone devices (Vos et al., 1994) or norgestomet ear implants (Van de Leemput, 1998; Vos et al., 1995), respectively. Olivera-Angel et al. (1984) found that following superovulation induced by 2000 IU PMSG combined with different methods of estrus cycle synchronization (Norgestomet), 48.9% had developed to the expected stage and 67.1% of these appeared normal.

In conclusion, the obtained results indicated that using CIDR device as progesterone source with 3000 IU of PMSG in superovulation protocol result in high ovulation rate and acceptable number of transferable embryos of excellent and good grades, which may consider as a useful tool for embryo transfer.

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استخدام البروجستيرون فى بروتوكولات التبويض الفائق لإنتاج الأجنة فى أبقار الفريزيان

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صممت التجربة لمعرفة تأثير استخدام بروتوكولات مختلفة للتبويض الفائق على ديناميكية تطور حويصلات المبيض والاستجابة للتبويض الفائق وانتاج الأجنة فى أبقار الفريزيان. اجريت هذه الدراسة على ٢٤ بقرة فريزيان تتراوح اوزانها بين (٤٥٠-٥٥٠ كجم) واعمارها بين (٣,٥-٥,٥ سنة) وفى الموسم (١-٣) . قسمت الابقار الى ٣ مجاميع (٨ حيوانات فى كل مجموعة). فى المجموعة الأولى (الكنترول) تم حقن الحيوانات عضليا بـ ٢ مل من هرمون البروستاجلاندين حتى تدخل فى شياع كى تبدأ فى دورات شياع جديدة . تم حقن جميع الحيوانات بجرعة واحدة قدرها ٣٠٠٠ وحدة دولية من هرمون الفرس الحامل (PMSG) فى اليوم العاشر من دورة الشياع ، يليها بـ ٤٨ ساعة الحقن العضلى بـ ٣ مل من هرمون البروستاجلاندين (PGF2α). اما فى المجموعة الثانية يتم زرع كبسولة البروجستيرون (SMB) بأذن الحيوانات عشوائيا فى أى يوم من أيام دورة الشياع مصحوبة بحقن ٥ ملجم من الاستراديول (Estradiol Benzoate) و ١٠٠ ملجم من البروجستيرون عضليا ، ثم حقن الحيوانات بـ ٣٠٠٠ وحدة دولية من هرمون الفرس الحامل (PMSG) و ٣ مل من هرمون البروستاجلاندين (PGF2α) عضليا فى اليوم السابع من زرع الكبسولة ثم تم ازالة الكبسولة فى اليوم التاسع. المجموعة الثالثة تم وضع جهاز البروجستيرون المهبلى (CIDR) فى الحيوانات عشوائيا فى أى يوم من أيام دورة الشياع

مصحوبة بحقن ٥ ملجم من الاستراديول (Estradiol Benzoate) وفى اليوم السابع من وضع الجهاز تم حقن الحيوانات بـ ٣٠٠٠ وحدة دولية من هرمون الفرس الحامل (PMSG) و ٣ مل من هرمون البروستاجلاندين (PGF2α) عضليا فى اليوم السابع مع نزع الجهاز. بعد ذلك تم تلقيح جميع الحيوانات التى أظهرت الشياع فى كل المجموعات ، ثم تم تجميع الأجنة بعد ٧ أيام من التلقيح و تحديد الإستجابة للتبويض عن طريق حساب عدد الأجسام الصفراء وعدد وقطر الحويصلات عن طريق جهاز التشخيص بالموجات فوق الصوتية. أوضحت النتائج أن معدل الشياع كان ١٠٠ % فى كل المجموعات. وحدوث الشياع بعد الحقن بهرمون البروستاجلاندين (PGF2α) كان متأخرا معنويا فى المجموعة الثانية والثالثة (٣,٢٥ و ٥,٧٥ يوم) على التوالى عن المجموعة الأولى (٢,٢٥ يوم). وكانت المجموعة الثالثة متأخرة بشكل معنوى فى اظهار الشياع (٥,٧٥ يوم) تليها المجموعة الثانية (٥,٢٥ يوم) ثم المجموعة الأولى (٢,٢٥ يوم) بعد نهاية المعاملة. كانت نسبة الأبقار التى أنتجت أجنة عالية معنويا فى المجموعة الثالثة (٦٢,٥%) عن المجموعتين الأولى والثانية (٣٧,٥%). فى يوم جمع الأجنة كان عدد الأجسام الصفراء لكل بقرة عالى معنويا فى المجموعة الثالثة (٧,٣٨) عنه فى المجموعة الأولى والثانية (٣,٥٠ و ٤,٦٢) على التوالى ، لكن كان عدد الحويصلات الغير مبوضة معاكس لذلك بشكل معنوى. معدل التبويض كان عالى معنويا فى المجموعة الثالثة (٧٠,٢٨%) عنه فى المجموعتين الأولى والثانية (٤٠,٥٠% و ٥٦,٨٩%) على التوالى. معدل استرداد الأجنة (٦٣,٣%) وعدد الأجنة لكل بقرة (٤,٧٥) وعدد الأجنة فى مرحلتى Early Morula و Compact Morula (١,٦ و ٢,٥) على التوالى أعلى معنويا فى المجموعة الثالثة عن المجموعة الثانية (٢٨,٤% ، ١,٢٨ ، ٠,٥ و ٠,٤/ بقرة) على التوالى و المجموعة الأولى (٤٣,٢% ، ١,٧٨ ، ٠,٥ و ٠,٥/ بقرة) على التوالى. الاختلافات بين اعداد الأجنة فى مرحلتى Early Blastocyst و Blastocyst غير معنوية. عدد الأجنة فى مرحلتى Morula و Blastocyst لكل بقرة عالى معنويا فى المجموعة الثالثة (٤,٧٥) عنه فى المجموعة الثانية والأولى (١,٢٨ و ١,٦٥) على التوالى. أعطت الحيوانات فى المجموعة الثالثة أعلى عدد من الأجنة القابلة للنقل بفرق معنوى عن باقى المجاميع (١ ممتاز ، ٢,٢٥ جيد لكل بقرة). وجد أن استخدام جهاز البروجستيرون المهبلى (CIDR) كمصدر للبروجستيرون فى بروتوكولات التبويض الفائق أعلى معدل للتبويض و عدد مقبول من الأجنة القابلة للنقل بجودة عالية (Excellent ,Good) ، والتى تعتبر وسيلة مفيدة فى نقل الأجنة.

الكلمات المفتاحية : الأبقار ، الحويصلات ، التبويض الفائق ، انتاج الأجنة ، جودة الأجنة.