Damanhour Journal of Veterinary Sciences 10 (1), (2023) 31-35

Journal homepage: https://djvs.journals.ekb.eg/

E-ISSN 2636-3003 | ISSN 2636-3011

Impact of different hormonal protocols for the treatment of smooth inactive ovaries (SIO) and the subsequent reproductive performance in dairy cows

Mohamed A. Elbehiry[1]

[1]Department of Theriogenology, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt

Abstract: Infertility caused by smooth inactive ovaries (SIO) is a common ovarian disorder that causes a major reproductive failure in dairy cows and has a significant economic impact on the dairy industry. The present study aimed to investigate the response of dairy cows with a syndrome of smooth inactive ovaries to different hormonal treatment protocols and their impact on reproductive performances. Lactating multiparous Holstein cows (*n*= 75) having smooth inactive ovaries diagnosed by two successive ultrasonographic examinations performed at 10 days interval were used in this study. Cows were assigned randomly into 5 groups (15 each). Group A, treated with a single injection of 250 µg GnRH, Group B with a single injection of 2500 IU HCG, Group C, in which cows received CIDR for 7 days and PGF2α at day 6 (24 hours before removal of CIDR followed by injection of 250 µg GnRH (at day 8) 24 hours after removal of CIDR (at day 8)(CIDR, PGF2α and GnRH), Group D in which cows received CIDR for 7 days and PGF2α at day 6 (24 hours before removal of CIDR) followed by injection of 2500 IU hCG 24 hours after removal of CIDR (CIDR, PGf2α, and HCG), and Group E in which cows did not receive any treatment and kept as a control group. The results showed that CIDR+ GnRH and CIDR+ hCG protocols achieved the full (100%) estrus induction rate (EIR). hCG group achieved 93% and GnRH group 80%, while the control group only achieved 20% EIR. The shortest treatment estrus intervals (TEIs) were recorded in CIDR+ hCG and CIDR+ GnRH protocols. Mean±SD of TEIs were 1 ± 0, 1.4 ± 1.1days 10.4 ± 5.4, 16.7 ±7.1 and 23.7± 2.9 days in CIDR+ hCG . CIDR+ GnRH, HCG, GnRH and control groups respectively. The CIDR+ hCG group had the highest pregnancy rate (86.7%), followed by hCG (79.9%) and CIDR+ GnRH groups (80%), and GnRH group with 73%. The control group showed a 13.3 % pregnancy rate however, GnRH group showed the highest total conception rate (91.6%). It was concluded that the highest EIR and shortest TEI were obtained in CIDR+ GnRH and CIDR+ hCG protocols. While the CIDR+ hCG group had the highest pregnancy rate (86.7%).

Keywords: *Inactive Ovaries; GnRH; CIDR; hCG; Estrus*

*Correspondence: Mohamed A. Elbehiry
Department of Theriogenology, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt
Email: mohamed.elbehiry2@vetmed.dmu.edu.eg

P ISSN: 2636-3003
EISSN: 2636-3011
DOI: 10.21608/DJVS.2023.207832.1114
Received: May 05, 2023; Received in revised form: June 01, 2023; Accepted: June 23, 2023.
Editor-in-Chief:
Prof Dr/Ali H. El-Far (ali.elfar@damanhour.edu.eg)

1. Introduction

Cows production depends primarily on reproduction, and its often measured by the number of births for each animal in a determined period (Monget and Monniaux 1995). Inactive ovaries cause sizeable economic loss when post parturient period to the first estrous becomes long (Ahmed, El-Khadrawy et al. 2010). One of the fundamental causes of depression of natural ovarian activities is impairment release or insufficient hormonal production of GnRH to grow and follicular development, or it might be because of no ovarian response to GnRH (Ahmed, El-Khadrawy et al. 2010), or hormonal imbalance between (LH) and (FSH) (Hafez and Hafez 2013). Inactive ovaries in lactation cows cause the increase of prolactin hormone, which depresses GnRH secretion (Arthur, Noakes et al. 1989). Using single GnRH in cows suffering from inactive ovary led to estrus appearance in 10 cows of 20 cows with a response rate of 50% (Hussein and Yaurb 2021).

Anovulatory anestrus type ll is the most common type in which the process of follicular growth and regression is repeated over and again in anestrus animals. The ovaries of such animals are small with the absence of a corpus luteum or ovulatory size follicles. Adequate LH pulse frequency is required for the growth and development of follicles after the emergence of the follicular wave. This condition is due to low LH pulse frequency (Kumar, Abadi et al. 2014).

The single intramuscular injection of GnRH analog (10 to 20 µg Buserelin) has been used effectively in the induction of estrus and concurrent ovulation with the variable response (45.5 to 87.5%) within 4–22 days (Markandeya and Patil 2003, Prahalad, Rao et al. 2010). Human chorionic gonadotrophin (hCG) has also been used for the management of anestrus with a fair degree of success (Dabas and Dwaipayan 2003).

Exogenous administration of progesterone mimics the luteal phase of the estrus cycle by exerting a negative feedback effect over the hypothalamus and pituitary for LH release. Upon withdrawal of progesterone, the normal follicular phase of the cycle is stimulated. To achieve better responses, the intravaginal devices or ear implants are generally used for 7 to 9 days, combined with other hormones (prostaglandins, GnRH, PMSG/eCG, and estradiol) towards the end of progesterone treatment. Estrus induction rate has been reported between 80 to 100% by most workers (Azawi, Ali et al. 2012).

Therefore, the main objective of our study was to assess CIDR+ GnRH, CIDR+ hCG, GnRH, or hCG for treatment of ovarian inactivity in dairy cows and evaluate their influences on subsequent reproductive performance.

2. Materials and Methods

2.1. Ethical statement

All treatment and animals care procedures were approved (approval number: DMU/VetMed: 2023/008) by the institutional animal care and use committee in AU-IACUC, Damanhour University, Egypt. The authors declare that the procedures imposed on the cattle were carried out to meet the directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals and birds used for scientific purposes.

2.1. Experimental design

2.1.1 Animals.

The study was conducted in a commercial dairy farm located in the Cairo Alexandria desert road, Egypt, from November 2020 to

July 2021. Cows are examined routinely at day 40 to 42 postpartum for reproductive assessment by ultrasound and considered SIO when there is no corpus luteum after 2 examinations 10 days apart. 75 Lactating multiparous Holstein cows having smooth inactive ovaries were used in this study. Cows were housed in free-stall barns, and individual pens were virtually identical in design, size, and several cows housed. Cows were fed 2 different diets as TMR (Total Mixed Ration) according to the stage of lactation, with an immediate postpartum diet-fed between 1 and 21 days in milk (DIM) and a lactating diet for the remainder of lactation. Cows were fed twice daily, and diets were based on corn silage, alfalfa hay, soybean meal, steam-rolled corn, whole cottonseed, calcium salts of palm oil, and a mineral, vitamin, and protein supplement. The 2 diets were designed to meet the National Research Council (NRC) requirements for lactating Holstein cows weighing 500 kg and producing 35 kg of milk/day, containing 3.5% fat (Council 2001). Cows had free access to water.

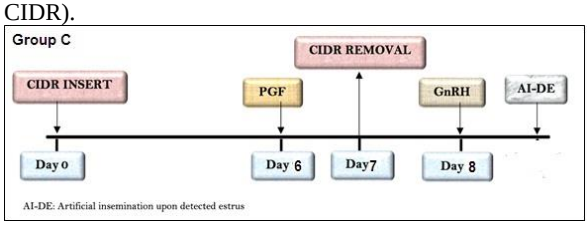
2.1.2. Hormones used in this study and their dosage.

- **Human Chorionic Gonadotropin**; 2500 IU of hCG (half a vial of **Epifasi®**, Eipico, Egypt".
- **Gonadotropin Releasing Hormone**; GnRH 250µg gonadorelin acetate (2.5ml; **Ovurelin®**, Bayer, NSW, Aust).
- **Progesterone:** Intravaginal progesterone-releasing insert (EAZI-BREED™ CIDR®-Zoetis animal production, USA) each insert is impregnated with 1.38 g of progesterone and is designed for intravaginal insertion,
- **ProstaglandinF2α** (PGF2α); 500µg Cloprostenol sodium (2ml of Ovuprost®, Bayer, Aust).

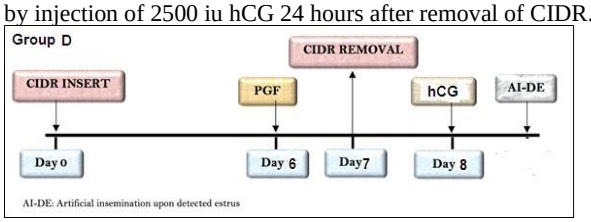
2.1.3. Animals grouping

In this experiment, seventy-five (n=75) multiparous postpartum cows having smooth inactive ovaries were allocated into five groups as follow:

- **Group A** (GnRH): (*n*=15) treated with 250 µg GnRH (2.5 ml Ovurelin® i.m).
- **Group B** (hCG): (*n*=15) treated with hCG 2500 IU i.m (half a vial of Epifasi).
- **Group C** (CIDR and GnRH) :(*n*=15) treated with (CIDR, PGF2α, and GnRH): Cows received CIDR for 7 days and PGF2α at day 6 (24 hours before removal of CIDR) followed by injection of 250 µg GnRH (24 hours after removal of CIDR).



- **Group D** (CIDR and HCG): (n =15) treated with (CIDR and PGF2α and hCG). Cows received CIDR for 7 days and PGF2α at day 6 (24 hours before removal of CIDR) followed by injection of 2500 iu hCG 24 hours after removal of CIDR.



- **Group E** (Control group): (*n*= 15) cows did not receive any treatment and were kept as a control group.

Animals were observed for estrus twice daily for 30 minutes and checked on SCR (Heat detection program). In addition, any observation of standing estrus during routine handling was recorded. Each animal in estrus was inseminated with frozen-thawed semen. Each animal that did not return to estrus was examined by ultrasound on day 28 after insemination for pregnancy diagnosis. A resident veterinarian made all inseminations.

2.2 .Statistical analysis

Data are presented as mean ± standard deviation of experiments. Each animal was considered as one experimental unit. GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA) was used for statistical analysis. Statistical significance between groups was detected using Student's t-test (for two groups) or one-way ANOVA followed by Tukey post hoc tests (for more than two groups). Results were statistically significant at $P < 0.05$. Moreover, Chi-square test was used to measure the variability in response between the different groups.

3. Results

Data on the distribution of estrus exhibition and estrus induction rate is presented in **Table 1**. Results showed that CIDR+GnRH and CIDR+hCG protocols achieved the full (100%) estrus induction rate (EIR). hCG group reached 93% and GnRH group 80%, while the control group only achieved 20% EIR. The results of EIR were statistically significant by chi-square test when compared to the control group.

Regarding the treatment estrous interval (from the beginning of treatment to estrous detection time), **Table 1** shows that treatment with hCG, CIDR+GnRH, and CIDR+hCG had significantly lower ($P < 0.05$) treatment to the estrous interval when compared to the control group with a Mean ± SD of (10.4 ±5.2), (1.4 ± 1.1), and (1 ± 0) respectively.

Table 2 shows the time to estrus exhibition from the end of treatment. It was classified as; short (<10 days), moderate (10- <20 days), or long (20- <30 days). All cows (100%) in CIDR+GnRH and CIDR+hCG groups exhibited estrous in the middle ten days after treatment. Almost half of the cows in the hCG group exhibited estrous in the 1st ten days after treatment, while almost half of the cows in the GnRH group exhibited estrous in the last ten days after treatment. All cows exhibited estrous late after 20 days of treatment in the control group.

Table 3 Illustrates that there was a statistically significant difference between groups was determined by one-way ANOVA (F (4,54) =11.16, P = 0.00) regarding the frequency of estrous exhibition from the end of treatment in days. Tukey post hoc test confirmed the previous statistically significant difference between each group and the control group except the GnRH group, as mentioned in **Table 2**.

Table 4 demonstrates that the conception rate of the first three artificial inseminations was highest in the GnRH group (91.6%), followed by the CIDR+hCG group (86.7%) and hCG group (85.7%), then the CIDR+GnRH group (80%). The control group showed (66.7%). The control group showed a 13.3 % pregnancy rate. The CIDR+hCG group had the highest pregnancy rate (86.7%), followed by hCG and CIDR+GnRH groups (almost 80%), and lastly GnRH group with 73%. About two-thirds (66.7%) of the cows in the CIDR+hCG group conceived at 1st insemination, while almost half of cows in CIDR+GnRH, GnRH, and hCG groups were designed at 1st insemination. Only one-third (33.3%) of the cows in the control group conceived at 1st insemination.

Table 5 shows the reproductive performance represented by treatment conception interval. The time interval between the end of treatment and the fertile artificial insemination was calculated and revealed that using CIDR plus hCG leads to a decrease in the interval by 2.5 times compared to the control group (10.6 ± 18.2) vs. (49 ± 38).

Table 6 demonstrates no statistically significant difference between the treatment groups regarding services per conception. However, treatment with CIDR+HCG showed the lowest number of services per conception with a mean and SD of (1.31 ± 0.63).

Table 1. Effects of different treatment protocols on the incidence of estrus and interval to the estrous exhibition from end of treatment

Treatment	**No. of cows within the group**	**Cows detected in estrous**		**Treatment estrous interval**		
		No.	%	Mean +/- SD	Test of significance	
					t-test	***P*** -value
GnRH	15	12	80%	16.7 ± 7.1	T= 1.6	***P*** = 0.13
hCG	15	14	93.3%	10.4 ± 5.4	T= 4*	***P*** = 0.001
CIDR+GnRH	15	15	100%	1.4 ± 1.1	T=13.2*	***P*** = 0.005
CIDR+hCG	15	15	100%	1 ±- 0	T=13.6*	***P*** = 0.005
Control	15	3	20%	23.7 ± 2.9	-	-

*Statistically significant result compared to the control group.

Table 2. Effects of different treatment protocols on the distribution of estrous exhibition from end of treatment in days

Treatment	No. of cows detected in estrous within the group	Distribution of estrus exhibition from end of treatment in days		
		<10 days	10- <20	20-<30
GnRH	12	3 (25%)	4 (33.3%)	5 (41.7%)
hCG	14	8 (57.1)	4 (28.6%)	2 (14.3%)
CIDR+GnRH	15	-	15 (100%)	-
CIDR+hCG	15	-	15 (100%)	-
Control	3	-	-	3 (100%) (a)

(a) = percent calculated from total detected in estrous within each group

Table 3. One way ANOVA

variable	F	df1	df2	***P*** -value
Frequency of estrous exhibition from end of treatment in days	11.16	4	54	0.00*

*Statistically significant result compared to the control group.

Multiple comparisons (Tukey post Hoc test)

Test	P-value
Control vs GnRH	0.1
Control vs hCG*	0.00
Control vs CIDR + GnRH*	0.00
Control vs CIDR + hCG*	0.00
GnRH vs HCG*	0.004
GnRH vs CIDR + GnRH*	0.003
GnRH vs CIDR + hCG*	0.001

Table 4. Effects of different treatment protocols on conception rate and pregnancy rate

Treatment	**No. of cows in estrus within the group**	**Conception at 1st AI**		**Conception at 2nd AI**		**Conception at 3rd AI**		**Total conception of 1st 3 AI**		Pregnancy rate
		No.	%(a)	No.	%(a)	No.	%(a)	No.	%	
GnRH	12	5	41.7%	4	33.3%	2	16.6%	11	91.6%	73.3%
hCG	14	8	57.1%	3	21.4%	1	7.1%	12	85.7%	79.9%
CIDR+GnRH	15	7	46.6%	3	20%	2	13.3%	12	80%	80%
CIDR+hCG	15	10	66.7%	2	13.3%	1	6.6%	13	86.7%	86.7%
Control	3	1	33.3%	---	---	1	33.3%	2	66.7%	13.3%

(a) percent calculated from total detected in estrous within each group

Table 5. Effects of different treatment protocols on treatment conception interval in days

Treatment	**No. of conceived cows**	**Interval to conception from end of treatment in days**	**Test of significance**	
		Mean ± SD	t-test	***P*** **-value**
GnRH	11	36.5 ± 22.3	T= 0.6	***P* = 0.5**
hCG	12	19.5 ± 16.9	T= 1.9	***P* = 0.07**
CIDR+GnRH	12	16.1 ± 18.1	T=1.2	***P* = 0.4**
CIDR+hCG	13	10.6 ±18.2	T= 2.5*	***P* = 0.02**
Control	2	49 ±38	-	-

*Statistically significant result compared to the control group.

Table 6. Effects of different treatment protocols on service preconception

Treatment	No. of conceived cows within the group	Services per conception	Test of significance	
		Mean±SD	t-test	***P* -value**
GnRH	11	1.73 ± 0.79	0.41	**0.6**
hCG	12	1.42 ± 0.67	1	**0.3**
CIDR+GnRH	12	1.58 ± 0.79	0.63	**0.5**
CIDR+hCG	13	1.31 ± 0.63	1.3	**0.2**
Control	2	2 ± 1.4	---	---

4. Discussion

Cows with true anestrus need sorts of management or hormonal interventions for induction of cyclicity and conception (Rahman 2010). In the current study, four different treatment protocols were used; Group (A) with a single injection of 250 µg GnRH, Group (B) with a single injection of 2500 IU hCG, Group (C) in which cows received CIDR for 7 days and PGF2α at day 6 (24 hours before removal of CIDR followed by injection of 250 µg GnRH 24 hours after removal of CIDR (CIDR and PGF2α and GnRH), and Group (D) in which cows received CIDR for 7 days and PGF2α at day 6 (24 hours before removal of CIDR) followed by injection of 2500 IU hCG 24 hours after removal of CIDR (CIDR and PGF2α and hCG).

According to this study, the combination of CIDR+GnRH and CIDR+hCG was associated with a 100% estrous detection rate. hCG alone was associated with a 93% estrous detection rate. GnRH alone had an 80% estrous detection rate, and cows in the control group showed a 20% estrous detection rate.

There was a statistically significant difference between the hCG, CIDR+GnRH, and CIDR+hCG groups when compared to the control group regarding the distribution of estrous exhibition from the end of treatment with a mean of (10.4 ± 5.4), (10.4 ± 1.1), and (10± 0.0) respectively**.**

Regarding pregnancy rates and total conception rates, both hCG and hCG combined with CIDR had the highest pregnancy rates (79.9% and 86.7%, respectively) and showed 85.7% and 86.7% total conception rates. CIDR combined with GnRH showed an 80% pregnancy rate and total conception rate, while GnRH alone showed a 73% pregnancy rate with the highest total conception rates (91.6%). The pregnancy rate in the control group was only 13%, with a 66.7% total conception rate.

The previous results are consistent with Canadas et al (2019), who concluded from his study that treatment of anestrous cows with GnRH, hCG or Progesterone-Ovsynch protocol increased ovulation rate compared with untreated Control cows, and the best reproductive performance results were obtained from the Progesterone-Ovsynch protocol. However, it had the highest cost.

Similar results were obtained from a study by Ravikumar, Asokan et al (2009). The use of CIDR plus Ovsynch was associated with 100% ovulatory response and 42% first service conception rate, which was higher than the results of Ovsynch protocol alone 83.33% and 33.33%. Dudhatra et al (2012) also demonstrated that administration of PGF2α or their analogs in early postpartum cows hasten early resumption of cyclic ovarian activity and thereby increased the reproductive efficiency

Progesterone released from CIDR inserted intra-vaginally in cows is absorbed through the vaginal wall into the circulation (Singh 2003). This increase in the circulatory concentration of progesterone exerts negative feedback on the hypothalamus and anterior pituitary. Following termination of progesterone therapy (after CIDR withdrawal by the day 7 after insertion), the rapid drop in the circulatory concentration of progesterone promotes the release of GnRH as the negative feedback of progesterone was abolished, followed by FSH and LH release with the subsequent resumption of ovarian cyclicity (Zerbe, Gregory et al. 1999) Also, the increased circulatory concentration of progesterone sensitizes the hypothalamic-pituitary system (Virendar, Malik et al. 2010). Likewise, progesterone increases the hypothalamus sensitivity to estrogen with a subsequent increase in the intensity of heat (Fabre-Nys and Martin 1991)

Furthermore, CIDR in combination with i.m. injection of PGF2α is more effective than CIDR alone in terms of an exhibition of estrus and conception rate (Singh 2003). This can be explained by the fact that PGF2α increases pituitary responsiveness to GnRH in the postpartum cow (Randel, Lammoglia et al. 1996). Hence, the released GnRH after CIDR removal effectively stimulates the pituitary gonadotropins with subsequent estrus induction in anestrous cows.

Treatment of anestrus in buffalo cows was studied by Bakr et al (2015) with different treatment protocols. The most efficient two treatment protocols were CIDR and CIDR+GPG (both had similar results) with 100% estrous detection rate in both compared to 85% in the control group, 81% total conception rate in both compared to 67% in the control group, higher first service conception rate 56%compared to 33% in the control group, and also lower treatment to the estrous interval (10.00 ± 0.65 vs. 28.67 ± 6.59 in control).

The use of CIDR in combination with Ovsynch protocol in postpartum anestrous buffaloes was associated with higher pregnancy rates (83%) when compared to Ovsynch alone (50%) and CIDR alone (50%) (Naikoo et al., 2010). This was also in line with Bhoraniya et al (2012) but slightly lower conception rates. The use of Ovsynch plus CIDR protocols in anestrus dairy cows showed 66.6% compared to the 50% overall conception rate in Ovsynch protocol.

Moreover, the application of an intravaginal progesterone-releasing insert with the Ovsynch protocol resulted in a higher estrous detection rate, higher first service conception rate, and higher pregnancy rate than the Ovsynch treatment (McDougall 2010).

According to Muneer et al (2009), hCG had an add-on effect when added to CIDR, compared to CIDR alone in postpartum anestrus crossbred cows. The combination of CIDR and hCG was associated with an 80% conception rate compared to a 70% conception rate with CIDR alone.

Garcia-Ispierto et al (2019) studied the effect of hCG compared to GnRH in a five-day progesterone-based fixed-time AI protocol on the fertility of anestrous dairy cows under heat stress conditions. They revealed that the ovulation failure rate was significantly lower, and the pregnancy rate was significantly higher in the (hCG+CIDR) group than the (GnRH+CIDR) group.

In his study, (Naglis et al (2018) compared the effect of hCG sv GnRH on the first service conception rates in anestrus dairy cows and revealed that hCG improved the 1st service conception rate to 72.2% compared to 64.4% in the GnRH group. These results are consistent with our results in which the 1st service conception rate was also higher in the HCG group than the GnRH group (57%vs41.5%). Also, (Portaluppi, Tenhouse et al. 2006) reported a higher ovulation rate in the hCG treated group (78%) compared to (60%) in GnRH treated group and both were significantly higher than the control group (2.4%).

In Egypt, Waheeb and Hatab (2017) reported the same estrous detection rate as the current study, 80% from a single injection of GnRH with 87.5% conception rate. Only a 40% conception rate in GnRH treated group (El-Shahat and Badr 2011). The variation between the different studies in the conception rates might be because of other environmental and managerial factors as days in milk, age, and season. The control group in Waheeb and Hatab (2017) showed a similar pregnancy rate of 10%.

The effect of GnRH is mediated through the stimulation of FSH secretion, which leads to the initiation of new waves of follicular growth, followed by LH surge and ovulation in response to elevated estradiol levels (Torrens, Snelling et al. 2009).

GnRH administration causes the large follicles to ovulate and induces the emergence of a new follicular wave within 3 to 4 days after treatment at any stage of the estrous cycle in cattle. Cows treated with GnRH showed a 75% estrus rate and 69% conception rate compared to 56% and 40% in the counterpart control group (Islam, Juyena et al. 2013). The differences in percentages between the previously mentioned study and the current study might be due to variation in the number and breed of animals, nutritional status, or season.

5. Conclusion

CIDR+GnRH and CIDR+hCG protocols achieved the full (100%) estrus induction rate (EIR), and the hCG group achieved 93% and GnRH group 80%. The Shortest TEI (1 ± 0 and1.4 ±1.1days) were obtained in CIDR+ GnRH and CIDR+ hCG protocols.

Conflict of interest: The authors declare no conflict of interest.

6.References

Ahmed, W., H. El-Khadrawy, A. Abd El Hameed and H. Amer (2010). "APPLIED INVESTIGATIONS ON OVARIAN INACTIVITY IN BUFFALO-HEIFERS." International journal of academic research **2**(1).

Arthur, G., D. Noakes and H. Pearson (1989). "Veterinary reproduction and obstetrics. theriogenology 6th ed." Baillier Tindall UK: 83-85.

Azawi, O., M. Ali, S. Oday, A. Salih, A. Al-Hadad, S. Mouayad and A. AbdulHussien (2012). "Comparative efficacy of different CIDR protocols for the treatment of postpartum anestrous in Iraqi buffaloes." Veterinary World **5**(4).

Bakr, M. M., M. Noseir and G. A. Amrawi (2015). "Effect of Exogenous Progesterone in Treatment of Ovarian Inactivity in the Egyptian Dairy Parturient Buffalo-Cows." Alexandria Journal for Veterinary Sciences **47**(1).

Bhoraniya, H. L., A. J. Dhami, M. Naikoo, B. C. Parmar and N. P. Sarvaiya (2012). "Effect of estrus synchronization protocols on plasma progesterone profile and fertility in postpartum anestrous Kankrej cows." Tropical animal health and production **44**(6): 1191-1197.

Canadas, E. R., M. Gobikrushanth, P. Fernandez, J. Kenneally, P. Lonergan and S. T. Butler (2019). "Evaluation of alternative strategies to treat anoestrous dairy cows and implications for reproductive performance in pasture-based seasonal calving herds: A pilot study." Theriogenology **127**: 130-136.

Council, N. R. (2001). Classroom assessment and the national science education standards, National Academies Press.

Dabas, Y. and B. Dwaipayan (2003). "Oestrus and fertility after gonadotropic treatment in anoestrus buffaloes." Intas Polivet **4**(2): 145-148.

Dudhatra, G., S. Mody, H. Patel, C. Modi, A. Chukewar, A. Kumar and M. Awale (2012). "Prostaglandins and its analogues: An approach for treatment of anoestrus and to enhance breeding efficiency." Veterinary World **5**(6): 378.

El-Shahat, K. and A. Badr (2011). "Comparative study on efficacy of different medicaments on postpartum anestrus dairy cows." Journal of Applied Biological Sciences **5**(3): 59-63.

Fabre-Nys, C. and G. Martin (1991). "Roles of progesterone and oestradiol in determining the temporal sequence and quantitative expression of sexual receptivity and the preovulatory LH surge in the ewe." Journal of endocrinology **130**(3): 367-379.

Garcia-Ispierto, I., F. De Rensis, X. Casas, F. Caballero, B. Serrano-Pérez and F. López-Gatius (2019). "Inducing ovulation with hCG in a five-day progesterone-based fixed-time AI protocol improves the fertility of anestrous dairy cows under heat stress conditions." Theriogenology **124**: 65-68.

Hafez, E. S. E. and B. Hafez (2013). Reproduction in farm animals, John Wiley & Sons.

Hussein, E. K. and M. Yaurb (2021). "Comparative Study for The Treatment of Inactive Ovary in Local Iraqi Breed Cow by Using Massage Method of The Ovaries and GnRH." Journal of Applied Veterinary Sciences **6**(1): 18-20.

Islam, M. R., N. Juyena, M. Bhuiyan, M. Rahman and R. Ferdousy (2013). "Treatment outcomes in postpartum anoestrus cows guided by transrectal ultrasonography." Progressive Agriculture **24**(1-2): 93-100.

Kumar, N., Y. Abadi, B. Gebrekidan and Y. H. Woldearegay (2014). "Productive and reproductive performance of local cows under farmer's management in and around Mekelle, Ethiopia." J. Agric. Vet. Sci **7**(5): 21-24.

Markandeya, N. and A. Patil (2003). "Studies on hormonal therapies for the induction of postpartum oestrus in buffaloes." Intas Polivet **4**(2): 167-169.

McDougall, S. (2010). "Effects of treatment of anestrous dairy cows with gonadotropin-releasing hormone, prostaglandin, and progesterone." Journal of dairy science **93**(5): 1944-1959.

Monget, P. and D. Monniaux (1995). "Growth factors and the control of folliculogenesis." Journal of Reproduction and Fertility-Supplements only(49): 321-334.

Muneer, S., K. Rao and K. Raju (2009). "Efficacy of GnRH-PGF2α-GnRH, PMSG and PMSG+ hCG in postpartum anestrous crossbred cows." Indian Journal of Animal Reproduction **30**(1): 7-9.

Naglis, G., I. Fasulkov, M. Karadaev, R. Vasileva, G. Petrovas and N. Vasilev (2018). "COMPARISON OF HCG VS GNRH EFFECTS IN DOUBLE OVSYNCH ON FIRST-SERVICE CONCEPTION RATES IN ANESTRUS DAIRY COWS." ANESTHESIA, CHEMICAL RESTRAINT AND PAIN MANAGEMENT IN SNAKES: 70.

Portaluppi, M., D. Tenhouse, A. Lloyd, D. Eborn, S. Kacuba, J. DeJarnette and J. S. Stevenson (2006). "Ovarian responses and conception rates in response to gNrh, Hcg, and progesterone."

Prahalad, P., K. S. Rao and K. Raju (2010). "Effect of GnRH, PMSG and Placentrex on reproductive performance of postpartum true anestrus Murrah buffaloes." Indian Journal of Animal Research **44**(3): 201-204.

Rahman, M. (2010). "Management and effective treatment of postpartum anoestrus cows guided by ultrasonography." MS in Theriogenology Thesis. Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Randel, R., M. Lammoglia, A. Lewis, D. Neuendorff and M. Guthrie (1996). "Exogenous PGF2α enhanced GnRH-induced LH release in postpartum cows." Theriogenology **45**(3): 643-654.

Ravikumar, K., S. Asokan and C. Veerapandian (2009). "Inclusion of CIDR in ovsynch protocol to improve fertility in postpartum subestrus buffaloes." Indian Journal of Animal Reproduction **30**(1): 29-32.

Singh, C. (2003). "Response of Anestrus Rural Buffaloes (Bubalus bubalis) to Intravaginal Progesterone Implant and PGF2alphaInjection in Summer." Journal of Veterinary Science **4**(2): 137-141.

Torrens, C., T. H. Snelling, R. Chau, M. Shanmuganathan, J. K. Cleal, K. R. Poore, D. E. Noakes, L. Poston, M. A. Hanson and L. R. Green (2009). "Effects of pre-and periconceptional undernutrition on arterial function in adult female sheep are vascular bed dependent." Experimental Physiology **94**(9): 1024-1033.

Virendar, S., R. Malik, P. Singh, R. Tuli, A. Verma and R. Chandolia (2010). "Induction of cyclicity in Murrah buffalo-heifers during summer using different hormonal protocols." Indian Journal of Animal Reproduction **31**(2): 11-14.

Waheeb, R. S. and S. A. Hatab (2017). "Efficacy of Administration of Supplemental and Hormonal Therapies for Treatment of Postpartum True Anestrous Dairy Cows." Alexandria Journal for Veterinary Sciences **52**(1).

Zerbe, H., L. Gregory and E. Grunert (1999). "Zur behandlung ovariell bedingter zyklusstorungen beim milchrind mit progesteron-abgebenden vorrichtungen." Tierärztliche Umschau **54**(4): 189-192.