Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
ISSN NO. 2320-5407
Article DOI: 10.21474/IJAR01/7847
DOI URL: http://dx.doi.org/10.21474/IJAR01/7847

***RESEARCH ARTICLE***

# COMPARISON BETWEEN ESTRADIOL AND GNRH-CIDR BASED PROGRAMS ON SUPER OVULATION IN HOLSTEIN COWS/HEIFERS.

**Oshba MR[1], Sosa GAM[3], Nossier MB[2], Fadel MS[1] and El-Raey M[3].**

1. Diagnostic Imaging and Endoscopy Unite, Animal Reproduction Research Institute, Al Haram.
2. Department of Theriogenology, Faculty of Veterinary Medicine, Alexandria University.
3. Department of Theriogenology, Faculty of Veterinary Medicine, Benha University, P.O: 13736, Tokh, Kaliobia, Egypt.

## Manuscript Info

***Manuscript History***
Received:09 August 2018
Final Accepted: 11 September 2018
Published: October 2018

***Keywords:-***
Cow, Gonadotropin, Oestradiol, Progesterone, Superovulation.

## Abstract

**Objectives:** The aim of the current study is to compare the response of GnRH-CIDR and estradiol-CIDR based programs on super ovulation outcomes in Holstein cows.

**Methods:** 12 Holstein cows/heifers were divided into two groups. Groups (A/N=7) synchronized with GnRH and group (B/N=5) synchronized with Estradiol. Both treated groups received progesterone releasing intravaginal device (CIDR) at day (0) and removed at day (7). Group (A) received GnRH (Receptal® /10 µg) at day (2 and 8) and Group (B) received estradiol benzoate at day (0). Both groups were super stimulated with Follicle-stimulating hormone (Follitropin-V) twice daily from day (4) to day (7). Cloprostenol (Estrumate®/500µg) was administrated at a day ($7^{PM}$& $8^{PM}$) in group (A) and at day ($6^{AM\&PM}$) for group(B). Cows/heifers were subjected to artificial insemination at day ($9^{AM\&PM}$and $8^{PM}$&$9^{AM}$) for group (A) and (B) respectively. The embryo flushing was done at day (16) for group (A) and at day (15) for group (B).

**Results:** The total number of recovered embryos was better in group (A/55) than group (B/45); although the recovered embryos per flush was 7.86 for group (A) which was lower than that of group (B/9). Even though, the embryo quality was better in group (A) as follow: 47.2% embryo grade I , 21.8% embryo grade II, 12.7% embryo grade III, 18.1% embryo grade IV, while they were 33.3%, 28.8%,11.1% and 26.6% respectively in group (B). The pregnancy rate in the recipient heifers were 30.90% and 24.44% for group (A) and (B) treated donors respectively.

**Conclusion:** GnRH-CIDR based program was better than estradiol-CIDRbased programon super ovulation in Holstein cows especially in terms of embryo quality, and pregnancy rate.



**Corresponding Author:-El-Raey M.**
Address:-Department of Theriogenology, Faculty of Veterinary Medicine, Benha University, P.O: 13736, Tokh, Kaliobia, Egypt

## Introduction:-

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)**. 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)**.

In the conventional superstimulation protocol, gonadotropin treatment is initiated during mid-cycle (8–12 days post-ovulation), coinciding approximately with the emergence of the second follicular wave in cows that have two-or three-wave cycles **(Ginther*et al*., 1989).**This approach required estrous detection prior to initiation of gonadotropin treatments. In addition, there was great individual variation in the precise timing of the second follicular wave.

Superovulation must be initiated on the day prior to, or on the day of follicular wave emergence, before the subordinate follicles begin the atresia process **(Adams, 1994 and Bo, 1995)**. This was because the presence of a dominant follicle during superovulation was known to decrease both super ovulatory response and embryo yield **(Bungartz and Niemann, 1994 and Kim *et al*., 2001)**. Synchronization of follicular wave emergence was achieved by removing the suppressive effect of the dominant follicle over the growth of the next follicular wave **(Bo, 1995)**.

One of the most promising strategies is the use of hormone treatments to synchronize the follicular wave so that it began at the start of superovulation **(Bo *et al*., 1993)**. Estrogen esters, such as estradiol benzoate (EB), Estradiol-17ß (E-17ß), or estradiol valerate, had been used to induce follicular wave emergence before super stimulation **(Bo, 1995 and Yaakub*et al*., 1998)**. Moreover, their use combined with subsequent superstimulatory treatments resulted in both superovulatory responses and embryo yields comparable to those initiated mid-diestrus**(Bo *et al*., 1996 and Andrade *et al*., 2002)**. Likewise, a number of studies had determined the efficiency of steroid hormone treatment in superovulatory protocols using progesterone/progestogen-treated cattle (**Nogueira*et al*., 2002; Andrade et *al*., 2003 and Colazo et *al*., 2005)**. On the other hand, there were been few reports on the effect of prior GnRH treatment on the superovulatory response or embryo yield, despite the fact that GnRH was shown to synchronize follicular wave emergence as effectively as estrogen esters following ovulation of a dominant follicle in cows **(Pursley*et al*., 1995 and Kim *et al*., 2005)**. However, in CIDR-treated cows, follicular wave synchronization with GnRH resulted in similar numbers of total ova and transferable embryos as synchronization with E-17ß **(Mitchell *et al*., 1998)**. Thus, further studies on these aspects of superovulation are warranted.

In the present study we compare the effects of EB and GnRH treatments prior to super stimulation with follicular stimulating hormone on the super ovulatory response and embryo yield in CIDR-treated, Holstein cows.

## Materials and Methods:-

### Animals and treatments

This study was performed from January to April 2016 at the Ellatar Dairy Farm Alex- Cairo desert road in Egypt. Twelve cows/heifers of body condition score 3.5±0.1 (1–5-point scale) **(Edmonson *et al*., 1989)** were selected. Chosen cows/heifers were subjected to clinical-gynecological examinations prior to experiment to ensure that they were healthy and of efficient reproductive system that free from diseases or pathological conditions. Animals were fed on total mixed ration (TMR) ration and had free access to water and mineral salts. The 12 cows/heifers were divided into two groups.Groups (A/ N=7) synchronized with GnRH according to table (1) schedule and Group (B/ N=5) synchronized with Estradiol according to table (2) schedule. All animals received a controlled, internal drug-release device containing 1.38 g progesterone (CIDR$^{TM}$, Zoetis) regardless to the stage of the estrous cycle and were assigned to the two treatments.

**Table 1:**-GnRH-CIDR based superovulation programschedule with timed insemination in the treated animals (group A).

| Days of treatment | 7 AM | 7 PM |
|---|---|---|
| 0 | CIDR insertion | |
| 2 | Receptal 10µg/ IM | |
| 4 | Folltropin 80 mg I/M | Folltropin 80mg I/M |
| 5 | Folltropin 60mg I/M | Folltropin 60mg I/M |
| 6 | Folltropin 40mg I/M | Folltropin 40mg I/M |

| | | |
|---|---|---|
| **7** | Folltropin 20mg I/M | Folltropin 20mg I/M +Estrumate (500 µg I/M) + CIDR remove |
| **8** | | Estrumate 500µg + Receptal( 10 µg I/M) |
| **9** | Timed AI | Timed AI |
| **16** | Embryo Flushing (non-surgical recovery of embryos). | |

**Table 2:-**Esteradiol Benzoate CIDR based superovulation programschedule with timed insemination in the treated animals (group B).

| **Days of treatment** | **7 AM** | **7 PM** |
|---|---|---|
| **0** | insertion of CIDR + Estradiol benzoate 2.5 mg I/M | |
| **4** | Folltropin 80mg I/M | Folltropin 80mg I/M |
| **5** | Folltropin 60mg I/M | Folltropin 60mg I/M |
| **6** | Folltropin 40mg + Estrumate 500µg | Folltropin 40mg + Estrumate 500µg |
| **7** | Folltropin20mg + CIDR removal | Folltropin 20mg |
| **8** | Cystorelin 100mg | Timed AI |
| **9** | Timed AI | |
| **15** | Embryos Flushing | |

(Folltropin-V®, Bioniche, USA); (Estrumate®, MSD); (Receptal ®, MSD); (Cystorelin®, Ceva)

Cows were artificially inseminated with superior genetic semen of USA origin (ABS Company). Which was equally distributed among the two experimental groups, (2) insemination was done (12) hours in between; for each insemination (2) doses of conventional semen were used. Embryos were recovered (7) days after the insemination by flushing with readymade flushing media (Complete flush, Agtech, USA.) with non-surgical flushing technique. On the day of recovery the recovered embryos were shifted to holding media (Agtech, USA) to be classified by using stereomicroscope (Meiji) according to the International Embryo Transfer Society Manual **(Wright, 1998)** by stage of development and quality. The number of total ova comprised unfertilized ova plus all embryos. Transferable embryos included morulae and blastocysts of quality 1, 2, 3 and 4. Recovered fresh embryos were transferred to 100 recipients Holstein heifers of age ranged between 14 and 18 month old and of good body condition score.

**Ultrasound scanning**

The ovaries of each cow/heifer were examined at 24 h after CIDR withdrawal (day 4 of super stimulatory treatments), 12 h after the 2nd insemination, and at embryo recovery by transrectal ultrasonography (Sonoscape A5.vet with 7.0MHz linear-array transducer; Sonoscape Co. Ltd., China). Examination involved counting the number of preovulatory follicles (≥8mm in diameters), ovulated preovulatory follicles, and CLs.

**Statistical analyses**

The statistical analysis was carried-out using ANOVA (Analysis of variance) for study the significance effect of the variables affecting the successful embryo transfer, quality grade of embryo and pregnancy rate. Also the Chi$^2$-test for study the percentage of pregnancy, embryo quality and grades among the factors affecting successful embryo transfer, the statistical analysis was carried-out according to (SAS, 2004).

## Results:-

Table (3) cleared that, the results of GnRH-CIDR based program is more effective than Estradiol-Benzoate CIDR program on superovulation rate and outcomes. Where, In GnRH-CIDR program the total number of recovered embryos reached to 55 and the recovered rate/Animal/flush was 7.86.

The total 55 recorvered embryos in GnRH-CIDR based superovulation program were classified according to its quality as follow: (26) for grade I embryos (47.2%), (12) for grade II (21.8%), (7) for grade III embryos (12.7%) and (10) for grade 4 embryos (18.1%).

In respect to the stage of embryo the harvested embryos werecategorized as follow: (50) for morula stage (90.9%), (4) for early blastocyst (7.2%) and (1) for blastocyst stage (1.8%). The pregnancy percentages on recipient heifers reached to 30.9 % in GnRH-CIDR based superovulation program.
While, the results of Estradiol- Benzoate CIDR based superovulation program cleared that, the total recovered embryos reached to (45), Recovered rate/Animal/flush was (9) which was higher than the first program but the embryo quality was (15) for grade I embryos (33.3%), (13) for grade II (28.8%), (5) for grade III embryos (11.1) and (12) for grade IV embryos (26.6%). The stage of embryos was 43 for morula stage (95.5%) 2 for early blastocyst stage (4.5%), and the pregnancy percentages on recipient heifers reached to 24.44 % in Estradiol Benzoate CIDR based superovulation program.

**Table (3):-**GnRH-CIDR versus Estradiol-Benzoate CIDR based programs effects on superovulation rate, embryo quality and pregnancy percentage of Holstein cows/heifers

| Parameters | | Group (A) | Group (B) | $Chi^2$ |
|---|---|---|---|---|
| | | | | |
| **Total animal** | | 7 | 5 | |
| **Polyparouscow/ Heifers** | | 5/2 | 4/1 | |
| **Total recovered embryos** | | 55 | 45 | 6.55** |
| **Recovering rate / Animal / flush** | | 7.86 | 9 | 3.55* |
| **Embryo quality** | **Grade I** | 26(47.27%) | 15(33.33%) | 8.58** |
| | **Grade II** | 12(21.81%) | 13(28.88%) | 4.25* |
| | **Grade III** | 7(12.72%) | 5(11.11%) | 5.25* |
| | **Grade 4** | 10(18.18%) | 12(26.66%) | 3.25* |
| | | | | |
| **Embryo stage** | **Morula** | 50(90.90%) | 43(95.55%) | 5.25* |
| | **Early blastocyst** | 4(7.27%) | 2(4.44%) | 8.25** |
| | **Blastocyst** | 1(1.818%) | 0 | 3.55* |
| **Recipient heifers pregnancy percentage** | | 17/55=30.90% | 11/45=24.44% | 4.55* |

* = Significant at *(P > 0.05)* ** = Significant at *(P < 0.01); Group (A):* GnRH-CIDR based superovulation program; *Group (B):* Estradiol-Benzoate CIDR based superovulation program

## Discussion:-

This study evaluated the effectiveness of super ovulatory protocols using EB or GnRH-induced synchronization of follicular wave emergence in CIDR-treated, Holstein cows. The current results proved that GnRH-CIDR-treated cows have super ovulatory responses and embryo yields comparable to EB-CIDR-treated cows/heifers.

In order to optimize the super ovulatory response the gonadotropin treatment must be initiated at the expected time of follicular wave emergence **(Baracaldo *et al.*, 2000 and Nasser *et al.*, 1993)**. Thus, in cattle, it was advisable to synchronize the emergence of the follicular wave prior to initiating of super stimulatory treatments **(Mapletoft*et al.*, 2002)**.Utrasonographic observations on the number of preovulatory follicles after super stimulation treatments across groups suggest that CIDR-treated cows was effectively synchronized by treatment with 2mg EB at 5 days or 100 mg GnRH at 3 days prior to super stimulation. These results support the observations, in which the treatment with 2.5 mg EB at the time of CIDR insertion resulted in synchronous emergence of a new follicular wave 3–4 days later (**Caccia and Bó, 1998)**. Similarly, previous studyclarified that treatment with 100 µg GnRH at the time of CIDR insertion resulted in synchronous emergence of a new follicular wave 2–4 days later **(Kim *et al.*, 2005)**. Taken together, these results indicated that in progesterone/progestogen treated cows, administration of either estrogen esters or GnRH prior to super ovulatory treatments, at any stage of the estrous cycle, effectively synchronizes follicular wave emergence, subsequent follicular development and ovulation.

The current study results concerning the measurable embryo yield outcomes (numbers of total ova, transferable embryos, degenerate embryos and unfertilized ova) were not agreed with the results of **(Andrade *et al*., 2003)**, who found that numbers of total ova, transferable embryos, degenerate embryos and unfertilized ova were the same when the follicular wave emergence was synchronized by 2mg EB in CIDR-treated cows as for a standard superovulation protocol. The current results were also disagreed with other reports**,** in which total ova and transferable embryos didn't differed between super ovulatory treatments, using 2.5 mg E-17ß and 100µg GnRH to synchronize the follicular wave in CIDR-treated cows **(Wright, 1998).**Another study reported improved superovulation protocols through super stimulation of a synchronized cohort of follicles 4 days after estradiol and progesterone, 2 days after follicle ablation, or 1.5 to 2 days after GnRH- induced ovulation **(Bó and Mapletoft, 2014)**, more than 95% of animals ovulated to the first GnRH administration and super ovulatory response, ova/embryo numbers and quality were similar to that obtained when estradiol was used to synchronize follicular wave emergence **(Bó*et al*., 2010).**Collectively these results support the concept that super ovulatory protocols using synchronization of follicular wave emergence induced by both estrogen esters and GnRH in progesterone/progestogen-treated cows resulting in an embryo yield comparable to a conventional superovulation protocol.

***In summary***, administration of both EB and GnRH prior to super stimulation in CIDR-treated Holstein cows at any stage of the estrous cycle, resulted in a super ovulatory response and embryo yield comparable to the conventional superovulation protocol in which super stimulatory treatments were initiated during mid-cycle. These new treatment protocols may minimize the costs associated with embryo production by permitting the use of a large number of donors over a short period of time. In the current study the GnRH-CIDR based protocol is better in term of embryo quality, stages and pregnancy rate in recipient heifers but Estradiol-CIDR based protocol is only higher at total embryo recovered per flush

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