Int.J.Curr.Microbiol.App.Sci (2020) *9*(1): 1511-1522

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 9 Number 1 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.901.169

Effect of Different Hormonal Protocols and Nutrient Supplementation on Reproductive Performance of Cattle under Different Field Conditions

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Keywords

Balanced feed, Conception rate, Hormonal protocols, Infertile cattle, Vitaminised chelated oxicareovn solution

Article Info

Accepted:
15 December 2019
Available Online:
20 January 2020

A B S T R A C T

Bovine infertility is a major issue in dairy industry and it is primarily addressed by hormonal therapy. In present study we compared ovarian structure palpation based minimum hormonal utilization approach (KVK-RDDC protocol) with other estrus synchronization protocols. This study was conducted on 315 infertile cows kept in different managemental conditions *i.e.* Commercial dairy farms, villages and *Gaushala's* in different areas of Udaipur district of Rajasthan. These 315 infertile cows were divided in three feed groups mainly balanced feed without mineral and vitamin mixture, balanced feed with vitaminised chelated mineral mixture and balanced feed with vitaminised chelated oxicareovn solution. These feed groups were treated with five therapy protocols *viz.* No hormone, Ovosynch(GPG48), Cosynch(GPG56), Cosynch+ progesterone (GPG56 + CIDR) and KVK-RDDC. The study revealed that the group 3 which fed balanced feed with vitaminised chelated oxicareovn solution have highest conception rate *i.e.* 61.0 % and followed by group 2 (Balanced feed with vitaminised chelated mineral mixture) and group 1 (Balanced feed *i.e* without mineral and vitamin mixture) *viz.* 44.8% and 29.5 %, respectively. On the basis hormone protocol, the animals which were treated with KVK-RDDC protocol have highest conception rate of 61.9 % and followed by GPG+CIDR (46.0%), GPG-56(46.0%), GPG-48(42.9%) and no hormone protocol (28.6%). On the basis of different managemental conditions, highest conception rate were of at commercial dairies *i.e.* 55.2% and followed by village animals (43.8%) and *Gaushala's* (36.9%).

Introduction

India's livestock sector is one of the largest in the world, distributed over 100 million households in approximately 600000 villages. It has 56.7% of world buffaloes and 12.5% world cattle comprising 109.85 million buffaloes and 192.49 million cattle (20th Indian livestock census, 2019). Rajasthan ranks second with 11% of the total livestock population in the country after Uttar Pradesh. The state has 13.32 million of cattle and 12.98 million of buffalo (19th Indian livestock census, 2012), possessing high yielding breeds of cattle (Rathi, Gir, Sahiwal) and buffalo (Murrah and Surti). In Rajasthan, Animal husbandry is not merely a subsidiary to agriculture but it is a major activity which plays a vital role in livelihood, employment, economy, food security and nutrition. This contributes to balanced growth, gender equality and in reducing rural poverty. Livestock sector accounts for 30-50% of economy of rural Rajasthan and contributes approximately 13% to the state GDP (World Bank, 2018).

Livestock productivity or production efficiency is to a large extent dependent on reproductive performance. Proper nutrition could encourage mediocre biological types to reach their genetic potential and also alleviate the negative effects of a harsh physical environment. Deficiencies of various trace minerals, inadequate vitamin intake, energy, protein imbalance and excessive protein intakes are mentioned as contributive to infertility and poor reproductive performance (Amin, 2014).

In Udaipur district, livelihood security despite erratic monsoon is largely contributed by livestock. However, the major problem faced by the farmers is infertility in most of breedable population. This infertile population is a serious burden on resource poor farmer. Solution to this serious problem can usher in prosperity for the tribal and other livestock rearing farmers.

Hormones play an important role in cattle reproduction. Number of infertility treatment protocols are available in cattle based on the use of various hormones like GnRH, $PGF_{2\alpha}$ (Pursley *et al.,* 1995, Geary *et al.,* 1998, Cartmill *et al.,* 2001, DeJarnette *et al.,* 2001, Kantharaj *et al.,* 2015, Barolia *et al.,* 2016), Progesterone (Melendez *et al.,* 2006, Chebel *et al.,* 2010), estrogen (Cavestany *et al.,* 2003) and their various combination (Geary *et al.,* 1998, Lemaster *et al.,* 2001, Cavestany *et al.,* 2003). However, Gonadotropin-releasing hormone (GnRH) and Prostaglandin used to increase pregnancy rate by inducing ovulation and improving the sperm transport in the female reproductive tract in dairy cattle and has been proven to be very successful (Odde, 1990).

Bovine infertility, silent estrus and repeat breeding are the major problems which reduce conception rate, increases inter-calving interval and dry period are major causes of loss and worry for livestock owners. Due to limited availability for feed, fodder, water, pasture and other resources, rearing of non-productive and low productive animals are very costly, challenging and become a burden for livestock owners. (Howlader *et al.,* 2019)

Scientist are now working on developing new estrus synchronization protocols which can reduce the ovulation time window post synchronization so as to practice insemination at a fixed time thereby obviating the need for heat detection which is a serious problem especially in buffaloes (Prakash *et al.,* 2012). A new ovarian structure palpation based estrus synchronization protocol for cows and buffaloes have been developed recently by Vidya Bhawan KVK, Badgaon Udaipur and Regional disease diagnostic center,

Department of AH, Udaipur. This protocol is an ovarian structure palpation based combination of GnRH – $PGF_{2\alpha}$ – GnRH injection which has been reported to considerably narrow down the ovulation time to a range of 24 hours to achieve the minimum rate with fixed time artificial insemination.

Present study we have evaluated the KVK-RDDC[3] protocol with other established hormonal protocol of estrus synchronization in various feeding supplement groups of infertile animals.

Materials and Methods

Animals selection

A total of 315 Infertile cows were kept under different managemental conditions *i.e.* in commercial dairies, in different village conditions (unorganized manner) and *Gaushala* were included in this study (Animal and Area details-Table 1)

Feed groups

In the present study on the basis of feed (composition-Table 2) provided to the cattle, the cattle were divided into three feed groups.

i) Group 1 - Control.-Balanced Feed without mineral and vitamin mixture
ii) Group 2- Balanced Feed + vitaminised chelated mineral mixture- Added 3.35% of vitaminised chelated mineral mixture with above balance feed.
iii) Group 3- Balanced Feed + vitaminised chelated oxicareovn solution provided by DSM Pvt. Limited– Added 3.35% of vitaminised chelated oxicareovn solution with above balance feed.

The feed was offered to each cattle for 60 days *i.e.* 2 kg feed/day/animal. The hormonal treatment was started after 30 days of feeding.

Protocol groups

Different hormonal protocols were used in these animals are as under.

i. No hormone: only balance feed with or without minerals and vitamins no other hormone
ii. GPG 48 (Ovosynch protocol)- First injection on day zero, seven days after GnRH injection $PGF_{2\alpha}$, 48 hours after $PGF_{2\alpha}$, II injection of GnRH repeated followed by Two AI at 12 hours apart.
iii. GPG 56 (Cosynch protocol)- First injection of GnRH on day zero, seven days after GnRH injection $PGF_{2\alpha}$, 56-60 hours after $PGF_{2\alpha}$, II injection of GnRH repeated followed by two[4] AI at 12 hours apart.
iv. GPG 56 + CIDR (Cosynch+progesterone therapy)- First injection of GnRH on day zero, with CIDR(intravaginal progesterone releasing device) seven days after GnRH injection $PGF_{2\alpha}$ and removal of CIDR 56-60 hours after $PGF_{2\alpha}$, II injection of GnRH repeated followed by Two AI at 12 hours apart.
v. Ovarian structure based on KVK- RDDC, Udaipur protocol.

The treatment plan is based on modifying the estrus cycle of the particular animal using hormonal treatment on the basis of presence and absence of specific structure on the ovaries.

This treatment is not synchronization of estrus in true sense because all animals selected will not be coming in estrus at the same time. Thorough rectal palpation will be done and ensure there is no abnormality and animal is not pregnant and based on ovarian structures, the selected animals are divided into two groups.

Group 1- Animals having prominent luteal structure
Group 2- Animals not having prominent luteal structure
These Animals of group 2 were examined after 7 days and subdivided into two groups depending upon the response of the treatment given.

Group 2A: If the prominent luteal structure is found after treatment
Group 2B: If no prominent luteal structure is found after treatment

Treatment details for Group 1: Animal having prominent luteal structure

S. N.	Days	Observation	Intervention
1	Day 01	Prominent luteal structure palpated	Inj. $PGF_{2\alpha}$ IVSM * or I/M**
2	Day 02 / 03 (Before 48 hrs.)	Some animals showed good signs of heat.	Inj. GnRH (Deep I/M) and perform A.I. and repeat A.I. after 12 hrs.
3	Day 03 (After 48 hrs.)	Animals not showed good signs of heat.	Inj. GnRH (Deep I/M) Perform A.I. (Fixed time A.I.) after 12 hrs and repeat A.I. after 8-12 hrs.
4	Day 05 to 10	Some of animals (5–10%) come in heat	Perform A.I. (if no functional CL is present)

Treatment details for Group 2: Animal not having prominent luteal structure (these animals may have prominent follicular structure or no palpable structures on ovaries)

S. N.	Days	Observation	Intervention	
1	Day 01	Prominent follicular structure OR No palpable structure	Inj. GnRH	2.5 ml I/M
		Follicular cyst	Inj. GnRH	5 ml I/M
2	Day 02 – 07	Some animal may come in heat	Perform A.I. and repeat AI after 8-12 hrs.	
Day 07 Perform rectal palpation to observe ovarian structures				

Treatment details for Group 2A: (If prominent Luteal structure is found)

S. N.	Days	Observation	Intervention	
1.	Day 07	Prominent luteal structure palpated	Inj. $PGF_{2\alpha}$	IVSM or I/M
2.	Day 08 / 09(Before 48 hrs.)	Some animals may show good signs of heat.	Inj. GnRH (Deep I/M) and perform A.I. and repeat AI after 12 hrs.	
3.	Day 09 / 10 (After 48 hrs.)	Animals may or may not show good signs of heat.	Inj. GnRH (Deep I/M) Perform A.I. (Fixed time A.I.) after 12 hrs and repeat AI after 8-12 hrs.	
4.	Day 11 to 15	Some of animals (5–10%) may come in heat	Perform A.I.	

Treatment details for Group 2 B**:** (If prominent follicular structure or no palpable structure is found)

S. N.	Days	Observation	Intervention	
1.	Day 07	Prominent Follicular Structure or No Palpable Structure is found	Inj. GnRH	2.5 ml I/M
Repeat same procedure as mention above using $PGF_{2\alpha}$ (Day-14), GnRH (Day-16) and followed by A.I. (Day 16 / 17).				

*IVSM (Intra vulvo submucosal route) **IM (Intra muscular)

Breeding: All cows were artificially inseminated with frozen semen of high fertility. AI was performed as per the plan after hormonal treatment.

Pregnancy diagnosis: All the inseminated cows which did not return to estrus were examined manually through rectum at day 35 - 45 and scanned ultra sonographically for the confirmation of pregnancy.

Parameters recorded: Conception rate (%)

Results and Discussion

Bovine infertility is a major concern to improve the economic status of Indian semi-organized dairy farmers. This is a single most factor responsible for abandon of the animals. So addressing this problem in a systematic and scientific manner is of prime importance. If we evaluate the cause of bovine infertility the major factor comes out is nutritional causes due to imbalance feed and feed with lack of micronutrient leads to disturbance of estrus cycle of cattle and buffalo results in repeat breeding. Further role of better management cannot be ruled out. Methods of address the infertility through hormonal therapy are documented very well (Roberts, 1986). With better nutrition this therapy gives higher conception rate. These all protocol Ovosynch, Cosynch and Cosynch+ progesterone therapy are blind protocols and require minimal animal investigation and moderate conception rate (Galvao and Santos, 2012).

But if we adopt the ovarian structure palpation based protocol with minimal hormonal intervention as required will definitely reduce the cost of therapy and improve the results in term of conception rate (Robert's 1986 2nd edition). Though it requires extensive animal examination but conception rate will be high so it will prove ultimately beneficial. Another benefit of ovarian structure based hormonal therapy is utilization of the intravulvo-submucosal route (to reduce the dose of $PGF_{2\alpha}$ up to one- fourth of the IM route) was considered. $PGF_{2\alpha}$ is fatty acid in nature. In nature, the secretion of $PGF_{2\alpha}$ is caused by the uterine horn epsilateral to the ovary having CL. This hormone when drained by the uterine vein diffuses into the ovarian artery forms a coiling mesh around this vein and thus the hormone directly reaches to ovary having CL to cause its luteolysis. This mechanism forms the basis for using lower doses of $PGF_{2\alpha}$ injection by epsilateral VSM route (Parmar *et al.,* 2016) which not only reduce the cost of $PGF_{2\alpha}$ but also improve its efficiency. One such protocol has been developed and tried on thousands of animals by Krishi Vigyan Kendra (KVK) and Regional Disease Diagnostic Centre (RDDC) Department of Animal Husbandry Udaipur Rajasthan (commonly termed as KVK-RDDC protocol).

Here in this study we incorporate the Ovosynch, Cosynch, Cosynch+CIDR and KVK-RDDC[6] protocols for treatment of bovine infertility under different nutritional and management conditions.

As described in materials and methods, we selected animals from different habitat i.e. organized commercial farms, semi-organized farms and at unorganized villages and *gaushala's*[7]. Further animal feed group were developed three as group-1 only balance feed, group -2 having balance feed+ vitaminised chelated mineral mixture from well reputed company and group-3 balance feed + chelated oxycareovn solution provided by DSM Pvt. Limited.

Minerals and vitamins play an important role with balanced feed and support to infertility by improving reproductive performance and health with little additional cost. Their imbalance causes various problems leading to lowered reproductive efficiency and resultant monetary loss to the dairy industry (Kumar *et al.*, 2011).

The cost of balance feed calculated on the basis of raw material cost in the open market (in pellet form and packaging) provided to feed group 1 was Rs. 20.79/kg whereas for feed group 2 and feed group 3, it was Rs. 27.09/kg and Rs. 30.77/kg, respectively. The additional cost incurred for mineral and vitamin mixtures in group 2 and group 3 animals would certainly be reimbursed with high production potentiality after calving and also higher reproductive efficiency. The effect of nutrient supplementation on conception rate (Table-4) showed that balanced feed with vitaminised chelated oxicareovn solution (feed group 3) has highest conception rate of 61.0% followed by 44.8% in balanced feed with vitaminised chelated mineral mixture (feed group 2) and 29.5% in balance feed with no minerals and vitamins (feed group 1) whereas Puvarajan and Vijayrajan (2013) and Mohapatra *et al.*, (2012) found 97.38% and 60% conception rate, respectively.

The higher conception rates in balanced feed with chelated oxicareovn solution observed may be due to supplementation of minerals as per NRC 2001 recommendation and also follow the optimum vitamin nutrition recommendations. In India, the main factors behind low production and suboptimal reproductive efficiency of our livestock is due to inadequate nutrition and that too particularly because of mineral deficiency because of their role in follicular dynamics, ovarian activity and fertility Minerals are the integral component of production in animal diet (Ansotegui *et al.,* 1999, Boland, 2003, Coran and Ives, 1991).

The effect of different hormonal protocols under feed group 3 (Table-3) revealed that the conception rate is highest in the ovarian structure based KVK-RDDC protocol *i.e.* 76.2%. As this protocol is developed and followed by us in and around Udaipur Rajasthan, so there is no data available to compare it yet in present study the conception rate is higher than our previous studies (unpublished data not shown here). The higher conception rate may be due to better micronutrient supplement in oxycare group-3. As compared to Cosynch (GPG 48) (61.9%), Ovisynch (GPG 56) (61.9%), and Ovosynch+ progesterone therapy (GPG 56 + CIDR) (57.1%) and no hormone (47.6%), the conception rate was better in our KVK-RDDC[8] protocol.

The conception rate observed by different researchers with Ovosynch protocol *viz.* Barolia *et al.,* (2016) 66.66%, Amle *et al., (2015) 60%,* Geary *et al.,* (1998) 59.0%, Geary *et al.,* (1998) 57.0%, Pursley *et al.,* (1995) 50.0%, Pursley *et al.,* (1997) 35.0% and Melendez *et al.,* (2006) found conception rate of 22.7%. The variation in our finding regarding the conception rate might be due to selection of animals from different management condition i.e. from *Gaushala's*, villages, and organized dairy farms.

Table.1 Description of selected areas, animals and treatments

Protocols / Feed group	No Hormone	GPG-48	GPG-56	GPG-CIDR	KVK- RDDC	Total
Balanced feed (No Mineralsand Vitamins)	Miraj (G)-7	Bansda (G)-7	Bansda (G) -7	Bansda (G)-4	Miraj (G)-7	
	Eklingpura (C)1	Eklingpura (C) -2	Eklingpura (C) -1	Miraj (G)-3	Infert. clinic (C)-1	
	Imperial (C) -6	KVK (C) -5	KVK (C) -5	Dabok (C)-7	KVK (C)-6	
	Gadwa (V) -7	Gadwa (V) -7	Debok (C) -1	Junawas (V)-7	Junawas (V)-7	
			Gadwa (V) -7			
Total	21	21	21	21	21	105
Balanced feed + Vitaminised chelated mineral mixture	Bansda (G)-7	Bansda (G)-7	Bansda (G)-4	Miraj (G)-7	Miraj (G)-7	
	Eklingpura (C) -1	Eklingpura (C)-1	Miraj (G)-3	Infert. clinic (C)-6	Infert. clinic (C)-7	
	Imperial (C) -6	KVK (C)- 6	Eklingpura (C)-2	Eklingpura (C)-1	Fatehpura (V)-7	
	Fatehpura (V)-2	Gadwa (V)-7	KVK (C)-5	Fatehpura (V)-7		
	Gadwa (V)-2		Fatehpura (V)-4			
	Junawas (V)-3		Gadwa (V)-3			
Total	21	21	21	21	21	105
Balanced feed + Vitaminised chelated oxicareovn solution	Miraj (G)-7	Miraj (G)-7	Miraj (G)-7	Miraj (G)-7	Miraj (G)-7	
	Imperial (C)-7	KVK (C)- 7	KVK (C)-7	Infert. clinic (C)-7	Debok (C)-1	
	Fatehpura (V)-7	Fatehpura (V)-7	Fatehpura (V)-7	Fatehpura (V)-6	KVK (C)-6	
				Gadwa (V)-1	Fatehpura (V)-7	
Total	21	21	21	21	21	105
Grand Total	63	63	63	63	63	315

C= Commercial dairy farm, V= Village, G= *Gaushala,*

Table.2 Balanced Feed Composition (Group1)

Ingredients	Qty (kg.)
Maize	20.0
Barley	13.0
Moong Churi	7.5
Molasses	4.0
Rice Polish	5.0
GNC	20.0
Guar Korma	10.0
By Pass Fat	1.5
DORB	10.0
DCP	3.0
Salt	1.6
Calcite	3.7
Na bi Carb	0.375
MgO	0.125
Toxin Binder	0.2
Total	100.0

Table.3 Effect of hormonal protocols under different nutrient supplement on conception rates in cows

Hormonal Protocols	Balanced feed (No Minerals and Vitamins)	Balanced feed + Vitaminised chelated mineral mixture	Balanced feed + Vitaminised chelated oxicare ovn solution	Total
	Conceived/total animals (%)			
No Hormone	4/21 (19.0%)	4/21 (19.0%)	10/21(47.6%)	18/63(28.6%)
GPG 48	5/21(23.8%)	9/21 (42.9%)	13/21(61.9%)	27/63(42.9%)
GPG 56	6/21(28.6%)	10/21 (47.6%)	13/21(61.9%)	29/63(46.0%)
GPG 56 + CIDR	6/21(28.6%)	11/21 (55.0%)	12/21(57.1%)	29/63(46.0%)
KVK-RDDC	10/21(47.6%)	13/21 (61.9%)	16/21(76.2%)	39/63(61.9%)
Total	31/105(29.5%)	47/105 (47.8%)	64/105 (61.0%)	142/315(45.0%)

Table.4 Effect of different nutrient supplementation on conception rates in cows

Feed Group	Number of animals	Number of animals conceived	Percent conception (%)
Balanced feed (No Minerals and Vitamins)	105	31	29.5
Balanced feed + Vitaminised chelated mineral mixture	105	47	44.8
Balanced feed + Vitaminised chelated oxicare ovn solution	105	64	61.0

Table.5 Effect of nutrient supplementation under different field conditions on conception rates in cows

Field Condition	Balanced feed (No Minerals and Vitamins)	Balanced feed + Vitaminised chelated mineral mixture	Balanced feed + Vitaminised chelated oxicare ovn solution	Total
	Conceived/total animals (%)			
Commercial dairies	13/35(37.1%)	20/35(57.1%)	25/35(71.4%)	58/105(55.2 %)
Village animals	10/35(28.6%)	16/35(45.7%)	20/35(57.1%)[18]	46/105(43.8 %)
Gaushala	8/35(22.9%)	11/35(31.4%)	19/35(54.2%)	38/105(36.2 %)

Table.6 Effect of hormonal protocols under different field conditions on conception rates in cows

Field Condition	No Hormone	GPG 48	GPG 56	GPG 56 + CIDR	KVK-RDDC	Total
	Conceived/total animals (%)					
Commercial Dairies	7/21(33.3%)	12/21(57.1 %)	12/21(57. 1%)	12/21(57.1%)	15/21(71.4%)	58/105(5 5.2%)
Village animals	6/21(28.6%)	9/21(42.9 %)	10/21(47. 6%)	9/21(42.9%)	12/21(57.1%)	46/105(4 3.8%)
Gaushala	5/21(23.8%)	6/21(28.6 %)	7/21(33.3 %)	8/21(38.1%)	12/21(57.1%)	38/105(3 6.9%)

The effect of different hormonal protocols under feed group 2 (Table-3) showed the highest conception rate of 61.9% in ovarian structure based KVK-RDDC protocol followed by GPG 56 + CIDR (55.0%), GPG 56 (47.6%), GPG 48 (42.9%) and no hormone (19.0%).

The effect of different hormonal protocols under feed group 1 (Table-3) showed the highest conception rate of 47.6% in ovarian structure based KVK-RDDC protocol followed by GPG 56 + CIDR (28.6%), GPG 56 (28.6%), GPG 48 (23.8%) and no hormone (19.0%).

In present study, in ovsynch and cosynch protocol the conception rate is somewhat lower than reported by earlier by Barolia *et al.*, 2016 (66.66%)[9] and similarly reported by Neglia *et al.*, (2003) & Baruselli *et al.*, (2001). It may be due to various feeding and managemental conditions of selected animal group because all above said workers reported that percentage of conception rate in same breed of cows and same feeding and managemental conditions. But our KVK-RDDC[10] protocol we received higher percentage of conception rate which was excepted as we monitored the development of ovarian structure and treated the animals accordingly.

During our study it has been observed that commercial farms have balanced availability of feed and green fodder, minerals and vitamins supplementation, scientific management conditions, better preventive health care *etc*. The effect of various treatments of nutrient supplementation under different field conditions revealed that in vitaminised chelated oxicareovn solution in commercial dairies have higher conception rate (71.4%) followed by 57.1% for village animals, whereas it was minimum (54.2%) under *Gaushala* condition(Table 5).

The availability of balanced feed and fodder and good management conditions favour's to increase production potentiality and reproductive efficiency of the bovine (NDDB, 2012). The effect of different hormonal protocol under different field conditions showed that KVK-RDDC Udaipur have highest conception rate in all field conditions followed by GPG 56, GPG 56 + CIDR, GPG 48 and no hormone(Table 6).[11]

Hence concluded in our study, we found that ovarian structure palpation based KVK-RDDC protocol to treat bovine infertility gave better conception rate followed by ovosynch (GPG48), cosynch(GPG56) and cosynch + progesterone(GPG56+CIDR)[12] protocols. Further animal fed on balance diet+ oxycareovn solution have better conception rate among all four treatment protocols. Better management also proved helpful in increasing the conception rate among selected animals.

Acknowledgement

The authors thankfully acknowledged DSM Nutritional Products India Private Limited for providing financially support for the trial and also to Vidya Bhawan Krishi Vigyan Kendra Udaipur for providing all the facilities required during the study.

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How to cite this article:

Praful Chandra Bhatnagar, J. L. Choudhary, B. Bhardwaj, Chandr Shakhar, Lokesh Gupta and Deepak Kumar Sharma. 2020. Effect of Different Hormonal Protocols and Nutrient Supplementation on Reproductive Performance of Cattle under Different Field Conditions. *Int.J.Curr.Microbiol.App.Sci.* 9(01): 1511-1522. doi: https://doi.org/10.20546/ijcmas.2020.901.169