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RESEARCH ARTICLE

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

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Abstract

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Objectives: The aim of the current study is to compare the response to GnRH-CIDR and Estradiol-CIDR based programs on super ovulation rate in Holstein cows/heifers. ***Methods:*** Twelve animals divided into two groups. Group (A, N= 7), while group (B, N=5). Both groups received progesterone injection (intra-vaginal progestrone-releasing device- CIDR) on day (0) and removed on day (7). On day 2, group (A) received GnRH (Receptal® 10µg), after that the animals stimulated using decreasing doses of follicle-stimulating hormone (Follitropin-V) twice daily (7am-7pm) starting from the day (4) to day (7). On day (7) at (7pm) the first group was injected with Estrumate®/500µg coinciding with the time of CIDR removal. On day (8) the first group received another dose of Estrumate 500µg, together with Receptal®10µg at 7:00 pm. Artificial insemination was set to be on day (9) at 7:00 am & pm. The second group, coinciding with CIDR application on day (0), they injected with Estradiol benzoate 2.5mg. After that, ovulation was stimulated by using decreasing doses of Follitropin-V twice daily (7am-7pm) from day 4 to day 7. On day (6) exactly at (7pm) animals received Estrumate 500µg. On day (8) at (7am) animals received a dose of Cystorelin 100mg, and then artificially inseminated at (7pm) on the same day. Again animals received fixed A.I at (7am) on day (9). Embryos were collected on day (16) for group (A) and on day (15) for group (B). ***Results:*** The total number of the recovered embryos was better for group A (55) than group B (45). Although the number of recovered embryos/flush/animal were (7.86) group A, which was lower than group B (9). Despite this, the embryos quality was better in group A. Where, it recorded (47.27%) grade (I), (21.81%) grade (II), (12.72%) grade (III), and (18.18%) grade (IV), while it was (33.33%), (28.88%), (11.11%), and (26.66%) for group B, respectively. The pregnancy rate for the recipient cow was (30.90%) and (24.44%) for group (A) and (B), respectively. Conclusion: for Holstein cows the program based on GnRH-CIDR was better than the program based on estradiol-CIDR concerning super ovulation rate, embryo quality, and pregnancy rate.

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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 super stimulation 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 super stimulatory treatments resulted in both super ovulatory 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 super ovulatory 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 Gn-RH treatment on the super ovulatory 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 Gn-RH resulted in similar numbers of total ova and transferable embryos as synchronization with E17ß **(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 Gn-RH 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) rat ion and had free access to water and mineral salts. The 12 cows/heifers were divided into two groups. Groups (A/ N=7) synchronized with Gn RH 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:-Gn-RH-CIDR based superovulation program schedule 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:-Estradiol Benzoate CIDR based superovulation program schedule 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 2^{nd} insemination, and at embryo recovery by trans rectal 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 Gn-RH-CIDR based program is more effective than Estradiol-Benzoate CIDR program on superovulation rate and outcomes. Where, In Gn-RH-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 Gn-RH-CIDR based superovulation program were classified according to its quality as follow: (26) for grade I embryos (47.27%), (12) for grade II (21.81%), (7) for grade III embryos (12.72%) and (10) for grade IV embryos (18.18%).

In respect to the stage of embryo the harvested embryos were categorized as follow: (50) for morula stage (90.90%), (4) for early blastocyst (7.27%) and (1) for blastocyst stage (1.81%). The pregnancy percentages on recipient heifers reached to 30.9 % in Gn RH-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 (33.33%), (13) for grade II (28.88%), (5) for grade III embryos (11.11) and (12) for grade IV embryos (26.66%). The stage of embryos was 43 for morula stage (95.55%), 2 for early blastocyst stage (4.44%), and the pregnancy percentages on recipient heifers reached to 24.44% in Estradiol Benzoate CIDR based superovulation program.

Table (3):-Gn RH-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	
Polyparous cow/ 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 IV	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(1.81%)	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):* Gn RH-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 Gn RH -induced synchronization of follicular wave emergence in CIDR-treated, Holstein cows. The current results proved that Gn RH-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)**.Ultrasonographic 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 Gn RH 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 study clarified that treatment with 100 µg Gn RH 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 Gn RH 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 Gn RH 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 Gn RH- 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 Gn RH-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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