STUDIES ON POST INSEMINATION SUPPLEMENTATION OF GnRH or hCG TO OVSYNCH PROTOCOL IN CROSSBRED COWS

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CERTIFICATE

Dr. A.LINGASWAMY has satisfactorily prosecuted the course of research and that the thesis entitled **"STUDIES ON POST INSEMINATION SUPPLEMENTATION OF GnRH or hCG TO OVSYNCH PROTOCOL IN CROSSBRED COWS"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

2/4/2018

Date: 2.4.2018
Place: Hyderabad

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CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON POST INSEMINATION SUPPLEMENTATION OF GnRH or hCG TO OVSYNCH PROTOCOL IN CROSSBRED COWS"** submitted in partial fulfillment of the requirements for the degree of **MASTER OF VETERINARY SCIENCE** of **P V NARSIMHA RAO TELANGANA VETERINARY UNIVERSITY, HYDERABAD** is a record of the bonafide research work carried out by **Dr. A.LINGASWAMY** under my guidance and supervision. The student's Advisory Committee has approved the subject of the thesis.

No part of the thesis has been submitted for any other degree or diploma. The author of the thesis has duly acknowledged all the assistance and help received during the course of investigation.

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Place: Hyderabad ***(AITHAGONI LINGASWAMY)***

Date: 27.12.2017

DECLARATION

I, Dr.A.LINGASWAMYhereby declare that the thesis entitled **"STUDIES ON POST INSEMINATION SUPPLEMENTATION OF GnRH or hCG TO OVSYNCH PROTOCOL IN CROSSBREED COWS"**submitted to **P V NARSIMHA RAO TELANGANA VETERINARY UNIVERSITYFOR THE DEGREE** of **MASTER OF VETERINARY SCIENCE** is a result of original research work done by me. It is further declared that the thesis or any part thereof has not been published earlier in any manner.

Date : 27-12-2017 **(A.LINGASWAMY)**

Place : Hyderabad

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ABSTRACT

The present investigation entitled "Studies on Post Insemination Supplementation of GnRH or hCG to Ovsynch Protocol In Crossbreed Cows" was undertaken on 50 post partum crossbreed cows to study the estrus pattern, estrus response and the efficacy of post AI supplementation of GnRH and hCG on conception rate. The 50 crossbreed cows were divided randomly into five equal therapeutic groups so that each group consist 10 crossbreed cows. The Ovsynch Group cows were injected with 10µg of GnRH(Buserelin acetate) IM on day 0, 500 µg of cloprostanol (PGF2α) IM on day 7 and 2nd dose of GnRH10µg IM on day 9. Fixed time AI was done after 16 hours of the 2nd dose GnRH of injection.Ovsynch+GnRH Group cows were injected with 10µg of GnRH IM on day 0, 500 µg of cloprostanol (PGF2α) IM on day 7 and 2nd dose of GnRH10µg IM on day 9.

Fixed time AI was done after 16 hours of the 2nd dose GnRH of injection.On day 6 of post insemination,GnRH@ 10µg IM was administered. Ovsynch+hCG Group cows were injected with 10µg of GnRHIM on day 0, 500 µg of cloprostanol(PGF2α) IM on day 7 and 2nd dose of GnRH10µg IM on day 9. Fixed time AI was done after 16 hours of the 2nd dose of GnRH injection. On day 6 of post insemination hCG@1500IU IM was administered. GPH+GnRH Group cows were injected with 10µg of GnRH(Buserelin acetate) IM on day 0, 500 µg of cloprostanol(PGF2α) IM on day 7 and hCG @1500IU,IM on day 9. Fixed time AI was done after 16 hours of hCG injection. On day 6 of post insemination GnRH@10µg IM was administered. Control Group cows were injected with 2.5 ml of NS IM on day 0, 7 and 9 and whenever the crossbreed cows exhibited estrus then cows were inseminated. On day 6 of post insemination 2.5 ml of NS IM was administered.

The estrus response was 90.00,100.00,100.00,100.00 and 30.00 per cent in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively. There was no significant difference ($p<0.05$) among the groups. The mean onset of estrus was recorded as 51.80±1.07, 50.90±1.20, 52.40 ±1.32, 52.40 ±1.32 and 96.00 ±13.86hrs in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively. The onset of estrus in therapeutic groups were not significantly ($p< 0.05$) different among the groups. Among 50 post partum crossbred cows, normal intensity of estrus was exhibited by 66.00 per cent of crossbred cows whereas weak and intense estrus was exhibited by 26.00 and 8.00 per cent of crossbred cows. The mean score for estrus intensity was recorded as 11.70±0.79, 12.50±0.83, 11.60±0.53, 12.30±0.77and 05.10±2.07, in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively. Statistical analysis revealed that the intensity of estrus in treatment groups were significantly ($p<0.05$) different with that of the control group. The mean duration of estrus was recorded as 21.90±0.69, 22.30±0.79, 21.80±0.73, 23.60±0.82 and 21.40±0.60 hrs in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively in the present study. The duration of estrus in theraputic groups was not significantly different among the groups.

The overall conception rate in therapeutic crossbred cows was recorded as 60.00, 50.00, 40.00, 60.00, and 20.00 per cent in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively. Statistical analysis revealed that the conception rates were higher in Ovsynch group, GPH+GnRH group and followed by group Ovsynch+hCG, Ovsynch+GnRH and control. However, the conception rates were significantly ($P<0.01$) lower in control group of crossbred cows than the treatment groups. The number of services required per conception was 1.83, 2.40, 2.75, 1.83 and 2.00 in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively. In conclusion, the present study revealed that the use of GnRH or hCG 6 days post insemination showed no significant improvement on the conception rate in crossbred cows over Ovsynch protocol.

LIST OF SYMBOLS AND ABBREVIATIONS

@	:	At the rate of
±	:	Plus or minus
>	:	Greater than
<	:	Less than
µg	:	Microgram
AI	:	Artificial Insemination
CIDR	:	Controlled Internal Drug Release
CL	:	Corpus Luteum
e*tal*	:	“and others”
FAO	:	Food and Agriculture Organization
FSH	:	Follicular Stimulating Hormone
FTAI	:	Fixed Time Artificial Insemination
gm	:	gram
GnRH	:	Gonadotrophic Releasing Hormone
hCG	:	Human Chorionic Gonadotrophin
hrs	:	hours
I M	:	Intramuscular
I U	:	International Unit
LH	:	Leuteinizing Hormone
MAPK	:	Mitogen Activated Protein Kinases

mg : Milligram

ml : Millilitre

NDDB : National Dairy Development Board

NS : Normal Saline

PGF2α: Prostaglandin F2 alpha

PRID : Progesterone Releasing Intra vaginal Device

SPSS : Statistical Packages for Social Sciences

TSLDA :Telangana State Livestock Development Agency

Vs:Versus

CHAPTER I

INTRODUCTION

Indian agricultural system is predominantly a mixed crop-livestock farming system, with the livestock segment supplementing farm incomes by providing employment, draught animals and manure. India ranks first in milk production, accounting 18.5 per cent of world production, Indian dairy industry has shown an unprecedented growth in milk production from about 55.6 million tonnes in 1991-92 to about 155.5 million tons in 2015-16 (NDDB, 2017) with growth rate of 6.28 per cent. Whereas, the Food and Agriculture Organization (FAO) reported a 1.6 per cent increase in world milk production from 789.1 million tons in 2014 to 816 million tons in 2016.

To maintain the steady growth rate in milk production for which reproduction is an important consideration,in the economics of cattle production. In the absence of regular breeding and calving at the appropriate time, cattle rearing is not profitable. In dairy cattle farming, it is necessary to have a calf once a year and to ensure that the postpartum cows are pregnant at the first service. For this purpose, there should not be any problem in the herd regarding reproduction. Many diseases and environmental factors can decrease fertility in postpartum cows. For cows not detected in estrous; sub estrous, anestrous, delayed ovulation, early embryonic death, high milk yield, negative energy balance, high environmental heat and various diseases are present as the reasons of decrease in fertility.

Synchronization of ovulation with FTAI is an effective management tool in lactating crossbred cows to increase reproductive efficiency (De La Sota *et al*., 1998). The Ovsynch protocol, which combines GnRH-PGF2α-GnRH treatments, has made planned breedingprogramme more effective (Pursley *et al*.,1997). Although fertilization rates in cattle are reported to be greater than 90 per cent, the majority of embryonic mortality (70 to

80 per cent of the total loss) occurs between days 8 and 16 after insemination (Dunne *et al*., 2000). Maintenance of pregnancy, in part, is dependent on progesteroneduring early pregnancy (Mehni *et al*., 2011). A significant proportion of infertility in cattle has been attributed to improper functioning of the CL, which is the major source of progesterone in the pregnant cow (Santos *et al*., 2001). Poor luteal activity has been associated with infertility in cattle (Mann and Lamming, 2001), and low circulating progesterone after breeding may impact embryonic development and maternal recognition of pregnancy (De Rensis *et al*., 2008). Furthermore, recent study reported that animals on day 5, with higher plasma progesteroneconcentrations, had developmentally more advanced and viable embryos (Green *et al*., 2012).

However, relatively few studies have been undertaken to investigate the strategy of GnRH replacement with hCG in FTAI protocol to improve ovulation and conception rates and to induce the formation of accessory CL, and subsequent plasma progesterone concentrations. Therefore, the objective of this study was to evaluate the efficacy of post insemination supplementation of GnRH orhCG in FTAI protocol on estrous response, estrous pattern and conception rates in post partum crossbredcows.

CHAPTER II

REVIEW OF LITERATURE

The literature available from India and abroad dealt with the study on incidence of reproductive disorders, estrous response,estrous patterns and conception rate in response to ovsynch and ovsynch followed by post insemination supplementation of GnRH and hCGwere reviewed.

2.1 INCIDENCE OF REPRODUCTIVE DISORDERSIN CROSSBRED COWS.

Incidenceof various types of reproductive disorders reported by different authors in crossbred cows were reviewed hereunder.

Shiferaw *et al*. (2005) reported that the principal postpartum reproductive disorders were retained fetal membranes (14.7 per cent) and uterine infection (15.5 per cent). Anoestrous was the major postpartum reproductive problem in the mixed crop–livestock production system (38.6 per cent) and was significantly associated with the production system. Apart from anoestrous, the occurrence of reproductive disorders was not significantly associated with a production system. Most of the reproductive disorders occurred as a complex rather than as a single abnormality. Two or more abnormal conditions were seen in 11.4 per cent of the cases.

The functional causes were reported to be the major incidence (68.64 per cent), the remaining being infectious (29.73 per cent) and anatomical (1.63 per cent) causes among Frieswal crossbred cows. The percentage of incidence of reproductive disorders were hypoplasia of ovaries and reproductive tract - 0.74, genital infantilism - 0.89, smooth ovaries - 8.88, post service anestrous - 6.66, postpartum anestrous - 13.31, silent ovulations - 2.66, anovulatory heat - 3.25, luteal persistency - 4.88, luteal cysts - 4.44, follicular cysts - 3.25, repeat breeder - 21.30, vaginitis - 1.18, cervicitis - 6.51, endometritis - 18.20, metritis- 2.66, pyometra - 0.59, salpingitis - 0.15 and bursal adhesions - 0.44 per cent in crossbred cows (Muneer *et al*., 2010).

Hadush *et al*.(2013) conducted a retrospective survey to determine major prepartum and postpartum reproductive problems of dairy cattle. Analysis of clinical data collected from 711 cows of farmers of different villages was recorded that 44.3 per cent of cows has major prepartum and postpartum reproductive problems. Among these, postpartum anestrous (12.9 per cent) was the major reproductive problem followed by repeat breeding (11.4per cent). A total of 104 cows were used in the regular follow up and 33.6per cent were found to be affected either with one or more of clinical reproductive problems. In those problematic animals, postpartum anestrous (12.5per cent) was found to be the leading reproductive problem followed by repeat breeding (10.6per cent), metritis (8.7per cent), abortion (6.7per cent), retained placenta (3.8per cent), dystocia (2.9per cent) and prolapse of uterus (1.9per cent).

Adane Haile *et al*. (2014) conducted a survey using questionnaires and observations in urban and per urban area of Hossana with the objectives of determining the prevalence of major reproductive health problems of dairy cattle and found that cows were affected either with one or more of reproductive problems.The incidence of repeat breeder, anoestrous, retained fetal membrane (RFM), and dystocia were 13.08, 12.06, 7.18 and 5.9 per cent, respectively and incidence of vaginal prolapse, abortion, mixed and uterine prolapse having 3.44, 2.56, 1.03, and 0.76 per cent, respectively.

In an survey conducted by Barua and Sarma(2016) in Golaghat district of Assam on incidence of various reproductive problems in Jersey crossbred dairy cattle, they recorded various reproductive disorders as anovulatory heat (3.00 per cent), sub-oestrous (1.25 per cent), true anoestrous (2.50 per cent), persistant corpus luteum (0.75 per cent), endometritis

(1.50 per cent), mucometra (0.25 per cent), follicular cyst (0.50 per cent) and luteal cyst (0.50 per cent).

Out of the total animal affected with reproductive disorders, the incidence of anestrous, repeat breeder, retention of fetal membrane, and abortion was found to be 31.79, 24.61, 14.35 and 11.25 per cent, respectively in crossbred cows. In addition, dystocia (5.12 per cent), prolapse (1.53 per cent), endometritis (4.61 per cent), and pyometra (6.66 per cent) were minor clinical reproductive problems reported by Khan*et al.* (2016)

Sujit Das *et al.* (2016) evaluated the survey on reproductive disorder distribution and revealed, that the incidence of reproductive problems were 33.4 per cent in cross bred cows and 51.2 per cent in heifers. Out of the total reproductive disorder cases, 26.62, 2.52and 4.32 per cent of cases were recorded as repeater, anoestrous and others, respectively in crossbred cows and the corresponding reproductive disorders were recorded as 12.24, 37.41 and 1.36 per cent, respectively among the crossbred heifers. Among anoestrous animals, the percent of non-functional ovary was found to be 44.0 per cent in cows. The presence of sub active ovary was 50.66 per cent among anoestrous cows and 27.96 per cent among repeat breeding cows.

The overall prevalence of reproductive health problems was 54.6 per cent and the major ones were found to be prolonged anoestrous, abortion, still birth and retained fetal membrane accounted for 48.5, 28.9, 14.4 and 5.2 per cent prevalence, respectively (Siyoum *et al.*, 2016).

2.2. ESTROUS RESPONSE.

The estrous responsewas calculated as the percentof treated cows andwhich exhibited estrousand the available literature were reviewed.

Crossbred and nondescript cows in India were treated with two injections of PGF2α at 11 days apart and showed estrous appearance rate as 100.00 and 80.00 percent, (Sahatpure and Patil, 2008). Atotal of 48 repeat breeder cows were divided into three groups viz., group I, II and III. Cows in group I and II were treated with 0.98mg of Triaprost (PGF2α) on day 10 following natural oestrous (day 0) and there was cent per cent oestrous response observed in PGF2α treated cows by Selvaraju *et al*.(2008**).**

Yendraliza *et al*.(2011) noticed the effect of administration of GnRH and PGF2α on onset of estrous and conception rate in different postpartum swamp buffaloes.The results showed that there was no significant difference ($p>0.05$) among treatments in induction of estrous.

The estrous induction response was noted as 100 per cent in CIDR, Ovsynch and Co-synch protocols treatment groups (Ramakrishnan *et al*.,2012), but the behavioural signs were observed in 83.33, 83.33 and 100.00 per cent of cows under CIDR, Ovsynch and Co-synch protocols, respectively.

Navrange *et al.*(2012) observed 100 percent estrous response in buffalos synchronized with Ovsynch protocol with injection GnRH 2.5 ml intramuscularly on day 0 and 9 while injection PGF2α 2 ml intramuscularly on day 7.

Ion Caraba and Serafin Velicevici(2013) studied on 15 dairy cows which were synchronized by Ovsynch and Cosynch programs. The estrous response for cows in Ovsynch protocol was 63 per cent and in Cosynch protocol it was 57 per cent.

Mohd Alyas *et al*. (2013) evaluated the efficiency of supplementation of progesterone in Ovsynch protocol to improve fertility in post-partum anestrous buffaloes. Twelve healthy lactating anestrous buffaloes were equally divided into two groups. Group-I buffaloes were treated with Ovsynch protocol and group-II buffaloes were treated with Ovsynch plus CIDR protocol. Timed artificial insemination was done at 12 and 24 hrs after second GnRH injection in all treated animals. The efficacy of treatment in terms of estrous induction rate was 100 per cent in both groups, however the first service conception rate in Ovsynch and Ovsynch plus CIDR treated buffaloes was 33.00 and 66.67per cent. It was significantly higher ($P<0.05$) in Ovsynch plus CIDR treated than Ovsynch treated buffaloes.

Atotal ofsixteen normally calved cows were selected at 35-50 days postpartum and equally divided into two groups. Group I cows were treated with ovsynch protocol between days 50 and 65 post-partum and group II as contol.Estrous response in group I and II was 100 and 62.5 per centrespectively (Velladurai *et al*., 2014).

Buhecha *et al.* (2015) carried out a study to evaluate the clinical response and monitor the peripheral plasma progesterone profile in 46 postpartum anoestrous crossbred cows(>90 days) treated with Triu-B/ PRID, Ovsynch and Heatsynch, keeping untreated anoestrouscows incontrol group and the results were compared with normal cyclic control group. All the cows(100 per cent) under three protocols exhibited estrous.

Dhami *et al*. (2015) reported that the use of CIDR, Ovsynch, and Norgestomet ear implant protocols resulted in 100 per cent estrous induction in crossbred cows.

WhenOvsynch treatment was initiated on day 6 of estrous cycle, all the cows (100 per cent) responded to Ovsynch and Ovsynch based GnRH treatments with expression of behavioral and physical signs of estrous. (Ahmed *et al*., 2016)

2.2.1 Onset of Estrous

The onset of estrous was calculated in hrs (h) from the time of PGF2α administration to the time of the first appearance of estrous signs in therapeutic crossbreed cows and the available literature were reviewed.

Selvaraju *et al*.(2008**)**observedthe mean onset of estrous was 59.38±0.81 and59.63±0.74 hrs in group I and II (both groups treated with PGF2α) respectively, in repeat breeder cows.

Onset of estrous ranged between 37.4 to 38.4 hrs in post partum swamp buffalo by treating with ovsynch protocol (Yendraliza *et al.*, 2011).

Patiland Pawshe. (2011) noticed the average time required for onset of estrous was significantly lower in cows (62.2±1.56 hrs) than heifer (64.66±1.76 hrs) after PGF2α injection.Navrange *et al.* (2012) observed 100 percent estrous response in buffaloes synchronized with Ovsynch protocol with injection GnRH 2.5 ml intramuscularly on day 0 and 9 while injection PGF2α 2 ml intramuscularly on day 7.

A total ofsixteen normally calved cows were selected at 35-50 days postpartum and equally divided into two groups as group I and II. Group I cows were treated with ovsynch protocol between day 50 and 65 post-partum and group II as contol. Recorded the mean interval of onset of estrous in group I and II were 47.97±2.65 hrs and 23.50±4.24 days,respectively(Velladurai *et al*., 2014).

Buhecha *et al.* (2015) carried out a study to evaluate the clinical response and monitor the peripheral plasma progesterone profile in 46 postpartum anoestrous crossbred cows(>90 days) treated with Triu-B/ PRID, Ovsynch and Heatsynch, keeping untreated anoestrouscows as control group and the results were compared with normal cyclic control group. All the cows(100 per cent) under three protocols exhibited estrous within mean intervals of 67.00±1.74, 69.83±0.87 and 68.17±1.24 hrs, respectively, from PGF2α injection.

In a study, Senthilkumar and Chandrahasan, (2015) randomly dividedcyclic crossbred cows into 4 groups comprising of 8 animals in each group namely control (Group l), PGF2α (Group-II), PGF2α+GnRH (Group-III), and PGF2α+hCG (Group-IV). The time taken for the onset of oestrous was 70.50 ± 3.27, 68.00 ± 3.17 and 68.25±2.21 hrs in Group (II), Group (III) and Group (IV), respectively.

Ahmed *et al*. (2016) recorded the mean onset of estrous as48.75±0.71 and 51.47±1.99hrs in Ovsynch and Ovsynch based GnRH treatment groupsrespectively,in crossbred cows and the difference was not significant.

Ahmed *et al*. (2017) reported mean onset of estrous as 48.80±5.74, 53.25±3.02 and 46.40±4.33 hrs,in treatment groups of Ovsynch, Ovsynch plus CIDR and G6G {PGF2∝(day 0) – GnRH(day 2)- GnRH(day 8) -PGF2∝(day 15) –GnRH(day 17) -A.I(12-24 hrs after last GnRH)}protocols, respectivelyin anestrous crossbred cows.

2.3 ESTROUS PATTERN

The literature available on types of estrous, intensity of estrous and duration of estrous in induced estrousin crossbred cows were reviewed.

2.3.1 Types of Estrous

Rao and Rao (1970) reported that the reproductive rhythm to be a continuous activity throughout the year with periods of peak and lean in the buffaloes. While no significant difference among seasons on the occurrence of estrous cycle was observed in white cattle(Lakshminarayana and Rao, 1983).

Kumar *et al.* (1988) observed the incidence of split estrous was 62.02, 40.98, 20.03, and 10.97 per cent in crossbred cows, indigenous cows, crossbred heifers and indigenous heifers, respectively, where as the incidence was found to be 5.01 and 26.1 per cent in buffaloe cows and buffaloe heifers, respectively (Roy *et al.* 1990). The incidence of silent estrous was reported to be 20 to 40 per cent in buffaloes (Kaikini, 1992).

Suryaprakasam and Rao (1998) reported that the incidence of split estrous was 0.77, 1.33 and 0.81 per cent in zebu, crossbred cows and buffaloes, respectively under village conditions.

Srinivasreddy (2014) reported the percentage of normal, silent and split estrous was 90.48, 3.17 and 6.35respectively in repeat breeding crossbred cows.

2.3.2 Intensity of Estrous

Intensity of estrouswas assessed by the symptoms exhibited by the cows and buffaloes during estrous. The intensity of estrous was studied by Rao and Kodagali (1983) and Singh *et al.* (1984) and theyreported that prominent estrous symptoms are bellowing (68.8 and 58.7 per cent), congestion of vulva (86.8 and 66.3 per cent) and mucus discharge (83.7 and 79.3 per cent) in cows and buffaloes, respectively.

Sahatpure *et al.* (2008) observed intensity of estrous in non-descriptive cows was intense in 25.00 per cent cows, intermediate in 50.00 per cent cows and weak in 25.00 per cent cows where as intensity of estrous in crossbred cows was intense in 41.67 per cent and intermediate in 58.33 per cent.

Selvaraju *et al.*(2008) observedthat the percentage of very good, good, fair and poor estrous intensity was 81.25, 18.75, 0.00 and 0.00 in cows treated withnorgestomet ear implant, whereas incontrol group (without any treatment) it was25.00, 75.00, 0.00 and 0.00per centrespectively.

Anjum *et al*. (2009) studied the estrous behaviour in Group A (control) and B (Treated with GnRH analogue at the time of AI) in crossbred cows. In group A, 40, 44 and

16 per cent females showed intense, moderate and weak estrous behaviour, respectively. In group B, 47, 41and 12 per cent females exhibited intense, moderate and weak estrous behaviour,respectively.

Lu Meng Chao *et al.* (2010) demonstrated that the estrous intensity of cows in estrous was different between natural estrous and induced estrous and also between the methods of the synchronization, but no difference was observed in the conception rate among the three groups.

A total sixteen normally calved cows were selected at 35-50 days postpartum and equally divided into two groups as group I and II. Group I cows were treated with ovsynch protocol between days 50 and 65 post-partum and group II as control and recorded the percentage of intensity of estrous was higher in group I than group II cows(Velladurai *et al*., 2014).

Senthilkumar and Chandrahasan.(2015) reported that the synchronized cows exhibited weak, normal and intense estrous as 8.33, 58.33 and 33.33 per cent, respectively.

Ahmed *et al*. (2016) recorded the intense, intermediate and weak estrous as 16.67, 50.00 and 33.33 per cent, respectively in control group, 33.33, 33.33 and 33.33 per cent, respectively in GnRH treatment on day 6 post-estrous group,16.67, 66.67 and 16.67 per cent, respectively in Ovsynch group 33.33, 50.00 and 16.67 per cent, respectively in Ovsynch based GnRH treatment group in crossbred cows. The incidence of intermediate estrous intensity was found to be highestin all the groups.

2.3.3 Duration of Estrous

Tanabe *et al*. (1994) studied and revealed a significant difference in the duration of estrous between first breeding (18.2 hrs) and repeat breeding (20.2 hrs) cows. The duration of estrous was ranged from 8 to 32 hrs.

Selvaraju *et al*. (2008**)** observed the mean duration of induced estrous was 28.50±0.56 and27.50±0.70 hrs in group I and II(both groups treated after PGF2α), respectively. In control, the mean duration of estrous was 29.38±0.77 hrs in repeat breeder cows.

Das *et al*. (2009) reported the mean duration of estrous as 25.20 ± 1.72, 33.27 ± 1.56 and 18.90 ± 1.05 hrs in anovulatory, delayed ovulatory and normal ovulatory crossbred cows, respectively.

Anjum *et al*. (2009) studied the duration of estrous in Group A (control) and B (Treated with GnRH analogue at the time of AI) in crossbred cows. The average estrous duration was 15 hrs in group A and it ranged from 7 to 24 hrs. Whereas in group B the estrous duration average was also 15 hrs and range was 8 to 24 hrs.

Honparkhe *et al*. (2010) reported that during GnRH pre-treatment to estrous cycle, animals showed the extended duration of estrous than normal values of 24-36 hrsunder the effect of suprabasal progesterone.

Yendraliza *et al.* (2011)recorded duration of estrous rangedfrom 16.8 to 18.2 hrs in post partum swamp buffalo by treating with ovsynch protocol.Likewise the average duration of estrous was 19.2±0.86 hrs in cows and 15.6±0.88 hrs in heifers by using single PGF2 α injection in crossbred cows(Patil and Pawshe, 2011).

Bhat and Battacharya. (2012) reported that the duration of estrous period in repeat breeding cows with different conditions of anovulation, delayed ovulation, normal ovulation with infection and control group were 26.33 ± 2.20, 34.43 ± 3.67, 18.43±0.48 and 18.71 ± 1.40 hrs, respectively.

A total sixteen normally calved cows were selected at 35-50 days postpartum and equally divided into two groups as group I and II. Group I cows were treated with ovsynch protocol between days 50 and 65 post-partumand group II as control.The mean duration of estrous in group I and II were 29.84±0.67and 28.78±0.67 hrs, respectively(Velladurai *et al*., 2014).

The cyclic crossbred cows were randomly divided into 4 groups comprising of 8 animals in each group, namely control (Group l), PGF2α (Group-II), PGF2α+GnRH (Group-III), and PGF2α+hCG (Group-IV). The duration of estrous in Group (I), Group (II), Group (III) and Group (IV) were 20.50 ± 0.41, 21.00 ± 0.47, 19.50 ± 0.33 and 19.25 ± 0.56 hrs,respectively (Senthilkumar and Chandrahasan,2015).

Ahmed *et al*. (2016) recorded the duration of estrousas 22.04±0.94, 21.45±1.10, 21.08±0.78 and 20.07±0.86 hrs, respectively, in control, GnRH treatment on day 6 post-estrous, Ovsynch and Ovsynch based GnRH treatment groups. There was no significant difference in duration of estrous among the groups.On other hand,Leaflet (2016) recorded the mean duration of estrousas 21.9 ± 1.1 hrs in crossbred cows.

Senthilkumar *et al*. (2017) noticed the duration of estrum in treatment groups was significantly less than control group in repeat breeding dairy cattle by using different hormonal treatment regimens.

2.4 POST INSEMINATION THERAPEUTIC EFEICACY ON CONCEPTION RATE.

Conception rate is a measure of a cow's fertility at service. The ideal first service conception rate should be more than 50 to 55per cent. Other strategies that try to improve conception rate by focusing on minimizing the embryonic mortality. Ovulation and fertilization occur in about 76to 90 per cent of treated animals with various synchronistion protocols (Diskin and Sreenan, 1980, Santos *et al.*,2001); hence on early post-insemination treatment with GnRH, human chorionic gonadotropin (hCG)and progesterone and the available literature was reviwed.

2.4.1 Post Insemination Treatment Efficacy of GnRH.

Mechanism of Action: Gonadotrophin-releasing hormone (GnRH)is a key integrator between the neural and endocrine systems. This decapeptide is synthesized in hypothalamic neurosecretory cells then released in a pulsatile pattern into the hypothalamo-hypophyseal portal circulation. This pulsatile pattern provokes the secretion of the gonadotropins, LH and FSH, from the anterior pituitary. Gonadotropins, in turn, control the function of the gonads; LH stimulates ovulation and corpus luteum formation in females and androgen secretion in males, whereas FSH stimulates the growth and maturation of ovarian follicles in females and spermatogenesis in males. (Hazum and Conn,1988)

Studies by Rajamahendran and Sianangama, (1992) demonstrated that supplementing GnRH or hCG between day 5 to 7 of the estrous cycle can induce the formation of an accessory CL, thereby increasing progesterone concentrations and possibly reducing embryonic loss. Treatment with GnRH or hCG given between day 5 to 7 post-AI, focuses on enhancing CL function, ovulating the first-wave dominant follicle, and increasing progesterone secretion. Ovulation of a dominant follicle at this time also results in a decrease in E2 production, which in turn results in an inhibition of up-regulation of oxytocin receptors and consequent inhibition of PGF2α secretion (Peters *et al.*, 1999). Up-regulation of oxytocin stimulates the synthesis of progesterone.

Administration of GnRH on day 12 post-AI was found to have no effect on embryonic mortality in cows (Tefera *et al*., 2001).

But, Batavani and Eliasi. (2004) carried out field trails to assess the effect of Gonadorelin on pregnancy rate of river buffaloes. Wherein, 84 lactating cyclic buffaloes were randomly allotted to three groups. (1) Untreated control, (2) injection of 25 μg of Gonadorelin IM at the time of artificial insemination and (3) injection of 25 μg Gonadorelin IM on days 11 to 13 post-insemination and reported pregnancy rate to first service (AI) in group 1, 2 and 3 as 25, 55 and 58 per cent, respectively.

Peters(2005) suggested the use of GnRH in cattle as an effective means of increasing pregnancy rates when given either at the time of insemination (first or repeat) or between days 11 and 14 after insemination. An overall increase of 18.0 per cent in pregnancy rate was recorded in cattle.

Howard *et al*. (2006) summarized the results of his study and stated that GnRH administration 5 days after AI increased serum progesterone by developing an accessory CL but did not improve conception rates in dairy cattle.

Stevenson *et al*. (2006) observed that 162 cows were assigned randomly to serve as control or to receive a CIDR insert for 7 days, 100 μg of GnRH or 3,300 IU of hCG. Higher number of cows were induced to ovulate in response to GnRH (60 per cent) and hCG (78per cent), compared with control (2.4per cent). Compared with control, cows treated with GnRH or hCG had more induced CL (day 7).

Lactating Holstein cows were assigned randomly to treatments to improve fertility after first postpartum timed artificial insemination (TAI). The study concluded that the treatment with CIDR inserts after TAI had no effect on pregnancy per AI, whereas treatment with GnRH 5 days after TAI improved pregnancy per AI for noncycling, but not for cycling cows (Sterry *et al.*, 2006).

Carvalho *et al*. (2007) observed the effect of supplementation of GnRH@25μg on 6^{th} day of AI in the 240 buffaloes synchronized with ovsynch protocol.The conception rate, number of accessory CL observed and birth rate were higher in GnRH treated on day 6 of post insemination than in control ($P<0.05$).

Vijayarajan *et al.* (2007) observed that with intramuscular injection of 10 µg of GnRH analogue (buserelin acetate) at mid cycle (day12) and inseminated in subsequent observed estrous (pre-A.I) buffaloes. Animals were inseminated at estrous and injected with 10 µg GnRHanalogue intramuscularly on day 12 (post-AI). The conception rate in pre-A.I., post-A.I., and control groups were 50, 40 and 30 per cent, respectively.

Dadarwal *et al.* (2007) administered intramuscular injection of buserelin acetate 20µg to crossbred cows 6 hrs before AI and second injection was given on day 12 post insemination. There was an increase of 18 and 35 per cent pregnancy rate over those observed in cows given single injection of buserelin (20µg) and control groups, respectively.

In Japan a study was designed to evaluate the reproductive performance of Japanese black cows following the 3rd injection of gonadotropin releasing hormone (GnRH) analogue administered concurrently with Ovsynch-based treatment on day 6 (day 1 = the day of ovulation)Abdurraouf Omar Gaja*et al.*(2008). In Experiment 1, 12 cows were allocated into three groups: a control group that was subjected to Ovsynch treatment and then injected with a placebo on day 6; group 1 (Ovsynch + GnRH), which was subjected to Ovsynch treatment and was injected with GnRH analogue on day 6 and group 2 (Ovsynch + controlled internal drug-release (CIDR) + GnRH), which received Ovsynch-CIDR treatment and was injected with GnRH analogue on day 6. Both treatments induced the formation of an accessory corpus luteum and significantly increased the cross-sectional area of the luteal tissue when compared to the control. However, the pregnancy rates on day 45 did not differ among groups. The study recorded that the administration of GnRH analogue on day 6 following Ovsynch-based treatment did not improve the reproductive performance of Japanese black cows, even though the progesterone concentration was higher in groups that received the GnRH.

Beltran *et al. (*2008) treated lactating Holstein cows, at 213±112 days post partumwith milkyield of 26±9kg per day, and these cows were randomly assigned to one of three treatment groups: control, GnRH (100µg gonadorelin), and hCG (2500IU) given five days after artificial insemination (AI). Pregnancy was determined between 42 and 49 days after AI. The study revealed increased concentrations of progesterone between days 7 and 12 in cows treated with GnRH and hCG; but not in control cows, suggesting that a new CL was formed. Treatments with GnRH or hCG on day 5 after AI increased conception rates in lactating cows.

Gordan *et al.* (2009) evaluated two stratagies to improve pregnancy rate following ovsynch timed artificial insemination in lactating dairy cows.The cows were assigned randomly to receive one of three treatments: Ovsynch protocol (GnRH 7 days before and 48 hrs after one PGF2α treatment), Presynch_Ovsynch (two treatmentsof PGF2α 14 days apart followed by Ovsynch 14 days later), or Ovsynch_Post-AI GnRH (GnRH 6 days after Ovsynch FTAI) for first service breeding. Pregnancy rates among treatments were not differed in lactating cows (42.5, 48.0, and 44.9 per cent) for Ovsynch, Presynch_Ovsynch, and Ovsynch_Post-AI, respectively.

Keskin *et al*. (2010)observed the study of Ovsynch protocol began with GnRH (10 µg) in the GPG group (GnRH-7d-PGF2α-56h-GnRH-18h-AI), whereas in the HPG group, the first GnRH of the Ovsynch was replaced with 1500 IU hCG (hCG-7d-PGF2α-56h-GnRH-18h-AI).The conception rate was found to be different between the HPG (37.6 pe rcent) and the GPG groups (48.4 per cent). Thus, beginning of the Ovsynch protocol with hCG did not increase ovulation and conception rate in lactating dairy cows, suggesting that hCG is not a suitable replacement of the first GnRH of Ovsynch. However, the results did notshowed increased ovulation rate in response to the first hormonal administration of Ovsynch can have a significant effect on conception rate.

Mahesh *et al.* (2010) reported that GnRH therapy irrespective of day of administration during the estrous cycle resulted in an overall enhancement of conception rate of 83.33 per cent as against 33.33 per cent in control group of cows.

Atotal 75 cowsaged from 2 to 3 years old were divided into five groups randomly and inseminated in the first estrous period. The first group received 10μg buserelin acetate both during AI and on the 12th day of AI. The second group was treated with 10 μg buserelin acetate during AI and 1.500 IU hCG on the 12th day after AI. In the third group, 1.500 IU hCG was injected immediately and on the 12th day after AI. Cows in the fourth group was administered 1.500 IU hCG immediately and 10 μg buserelin acetate on the 12th day after AI. The last group was left as control.The pregnancy rate was 40.0 per cent for group 1, 4 and 5, 46.7 per cent for group 2 and 3. And concluded that GnRH and hCG administration during and at the 12th day after AI failed in increasing pregnancy rate (Zahid Paksoy andCahit Kalkan, 2010).

Khoramian *et al*. (2011) compared the three different treatments to improve pregnancy per artificial insemination (P/AI) in repeat-breeder (RB) dairy cows. All cows were assigned to one of four groups: (1) gonadotropin-releasing hormone (GnRH); (2) human chorionic gonadotropin (hCG); (3) once-used controlled internal drug release (CIDR) device; and (4) control. All treatments performed 5–6 days after artificial insemination (AI). Pregnancy per AI on day 45 in hCG and CIDR groups were significantly higher than GnRH and control groups (60.0and 56.0per cent vs. 26.9and 29.6per cent, respectively).

Vasconcelos *et al.* (2011) studied the effects of treatments with human chorionic gonadotropin (hCG) or GnRH 7 d after induced ovulation on reproductive performance of lactating dairy cows presented to timed artificial insemination (TAI) or timed embryo transfer (TET). Postbreeding treatment affected the percentages of pregnant cows at TET on d 28 (control: 38.1 per cent; GnRH: 52.4 per cent; hCG: 45.1 per cent) and on day 60 (control: 32.5 per cent; GnRH: 41.1 per cent; hCG: 38.5 per cent), but postbreeding treatment did not affect percentages of pregnant cows at TAI on day 28 (control: 30.1 per cent; GnRH: 32.2 per cent; hCG: 32.4 per cent) or on d 60 (control: 25.6 per cent; GnRH: 27.1 per cent; hCG: 29.8 per cent).In nutshell, treatment with GnRH or hCG 7 day after induced ovulation increased conception rates in lactating dairy cows presented to TET, but not in cows presented to TAI.

The studies were aimed at synchronization of estrous in postpartum anestrous Kankrej cows of zebu cattle by use of different hormone protocols, viz., Ovsynch, CIDR (controlled internal drug release), Ovsynch plus CIDR, and Heatsynch(Bhoraniya *et al*., 2012). Thirty selected anestrous animals were divided into five equal groups (four treatment and one control), and the findings were compared with the normal cyclic control group of six cows. All the protocols were initiated in cows with postpartum anestrous period of more than 4 months. The animals were bred by fixed time artificial insemination. Pregnancy was confirmed per rectum on day 60 post-AI in non-return cases. The conception rates at induced heat in Ovsynch, CIDR, Ovsynch + CIDR, and Heatsynch protocols were 33.33, 66.66, 50.00 and 16.67 per cent, respectively. The corresponding overall conception rates of post-treatment were 50.00, 100.00, 66.66 and 50.00 per cent, respectively.

Ergene (2012) concluded the application of PRID and GnRH after artificial insemination did not significantly improve pregnancy rates, despite the fact that serum progesterone concentrations were higher in treatment groups.On the other hand, Navrange *et al.* (2012) reported the first service conception rate was 41.66 per cent showing augmentation of postpartum fertility using Ovsynch synchronization protocol in buffaloes under filed conditions.

Krishnakumar and Chandrahasan.(2012)studied on repeat breeder cowsand randomly allotted into four groups of were induced to estrous with 25mg ofPGF2α IM on day 10 of the estrous cycle. After estrous induction, group I was not given any treatment.Group II, III and IV cows were treated with 20µg of GnRH agonist IM at 48 and 72 hrs after PGF2α administration and day 5 post-insemination, respectively. The conception rate in the first service was higher in group III, the second service andoverall conception rate was higher in group IV. Concluded that PGF2α synchronizedestrous combined with GnRH 72h after PGF2α administration and on day 5 post-insemination improved overall conception rate in repeat breeder cows.

A total 531 dairy cows were inseminated with the aim to study their reproductive performance following administration of GnRH analogue on different days of estrous cycle with different doses. These cows were divided into six treatment and one control group. Depending upon different treatment groups, Buserelin acetate was injected at a dose of 10.5 µg or 21.0 µg on different days (0, 5 or 12) of estrous cycle in these cows. Control cows were inseminated without any treatment. Buserelin acetate improved conception in cows if administered either on day 0 along with AI or on Day 5 or 12 post insemination. The highest conception rate was recorded when 10.5 μg GnRH analogue was administered on day 12 post AI. The doses of 10.5 μg or 21.0 μg were equally effective when inseminatedon day 12 post AI (Jaswal *et al*., 2013).

A field research trial in which lactating Holstein cows from 6 commercial dairy herds were stratified by parity and breeding number by Nascimento*et al*. (2013) and these cows were randomly assigned to one of 2 groups: control (no further treatment) or hCG [Chorulon: 2,000 IU or 3,300 IU] on day 5 after a timed AI (ovulation synchronized with Ovsynch, Presynch-Ovsynch, or Double-Ovsynch).Treatment with hCG increased progesteronelevel on day 12 (4.3 vs. 5.3 ng/mL). Pregnancy per AI were greater in cows treated with hCG (40.8 per cent) than control (37.3 per cent) cows. Thus, targeted use of hCG on day 5 after TAI enhances fertility about 3.0to 3.5 per cent.

Ion Caraba and Serafin Velicevici. (2013) studied the efficacy of Ovsynch and Cosynch programson 15 dairy cows. The estrous response for cows in Ovsynch protocol was 63 per cent. Pregnancy per insemination at 60 days was 25 per cent. Estrous response for cow in Cosynch protocol was 57 per cent. Pregnancy per insemination at 60 days was 57 per cent

Dirandeh *et al*. (2014) studied the effects of two different timed GnRH injectionafter AI on reproductive performances of Holstein cows during the warm season. Cowswere randomly assigned to treatments 1) No GnRH injection (control), 2) GnRH injection at day 6 after AI and 3) GnRH injection at day 12 after A.I. InGnRH injection administered on day 6 after AI, 25.0per centof cowswere pregnant at 32 days after resynchronized AI compared tocontrol cows (19.3 per cent) and GnRH injection administered on day 12 after AI cows (20.0 per cent). The risk of pregnancy loss between day 32 and 60 of gestation was affected by treatments and decreased in cows received

GnRH injection after AI compared to control cows,and reported that administration of GnRH on day 6 after AI improved reproductive performance in dairy cows.

Musilova Darja *et al*. (2014) studied the effect of gonadotropin-releasing hormone on induction of accessory corpus luteum, progesterone concentrations and pregnancy in dairy cows. GnRH0.05 mgwas administrated intramuscularly to cows with one corpus luteum on day 5–7 (Group E1) or 11–13 (Group E2) after inseminationand control groups C1 and C2 were not treated. Detection of pregnancy by ultrasound examination and measurement of serum progesterone concentration was performed on day 25–39 and around day 90 after insemination. The occurrences of two corpora lutea in groups E1 and E2 versus C1 and C2 were 43.9and 33.5 per cent vs. 3.7and 9.8 per cent ($P < 0.001$). Progesterone concentrations were 5.9 ± 2.92 and 7.8 ± 2.35 ng/ml in untreated and treated cows, respectively.

A total of sixteen normally calved cows were selected at 35-50 days postpartum and were equally divided into two groups. Group I cows were treated with ovsynch protocol between day 50 to 65 post-partumand group II was control. The first service, second service and overall conception rate observed were 37.50, 50.00 and 87.50 per cent in group I and 25.00, 37.50 and 62.50 per cent in group II cows, respectively(Velladurai *et al*.,2014).

Dhami *et al.* (2015) reported that the use of CIDR, Ovsynch, and Norgestomet ear implant protocols resulted in 100 per centestrous induction with conception rates at induced estrous of 60, 50, and 50 per cent respectively.The overall conception rates for three cycles were 80, 80, and 70 per cent. In untreated anestrous control, only three cows exhibited spontaneous estrous within 90 days of follow-up and conceived with the first service and overall conception rates of 66.66and 30.00 per cent, respectively.

Birhanu *et al*. (2015) conducteda study to determine outcome of gonadotropin releasing hormone analogue given on 12 days post AI and double inseminations was done during estrous period in dairy cattle and observed improvement of conception rate. A total of sixty three repeat-breeding cows from twenty eight herds were selected and assigned randomly in to three equal groups (G: 1, 2 and 3); two treated and one control group. Group1 cattle were treated with injection 10 µg buserelin acetate IM on 12 days post AI. Group2 cattle were inseminated twice during estrous exhibition with 6-8 hrsinterval. Group3 cattle were received no treatment, inseminated once as heat was detected and considered as a control group. Dairy cows were examined for pregnancy after three months through rectal palpation. Pregnancy rates recorded were 52, 38and 28per cent in G1, G2 and G3 respectively. Conception rates of treated groups (G1 and G2) exceed control group by 24and 10 per cent, respectively.

Prajapati*et al*. (2015) studied on 20 repeat breeding crossbred cows to evaluate the use of ovsynch protocol in improving the reproductive efficiency and concluded that the conception rate in first, second, third service and overall conception rate was 30.0, 20.5, 5.0 and 55.0 per cent, respectively.

Ahmed *et al.* (2016) evaluated the success of ovsynch protocol by initiating the protocol on day 6 after oestrous onset with additional GnRH treatment during Post insemination (early luteal Phase) in repeat breeder crossbred cattle. Twenty four repeat breeder cattle were allocated into four groups, Gp I, control; Gp II, GnRH treatment on day 6 post estrous onset; Gp III, Ovsynch treatment initiated on day 6 post estrous onset; Gp IV, same as in Gp III with an additional GnRH on day 6 after 2nd GnRH of ovsynch. The conception rate for Gp I, II, III and IV was 0.0, 16.7, 50.0 and 50.0 per cent, respectively.

In group II and IV, serum progesterone was high ($p<0.01$) on day 12 post AI in comparison to Gp I and III, respectively.

Jae Kwan Jeong *et al*. (2016) evaluated the effect of a single GnRH administration or treatment with a CIDR insert after FTAI on the pregnancy rates of dairy cows. Cows treated with a CIDR insert at days 3.5 to 18 after FTAI had a higher probability of pregnancy and an increased circulating progesterone concentration during the early luteal phase than non-treated control cows. However, GnRH treatment had no beneficial effects on pregnancy outcomes, although the percent of cows with an accessory CL after FTAI was higher among GnRH-treated cows than untreated cows.

Kunde*et al*. (2017) studied in repeat breeder cattle thatwere synchronized using intravaginal progestational device (TRIU-B) based Co-Synch protocol. On day 6 post artificial insemination (AI), all the animals were administered either 10µg GnRH analogue, 1500 IU hCGor normal saline. Pregnancy diagnosis was carried out on day 45 post-AI. In GnRH, hCG and control group, the respective conception rate was 40, 46.7 and 33.3 per cent. In brief, administration of hCG on day 6 post AI improves subsequent luteal profile with tendency for better conception rate in repeat breeder cattle.

Lactating Holstein cows from two herds wererandomly divided in to four treatment groups: (1) control, (2) GnRH treatment at AI,(3) GnRH treatment 5 days after post-AI and (4) GnRH treatment at AI and 5 days after post-AI. Among cows diagnosed pregnant at day 36, progesterone concentration on day 5 was not affected ($P \geq 0.24$) by treatments with GnRH at AI, 5 days after AI, or both, but progesterone concentration on day 12 was greater for cows treated with GnRH at AI and 5 days after AI compared with GnRH treatment at AI cows(Mendonça *et al*., 2017). Lactating dairy cows in their third or greater lactation exposed to heat stress conditions with compromised fertility may have increased pregnancy rate after treatment with GnRH 5 days after AI or the combined treatment of GnRH at AI and 5 days after AI because of reduced early embryonic loss.

2.4.2Post Insemination Treatment Efficacy of hCG

Mechanism of Action: This glycoprotein is composed of two polypeptide chains with carbohydrates attached to each.Human chorionic gonadotropin is produced by the trophoblast of the blastocyst and can be detected in the peripheral circulation as soon as d 8 to 10 of gestation. The hormone maintains the CL of pregnancy and is used to detect early pregnancy (Jameson and Hollenberg, 1993). Once hCG binds to the LH-CG receptor it directs the CL to produce different hormones including progesterone and estrogen. Over a time, the CL becomes less sensitive to hCG, but increasing concentrations of the hormone maintain its functional capacity until approximately 7 weeks of pregnancy (Jameson and Hollenberg, 1993).

Rajamahendran and Sianangama, (1992) demonstrated that administering GnRH or hCG between day 5 to 7 of the estrous cycle can induce the formation of an accessory CL, thereby increasing progesterone concentrations and possibly reducing embryonicloss. Treatment with GnRH or hCG given between day 5 to 7 post-AI, focuses on enhancing CL function, ovulating the first-wave dominant follicle, and increasing progesterone secretion. Ovulation of a dominant follicle at this time also results in a decrease in E2 production, which in turn results in an inhibition of up-regulation of oxytocin receptors and consequent inhibition of PGF2α secretion (Peters *et al.*, 1999). Up-regulation of oxytocin stimulates the synthesis of progesterone.

Rajamahendran and Sianangama. (1992) observed that thirty-four lactating Holstein cows were randomly assigned to four groups for treatment with human chorionic gonadotrophin (hCG, 1000 IU) at insemination day 0, 7 and 14 after insemination, and with no hCG treatment as control. Three cows in the control group were diagnosed pregnant by ultrasound scanning and rectal palpation. In the hCG-treated groups 4, 7 and 4 cows were diagnosed as pregnant for cows treated on day 0, 7 and 14, respectively.

In an experiment Schmitt *et al*. (1996) reported the induction of an accessory CL with hCG on day 5 or 6 after insemination increased progesterone concentrations, but did not increase pregnancy rate in fertile heifers or lactating dairy cows during summer heat stress.

Santos*et al*. (2001) stated that treatment with hCG on day 5 resulted in 86.2per cent of the cows with more than one CL compared with 23.2 per cent in control.Plasma progesterone concentration were increased by 5.0ng/mL in hCG treated cows. Conception rates were higher for hCG treated cows on day 5 after AI.

Tefera *et al*. (2001) found no effects on the rate of embryonic mortality with the administration of hCG on day 4 or GnRH on day 12 post-AI.

Selvaraj *et al*. (2003) observed that conception rates as 66.66,33.33 and 66.66 per cent after intramuscular injection of hCG 1500 IU on day 0,7 and 14 of estrous cycle and 16.66 per cent in control group of repeat breeder crossbred cows.

Funston *et al.* (2005) summarized that the administration of hCG 5 to 6 d after AI did not improve conception or pregnancy rates at two out of three locations evaluated, suggesting insufficient progesterone is not a major factor contributing to early pregnancy failure in beef heifers.

Hanlon *et al.* (2005) reported that administration of 1500 IU of hCGon day 5 after insemination did not improve first service conception rate in anovulate anoestrous dairy cows. Stevenson *et al*. (2006) observed that 162 cows were assigned randomly to serve as control or to receive a CIDR insert for 7 days, 100 μg of GnRH, or 3,300 IU of hCG. More cows were induced to ovulate in response to GnRH (60 per cent) and hCG (78per cent), compared with control (2.4per cent). Compared with control, cows treated with GnRH or hCG had more number of CL (day 7).

Shams *et al.* (2007) observed that the effect of administration of exogenous hCG (3000 IU) 5 days after artificial Insemination (AI)did not improve pregnancy rate in dairy cows, Where as serum progesterone concentration were higher in treatment group compared to control group.

Lactating Holstein cows, at an mean 213±112 days of lactation with milkyeildof 26±9kg per day, were randomly assigned to one of three treatment groups: control, GnRH (100μg gonadorelin), and hCG (2500IU) given five days after artificial insemination (AI) (Beltran *et al.*, 2008). Pregnancy was determined between 42 and 49 days after AI. The study revealed increased concentrations of progesterone between days 7 and 12 in cows treated with GnRH and hCG; but not in control cows, suggesting that a new CL was formed. Treatments with GnRH or hCG on day 5 after AI increased conception rates in cows.

Keskin *et al*.(2010) synchronized estrous in cows withOvsynch protocol began with GnRH (10 μg) in the GPG group (GnRH-7d-PGF2α-56h-GnRH-18h-AI), and in the HPG group, the first GnRH of the Ovsynch was replaced with 1500 IU hCG (hCG-7d-PGF2α-56h-GnRH-18h-AI). Conception rate was found to be different between the HPG (37.6 pe rcent) and the GPG groups (48.4 per cent). Thus, beginning of the Ovsynch protocol with hCG did not increase ovulation and conception rate, that hCG was not a suitable replacement of the first GnRH of Ovsynch. However, their results did not showed increased ovulation rate in response to the first hormonal administration of Ovsynch,but had a significant effect on conception rate.

A total of 75 cows ranging from 2 to 3 years old were divided into five groups randomly and inseminated in the first estrous period (Zahid PaksoyandCahit Kalkan., 2010). The first group received 10μg buserelin acetate both during AI and on the 12th day of AI. The second group was treated with 10μgbuserelin acetate during AI and 1.500 IU hCG on the 12th day after AI. In the third group, 1.500 IU hCG was injected immediately and on the 12th day after AI. Cows in the fourth group was administered 1.500 IU hCG immediately and 10 μg buserelin acetate on the 12th day after AI. The last group was left as control.The pregnancy rate was 40.0 per cent for group 1, 4 and 5, and46.7 per cent for group 2 and 3. The study concluded that GnRH and hCG administration during and at the 12th day after AI failed in increasing pregnancy rate.

Khoramian *et al*. (2011) compared the three different treatments to improve pregnancy per artificial insemination (P/AI) in repeat-breeder (RB) dairy cows. All cows were assigned to one of four groups: (1) gonadotropin-releasing hormone (GnRH); (2) human chorionic gonadotropin (hCG); (3) once-used controlled internal drug release (CIDR) device; and (4) control. All treatments performed 5–6 days after artificial insemination (AI). Pregnancy per AI on day 45 in hCG and CIDR groups were significantly higher than GnRH and control groups (60.0and 56.0per cent vs. 26.9and 29.6per cent, respectively).

Vasconcelos *et al.* (2011) studied the effects of treatments with human chorionic gonadotropin (hCG) or GnRH 7 d after induced ovulation on reproductive performance of lactating dairy cows submitted to timed artificial insemination (TAI) or timed embryo transfer (TET). Postbreeding treatment affected the percentages of pregnant cows at TET on d 28 (control: 38.1, GnRH: 52.4, hCG: 45.1 per cent) and on day 60 (control: 32.5, GnRH: 41.1, hCG: 38.5 per cent), but postbreedingTAI treatment did not affect percentages of pregnant cows on day 28 (control: 30.1, GnRH: 32.2, hCG: 32.4 per cent) or on day 60 (control: 25.6, GnRH: 27.1, hCG: 29.8 per cent).In conclusion, treatment with GnRH or hCGon day 7 after induced ovulation increased conception rates in lactating dairy cows submitted to TET, but not in cows submitted to TAI.

Navrange *et al.* (2012) reported the first service conception rate was 41.66 per cent showing augmentation of postpartum fertility using Ovsynch synchronization protocol in buffaloes under field conditions.

Nascimento*et al*. (2013) conducted a field research trial in which lactating Holstein cows from 6 commercial dairy herds were stratified by parity and breeding number and then randomly assigned to one of 2 groups: control (no further treatment) or hCG [Chorulon: 2,000 IU or 3,300 IU] on day 5 after a timed AI (ovulation synchronized with Ovsynch, Presynch-Ovsynch, or Double-Ovsynch). Treatment with hCG increased progesterone. Pregnancy rateswere greater in cows treated with hCG (40.8 per cent) than control (37.3 per cent) cows. Thus, targeted use of hCG on d 5 after TAI enhances fertility about 3.0to 3.5 per cent.

Dahlen *et al*. (2014) determined the effects of administering hCG 7 d after a fixed-time AI (FTAI) on ovarian response, concentrations of progesterone, and pregnancy rates in postpartum suckled beef cows. Their studies concluded that treatment with hCG increased the volume of luteal tissue on d 14 and concentrations of progesterone on d 14 and 33 after TAI. Treatment with hCG tended to increase pregnancy rates at 5 of 6 locations from 1.1 to 27 per cent points compared with saline, but cumulative pregnancy rates determined on d 68 after TAI were similar between treatments.

A study was conducted to evaluate the effect of human chorionic gonadotropin (hCG) administration 4 or 6 days after timed AI (TAI) on progesterone concentration and pregnancy outcomes in repeat breeding dairy cows(Alnimer and Shamoun, 2015). All cows were treated by Cosynch protocol and assigned into 3 groups. Control, did not receive hormonal treatments; group1, received 1500 IU hCG 4 days post TAI; and group2 received 1500 IU hCG 6 days post TAI and summarized the administration of hCG 6 days after TAI was beneficial in improving pregnancy rate either in summer or winter as a result of reducing pregnancy loss and increasing progesterone concentration.

Sanchez *et al*. (2015) recorded 40 per cent of embryonic loss in cattle occurs in the period from day 8 to day 16 of pregnancy. A significant proportion of embryonic loss must be due to inadequate circulating progesteroneconcentrations. The authors also stated that administration of hCG on day 2 or day 5 after FTAI would lead to an increase in pregnancy rates in dairy cattle. Pregnancy rate to AI was significantly higher in the hCG2 and hCG5 groups than in the control group (43.1 and 45.3 per cent only υ 27.7 per cent).

Manshadi *et al*. (2016) concluded that the eCG administration at the time of removal of CIDR device when using the CO-Synch + CIDR program only increased the growth rate of the largest follicle (LF) from implant removal to FTAI, the diameter of the LF at FTAI, and rates of ovulation and did not have any effect on pregnancy rates in dairy cows.

Kunde*et al*. (2017) synchronized repeat breeder cattleby using intravaginal progestational device (TRIU-B) based Co-Synch protocol. On day 6 post artificial insemination (AI), all the animals were administered either 10µg GnRH analogue, 1500 IU hCGor normal saline. Pregnancy diagnosis was carried out on day 45 post-AI. In GnRH, hCG and control group, the respective conception rate was 40, 46.7 and 33.3 per cent. In brief, administration of hCG on day 6 post AI improves subsequent luteal profile with tendency for better conception rate in repeat breeder cattle.

A total of 989 lactating Holstein cows with different parity and number of prior services were used. Cows were inseminated after observedestrous or at a fixed-time. Five days post-insemination, cows were randomly assigned to two treatment groups: hCG, which received 3500 IU of hCG by intramuscular injection; and the control group, which did not receive any treatment. Progesterone concentration was higher on days 11 and 15 in cows treated with hCG, compared to the control group ($P<0.05$). The pregnancy rates were 47.5 and. 37.4 per cent inhCGand control groups respectively (Gonzalez *et al*., 2017).

2.4.3 Services taken per conception.

In postpartum anestrous cows followingGnRH – $PGF_2\alpha$ – GnRH protocol, the number of services per conception was found to be 1.70 ± 0.18 (Naidu 2004).

Ansari (2007) reported that the number of services required per conception in postpartum anestrous crossbred cows treated with GnRH – $PGF_2\alpha$ - GnRH protocol was found to be 1.44.

CHAPTER III

MATERIALS AND METHODS

3.1 PLACE OF RESEARCH WORK

The present investigation entitled "Studies on Post Insemination Supplementation of GnRH or hCG to Ovsynch Protocol In Crossbred Cows" was undertaken in private dairy farm at Nagole, Rangaredy District.

3.2 INCIDENCE OF REPRODUCTIVE DISORDERS IN CROSSBRED COWS

The data on fertility cases available from 2012-2017 at District Veterinary and Animal Husbandry Office, Ranga Reddy district were utilized for the present study.

3.3SOURCE OF EXPERIMENTAL CROSSBRED COWS

The study comprised of 50post partum anoestrumcrossbred cows after completing 60 days post partum period and free from uterine infections.

3.4 MANAGEMENT OF CROSSBRED COWS

The crossbred cows were maintained under good nutritional and managemental conditions. In addition to this, lactating crossbred cows were allowed for grazing in open fields. Most of the cows were housed in asbestos roof sheds with concrete flooring with rubber sheetbedding onconcrete.

3.5 PERIOD OF EXPERIMENT

The experiment was carried out for a period of 6 months from March 2017 to August 2017.

3.6 SELECTION OF CROSSBRED COWS

The post partum anoestrumcrossbred cows after completing 60 days post partum period were selected aftersubjected to detailed gynaecological examination of genital tract by rectal palpation and screened by White side test to identify uterine infections.

3.6.1 Gynaecological Examination

Allpost partum anoestrumcrossbred cows after completing 60 days post partum period were subjected to detailed examination of the genital tract by rectal palpation for the examination of cervix, uterine body, uterine horns and ovaries to detect palpable abnormalities in the genital tract.

3.6.2 White Side Test

White side test was conducted to identify the subclinicalendometritis condition as per the procedure described by Pateria and Rawal (1990).

3.6.2.1 Collection of Mucus samples

Mucus samples from the cervix of all cows were collected by using sterilized glass catheter through aspiration technique.

3.6.2.2 Procedure of test

One ml of cervical mucus sample from post partum anoestrumcrossbred cows was collected into a clean, sterilized test tube. Added one ml of freshly prepared 5 per cent of sodium hydroxide solution to the mucus sample in the test tube and mixed well. Heated the test tube on the spirit lamp for 2-3 minutes to boiling temperature and the colour reaction was observed. If the mucus sample turned to yellow color, the test was positive for clinical or sub clinical endometritis. If it remains white color, the test was negative for endometritis.

3.7 TREATMENT REGIMEN

Out of 75 post partum anoestrum crossbred cows, 50 post partum anoestrum crossbred cows without any palpable abnormalities were selected for the present therapeutic study.All post partum anoestrum crossbred cows were selected after completing 60 days post partum period without any palpable abnormalities for the present study. The crossbredcows wererandomly divided equal into five therapeutic groups so that each group consist 10 crossbred cows. The treatment groups were designed as follows.

OvsynchGroup (1): In this group, cows were injected with 10µg of GnRH (Buserelin acetate-Receptal*) IM on day 0, 500 µg of cloprostanol (PGF2α) (Pragma**) IM on day 7 and 2nd dose of GnRH 10µg IM on day 9. Fixed time AI was done after 16 hrs of the 2ndGnRH injection.

Ovsynch+GnRH Group(2): In this group, cows were injected with 10µg of GnRH IM on day 0, 500 µg of cloprostanol (PGF2α) IM on day 7 and 2nd dose of GnRH 10µg IM on day 9. Fixed time AI was done after 16 hrs of the 2ndGnRH injection.On day 6 of post insemination,GnRH@10µg IM wasadministered.

Ovsynch+hCG Group (3): In this group, cows were injected with 10µg of GnRH IM on day 0, 500 µg of cloprostanol(PGF2α) IM on day 7 and 2nd dose of GnRH 10µg IM on day 9. Fixed time AI was done after 16 hrs of the 2nd GnRH injection. On day 6 of post insemination hCG(Chorulon***) @1500IU IM was administered.

*****Receptal®: Intervet Laboratories Ltd, Briahnagar, Wagholi. Each ml contains 0.004 mg of Buserelin acetate (GnRH analogue).

******Pragma®: Intas Pharmaceuticals Ltd, Matoda,Ahamadabad. Each ml contains 250 µg of cloprostanol

******* Chorulon®: Intervet Laboratories Ltd, Briahnagar, Wagholi. Each vial contains 1500 IU of hCG

GPH+GnRHGroup(4): In this group, cows were injected with 10µg of GnRH (Buserelin acetate-Receptal*) IM on day 0, 500 µg of cloprostanol(PGF2α)(Pragma**) IM on day 7 and hCG @1500IU,IM on day 9. Fixed time AI was done after 16 hrs of hCG injection. On day 6 of post insemination GnRH@10µg IM was administered.

Control Group(5): The cows were injected with 2.5 ml of Normal Saline IM on day 0,7,and 9 andwhenever the crossbred cows exhibited estrous, Cows was inseminated. On day 6 of post insemination 2.5 ml of Normal Saline IM was administered.

3.8 SOURCE OF FROZEN SEMEN

The inseminations were conducted by using 0.25 ml of 'French mini straw' containing good quality of imported (USA) crossbred bull semen supplied by TSLDA,Govt.of Telangana State.

3.9 INSEMINATION SITE AND TIME

The thawed semen was deposited at the base of the body of the uterus just front of the internal os of the cervix 16 hrs after 2^{nd} GnRH or hCG injection in treatment groups and natural estrous in control groups.

3.10 THERAPEUTIC EFFICACY

The therapeutic efficacy was expressed in terms of estrous response, intensity of estrous, duration of estrous and conception rates in the present investigation.

3.10.1 Estrous Response

The estrous responsewas calculated as the percentage of treated cows which exhibited estrous. Estrous was detected on the basis of behavioral,physical,and gynecological examination.The physical examination was carried out 24 h after the PGF2α injection and then every 12 h to confirm estrous characterized bymucus discharge, edema and congestion of vulva,mounting behavior and stand to be mounted in Ovsynch ,

Ovsynch+GnRH ,Ovsynch+hCG, GPH+GnRHand control groupcrossbred cows. Estrous response was expressed as per cent cows expressed estrous.

3.10.1.1 Onset of Estrous.

The onset of estrous was calculated in hrs (h) from the time of PGF2α administration to the time of the first appearance of estroussigns in treatment groups.

3.10.2Estrous Pattern

The estrous pattern in crossbred cows were stated in terms of type of estrous, duration of estrous and intensity of estrous. The dairy farm staff were explained about the behavioralestrous symptoms and the heat detection methods of crossbred cows. They were advised to observe the cows at least 2 times a day particularly in the early morning hrs and evening hrs for estrous symptoms.

3.10.2.1 Types of Estrous

Normal estrous was identified as estrous period that was best characterized as continuous with a high intensity at mid-estrous. Split estrous was identified as the initial period of standing to be mounted andinterrupted by a period of non-receptivity lasting a few hrs and then followed by another period of mounting.

3.10.2.2 Intensity of Estrous

The intensity of estrous was assessed in crossbredcows as per the score card adopted by Rao and Rao (1981). The intensity of estrous was graded as weak estrous, normal estrous and intense estrous by scoring less than 10, 10-15, and more than 15 points, respectively as per the modified score card (Table1).

3.10.2.3 Duration of Estrous

The duration of estrous was recorded as the interval in hrs between the onset and cessation of estrous. In crossbred cows, the duration of estrous was confirmed by the history collected from the technical staff of dairy farm on visual observation of external genital organs and rectal examination of internal genital organs for appearance (or) disappearance of estrous symptoms.

3.10.3 CONCEPTION RATE

The post partum anoestrumcrossbred cows in all the groups of present study were subjected to pregnancy diagnosis by per rectal examination after sixty days of insemination. Efficiency of therapeutic groups was expressed in terms of per cent cows conceived to total no.of cows in the group. Services taken per conception was also recorded.

3.10.4 STATISTICAL ANALYSIS

The conception rates in different groups were compared by Chi-square test. The data on intensity of estrous and duration of estrous were analyzed using one way analysis of variance (ANOVA)to compare variation between groups by SPSS software (Version 17.0).

Table 1. Score card for assessment of intensity of estrous in crossbred cows

Estrous Symptoms	Points allotted
Bellowing	2
Excitement (or) restlessness	2
Estrual mucus discharge	3
Edema, congestion and wetness of vulva	3
Raising of tail in response to placing of palm on the rump region	2
Micturition	2
Cervical relaxation	2
Uterine tonicity	3
Graafian follicle on the ovary	3
Temporary engorgement of teats	1
Decreased milk yield	1
Decreased feed intake	1
TOTAL	**25**
GRADING Weak estrous : < 10 Points Normal estrous : 10-15 Points Intense estrous : >15 Points	

CHAPTER IV

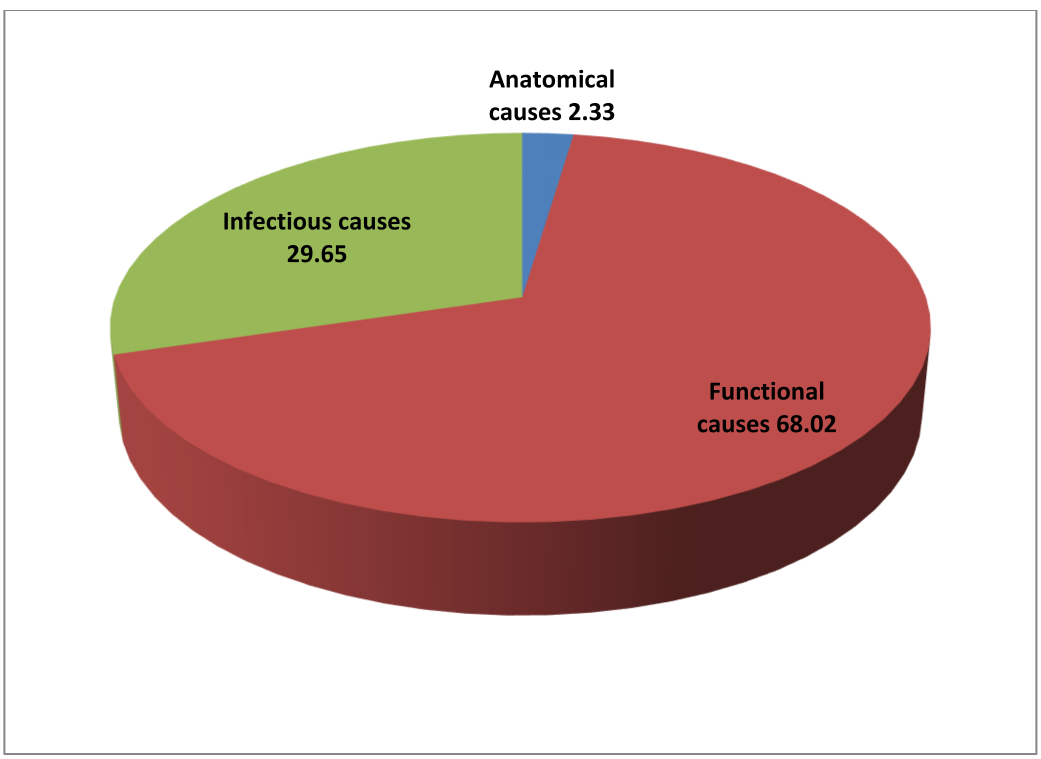
RESULTS

The present investigation entitled "Studies on Post Insemination Supplimentaion of GnRH or hCG to Ovsynch Protocol In Crossbred Cows" was studied in private dairy farm at Nagole, Ranga Reddy District. The results obtained were presented in tables and graphs.

4.1 INCIDENCE OF REPRODUCTIVE DISORDERS

The incidence of anatomical ,functional and infectious causes of infertility were recorded as 2.33, 68.02 and 29.65 per cent over 9217 crossbred cowsthatwere treated in last five years (Table.2 and Fig.1) in Ranga Reddy District.

Fig.1.Showing incidence of reproductive disorders.



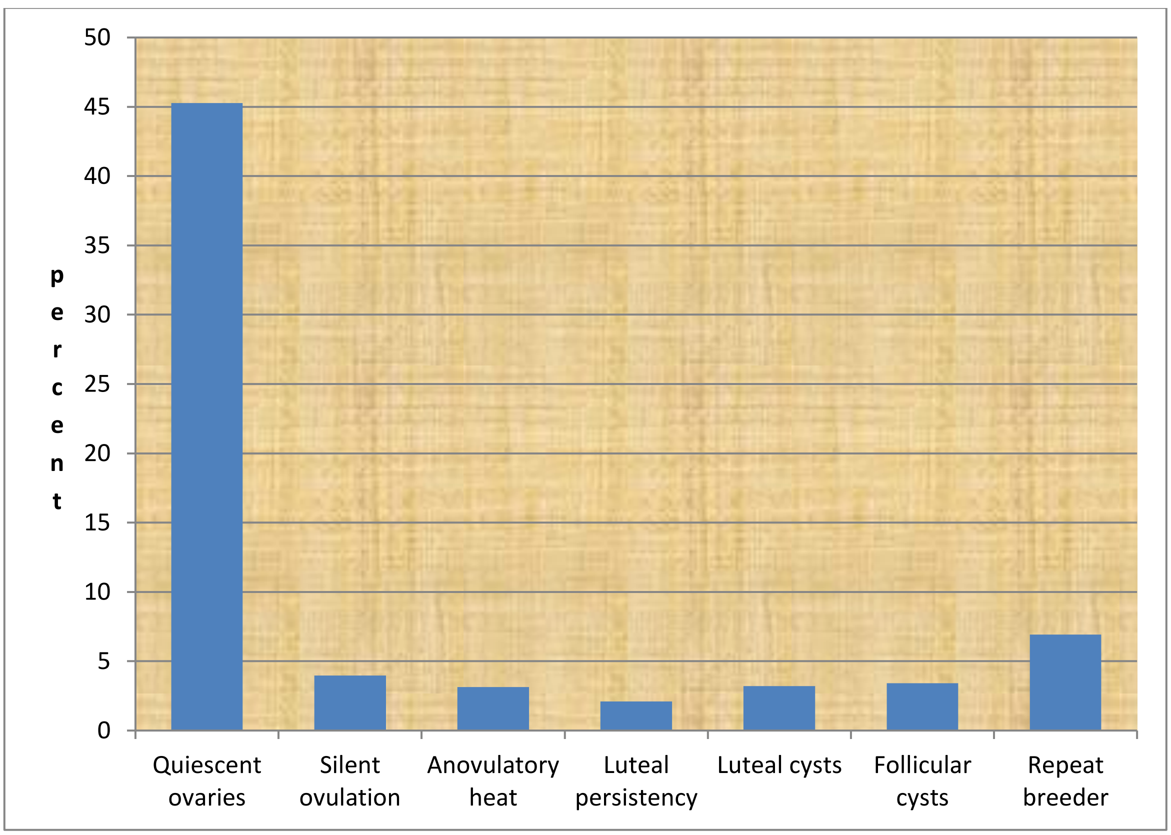
4.1.1 Anatomical causes

The overall incidence of anatomical causes was 2.33 per cent among the various reproductive disorders in crossbred cows. The incidence of hypoplasia of ovaries and genital infantilism was 1.47 and 0.86 per cent, respectively in the crossbred cows (Table 2).

4.1.2 Functional causes

The overall incidence of functional causes was 68.02 per cent among the various reproductive disorders in crossbred cows. The incidence of quiescent ovaries, silent ovulations, anovulatiory heat, luteal persistency, luteal cysts, follicular cysts and repeat breeder was 45.26, 3.98, 3.13, 2.1, 3.21, 3.42 and 6.92 per cent, respectively (Table.2 and Fig.2).

Fig.2.Showing incidence of different functional causes of infertility in crossbred cows.



Among the quiescent ovaries, the incidence ofpostpartum anoestrous, smooth ovaries and post service anestrous

were21.82,15.62 and 7.82 per centrespectively(Table.2 and Fig.3).

Fig.3.Showing incidence of different infertility causes of quiescent ovaries in crossbred cows.

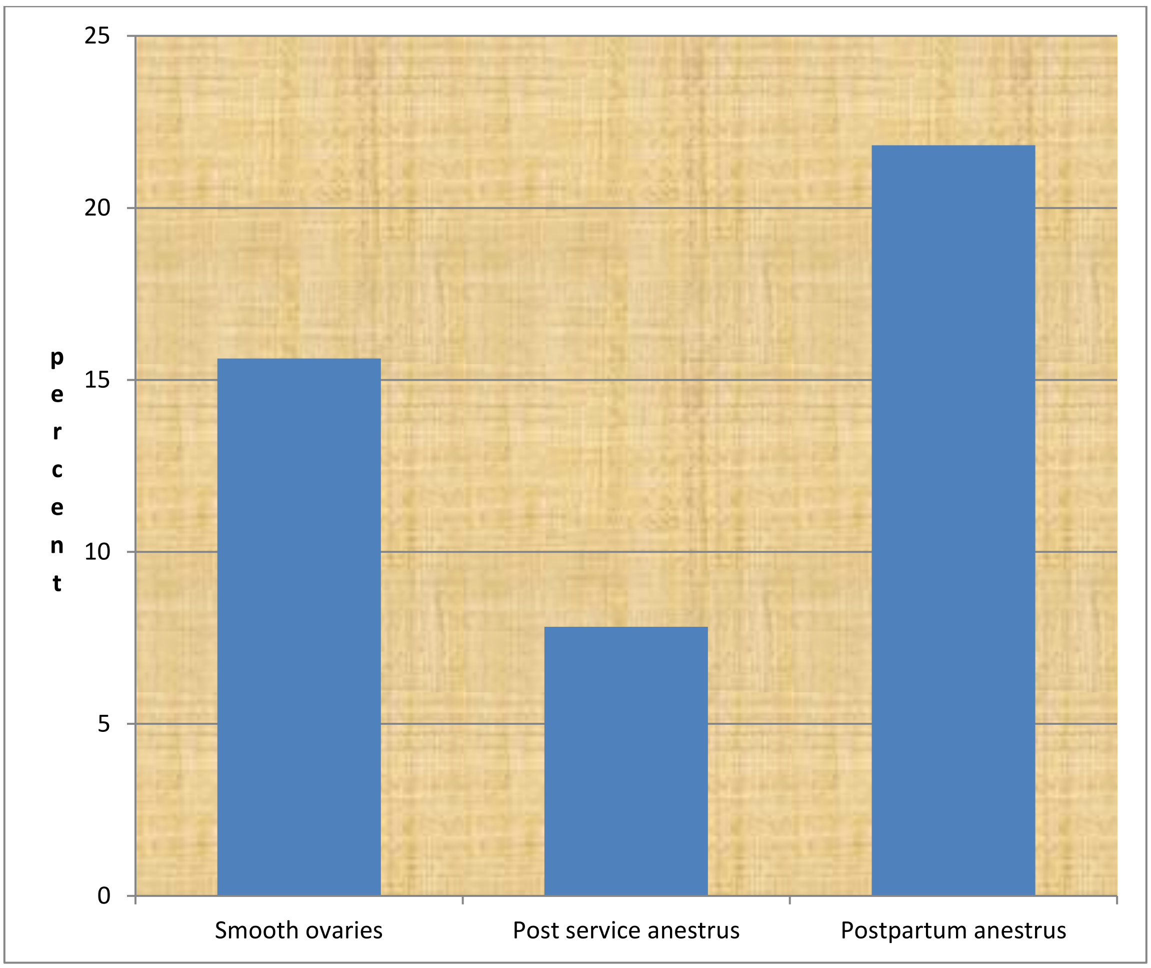


Table.2: Incidence Of Reproductive Disorders In Crossbred Cows.

S.No.	Name of reproductive disorder	Reproductive disorder (n)	Per cent of incidence
(A)	**Anatomical causes**	**214**	**2.33**
1	Hypoplasia of ovaries	135	1.47
2	Genital infantalism	79	0.86
(B)	**Functional causes**	**6270**	**68.02**
1	**Quiescent ovaries**	4172	45.26
a)	Smooth ovaries	1440	15.62
b)	Post service anestrous	721	7.82
c)	Postpartum anestrous	2011	21.82
2	Silent ovulation	367	3.98
3	Anovulatory heat	288	3.13
4	Luteal persistency	194	2.1
5	Luteal cysts	296	3.21
6	Follicular cysts	315	3.42
7	Repeat breeder	638	6.92
(C)	**Infectious causes**	**2733**	**29.65**
1	Vaginitis	397	4.31
2	Cervicitis	743	8.06
3	Endometritis	1405	15.24
4	Metritis	65	0.71
5	Pyometra	123	1.33
6	Salphingitis	--	--
7	Bursal adhesions	--	--
	Overall	**9217**	**100**

Source: District Veterinary and Animal husbandry office, Ranga Reddy district.

4.1.3 Infectious causes

The overall incidence of infectious causes was 29.65 per cent among the various reproductive disorders in crossbred cows. The incidence of vaginitis, cervicitis, endometritis, metritis, pyometra, salphingitis and bursal adhesions was recorded as 4.31, 8.06, 15.24, 0.71, 1.33, 0 and 0 per cent, respectively (Table.2 and Fig.1).

4.2 THERAPEUTIC EFFICACY

The therapeutic efficacy was recorded and expressed as estrous response rate, estrous pattern, duration of estrous and conception rates (Table.3 to 9 and Fig.4 to10)

4.2.1Estrous Response

The estrousresponse were 90.00, 100.00, 100.00, 100.00 and 30.00 per cent in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively. There was no significant ($p<0.05$) difference among the groups estrous response in the present study(Table.3 and Fig.4)

4.2.1.1 Onset of Estrous

The onset of estrous was calculated in hrs from the time of PGF2α administration to the time of the first appearance of estrous signs and the mean onset of estrous was recorded as 51.80±1.07, 50.90±1.20, 52.00 ±1.32, 52.40 ±1.32 and 96.00 ±13.86 hrs in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively in the present study. The onset of estrous in therapeutic groups were not significantly ($p<0.05$) differed among thetreatment groups. (Table.4 and Fig.5).

Table.3: Estrous response rate in therapeutic groups of crossbred cows.

Sl.No	Name of the group	Cows treated (n)	Cows exhibited estrous (n)	Per cent cows responded	Chi-square value
1	**Ovsynch**	10	9	090.00	**9.55**^{NS}
2	**Ovsynch+GnRH**	10	10	100.00	
3	**Ovsynch+hCG**	10	10	100.00	
4	**GPH+GnRH**	10	10	100.00	
5	**Control**	10	3	030.00	

***NS Non Significant**

Fig.4.Showing Estrous response rate in therapeutic groups of crossbred cows.

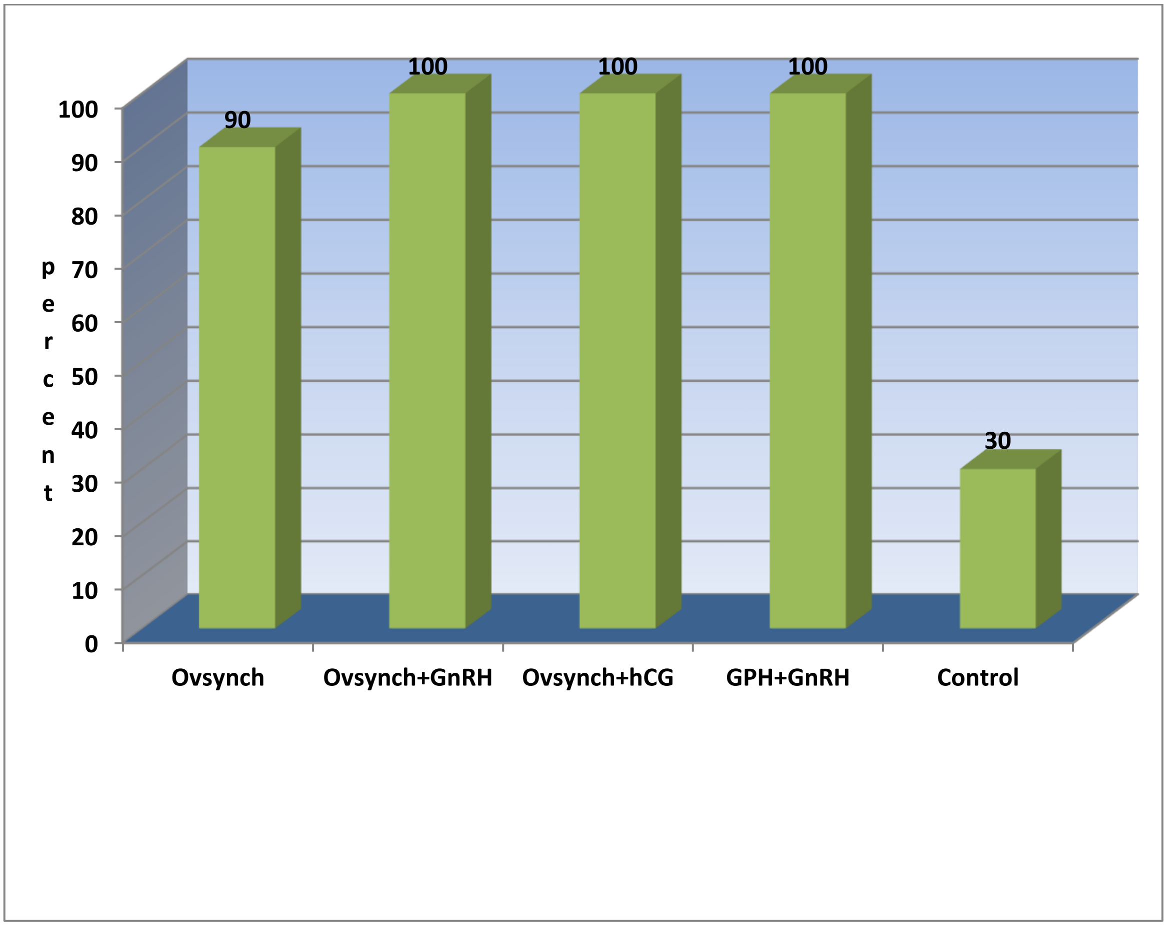
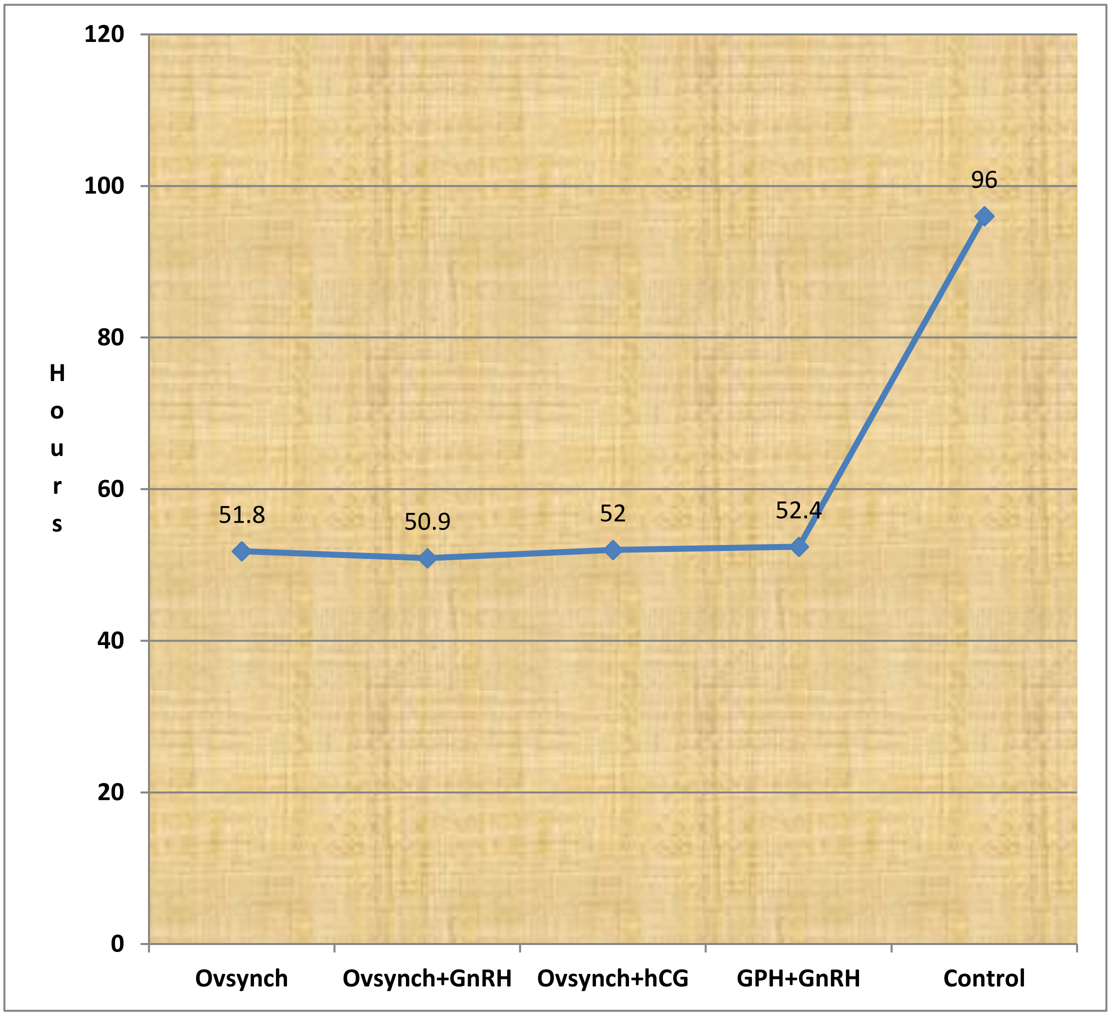


Table 4: Onset of estrous (hrs) in therapeutic crossbred cows after PGF2αinjection.

Sl.No	Name of the group	Cows treated (n)	Mean onset of estrous (hrs)
1	**Ovsynch**	10	51.80±1.07[a]
2	**Ovsynch+GnRH**	10	50.90±1.20[a]
3	**Ovsynch+hCG**	10	52.00 ±1.32[a]
4	**GPH+GnRH**	10	52.40±1.32[a]
5	**Control**	10	96.00±13.86[b]

Means bearing different superscripts differed significantly ($p<0.05$)

Fig.5. Showing Onset of estrous (hrs) in therapeutic groups of crossbred cows.



4.2.2 Estrous Pattern

The observed data pertaining to type of estrous, intensity of estrous and duration of estrous in crossbred cows was furnished in table.

4.2.2.1 Types of Estrous

The per cent of normal, silent and split estrous was 95.3, 0 and 4.7 in crossbred cows examined in the present study (Table.5 and Fig.6).

4.2.2.2 Intensity of Estrous

Among 50 post partumcrossbred cows, mean normal intensity of estrous was exhibited by 66.00 per cent of crossbred cows whereas weak and intense estrous was exhibited by 26.00 and 8.00 per cent of crossbred cows.(Table 6and Fig7).

The therapeutic crossbred cows exhibited intense estrous10.00, 10.00, 10.00, 10.00 and 00.00 per cent in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively. Statistical analysis revealed that the intenseestrous in treatment groups were significantly ($p<0.05$) different with that of the control group (Table.6).

The therapeutic crossbred cows exhibited normal estrous80.00, 70.00, 80.00, 80.00 and 20.00 per cent in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively. Statistical analysis revealed that the normalestrous in treatment groups were not($p<0.05$) significantamong the groups (Table.6).

The therapeutic crossbred cows exhibited normal estrous10.00, 20.00, 10.00, 10.00 and 80.00 per cent in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively. Statistical analysis revealed that the intenseestrous in treatment groups were significantly ($p<0.05$) different with control group (Table.6).

The mean score for estrous intensity were recorded as 11.70±0.79, 12.50±0.83, 11.60±0.53, 12.30±0.77and 05.10±2.07, in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively. Statistical analysis revealed that the intensity of estrous in treatment groups were significantly ($p<0.05$) different with control group (Table.7 and Fig.8).

4.2.2.3 Duration of Estrous

The mean duration of estrous was recorded as 21.90±0.69, 22.30±0.79, 21.80±0.73, 23.60±0.82 and 21.40±0.60 hrs in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively in the present study. The duration of estrous in theraputic groups was not ($p<0.05$) significantly differentamong the groups. (Table 9 and Fig 9).

4.2.2.4 Conception Rate

The overall conception rate in therapeutic crossbred cows was recorded as 60.00, 50.00, 40.00, 60.00, and 20.00 per cent in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively in the present study (Table.10 and Fig.10).

The first service conception rate was recorded as 44.44, 30.00, 30.00, 50.00 and 33.33 per cent in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively. The second service conception rate was recorded as 40.00, 28.57, 14.29, 20.00 and 50.00 per cent in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectivelyin the present study (Table.11 and Fig.11).

Statistical analysis revealed that the conception rates were significantly ($P<0.01$) higher in Ovsynch group,GPH+GnRHgroup and followed by group Ovsynch+hCG, Ovsynch+GnRH and control. However, the conception rates were significantly ($P<0.01$) lower in control group of crossbred cows than the treatment groups.

4.2.2.4.1 Number of services per conception

The number of services required per conception was found as1.83, 2.40, 2.75, 1.83 and 2.00 in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively in the present study (Table.12 and Fig.12).

Table.5: Types of estrous intherapeutic crossbred cows.

S. No	Types of Estrous	Crossbred cows	
		Number observed	**Per cent**
1	**Normal estrous**	41	95.3
2	**Silent estrous**	0	0
3	**Split estrous**	2	4.7
	TOTAL	**43**	**100.00**

Fig .6.Showing types of estrous intherapeutic crossbred cows.

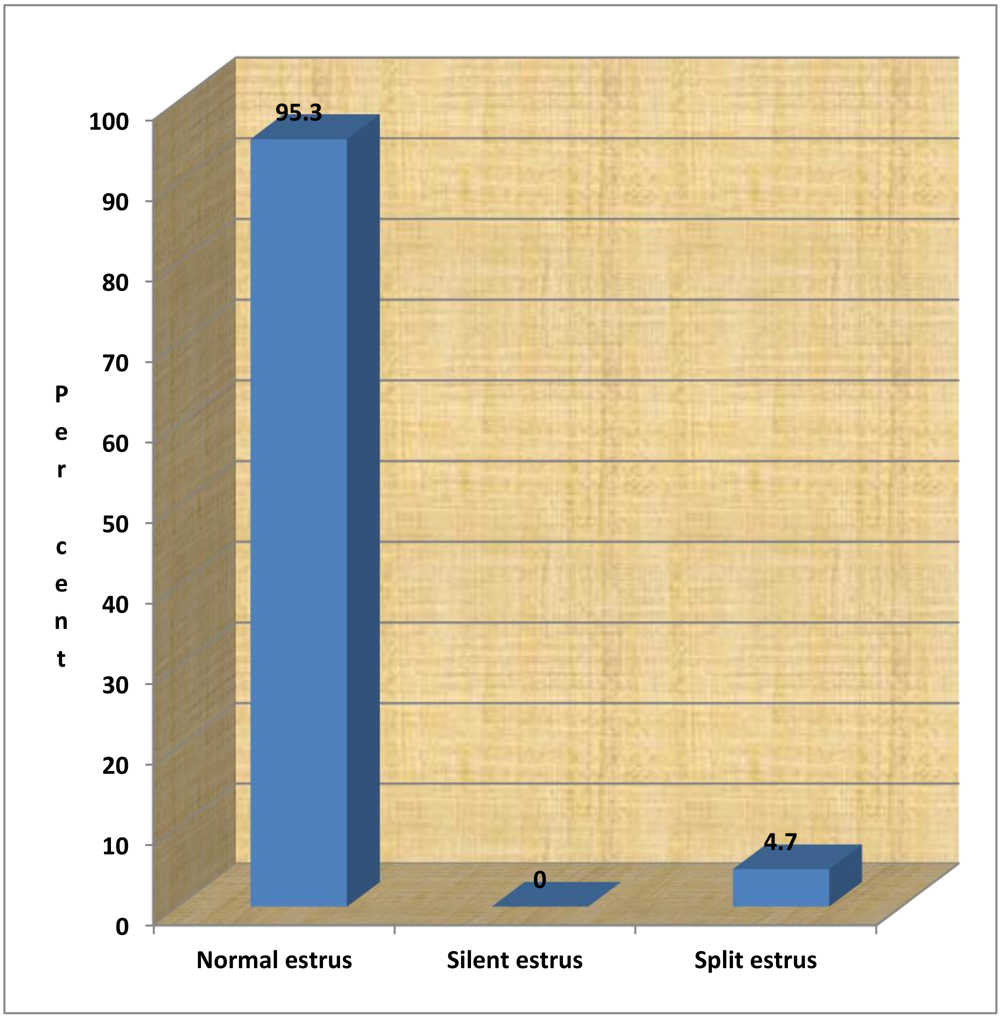


Table.6: Intensity of estrous in therapeutic groups of crossbred cows.

Sl.No	**Name of the group**	**Cows treated (n)**	**Percent**		
			Intense (n)	**Normal (n)**	**Weak (n)**
1	**Ovsynch**	10	10.00(1)	80.00(8)	10.00(1)
2	**Ovsynch+GnRH**	10	10.00(1)	70.00(7)	20.00(2)
3	**Ovsynch+hCG**	10	10.00(1)	80.00(8)	10.00(1)
4	**GPH+GnRH**	10	10.00(1)	80.00(8)	10.00(1)
5	**Control**	10	0.00	20.00(2)	80.00(8)
	Overall per cent		**8.00(4)**	**66.00(33)**	**26.00(13)**

Fig.7.Showing intensity of estrous in therapeutic groups of crossbred cows.

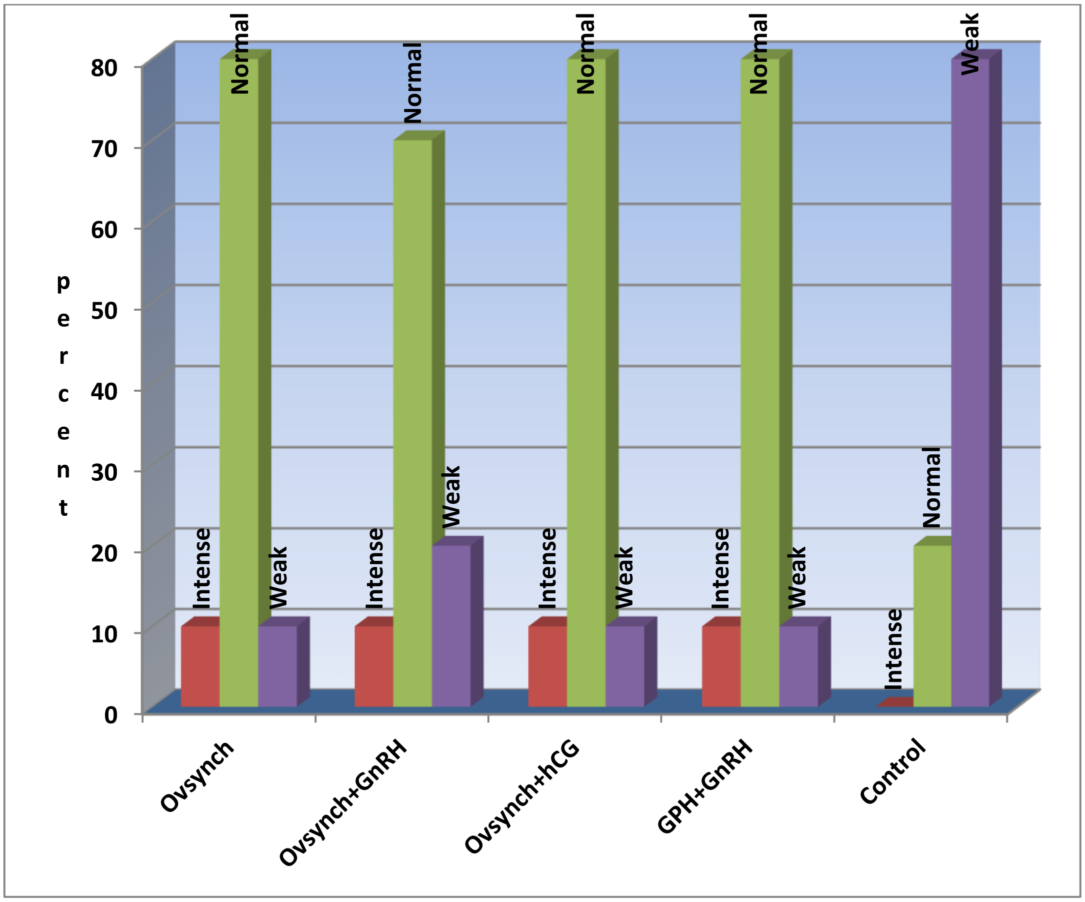


Table.7: Mean estrous score in therapeutic groups of crossbred cows.

Sl.No	Name of the group	Cows treated (n)	Cows exhibited estrous (n)	Mean estrous score
1	**Ovsynch**	10	9	11.70 ± 0.79^{a}
2	**Ovsynch+GnRH**	10	10	12.50 ± 0.83^{a}
3	**Ovsynch+hCG**	10	10	11.60 ± 0.53^{a}
4	**GPH+GnRH**	10	10	12.30 ± 0.77^{a}
5	**Control**	10	3	05.10 ± 2.07^{b}

Means bearing different superscripts differed significantly ($p<0.05$)

Fig.8. Showing meanestrousscore in therapeutic groups of crossbred cows.

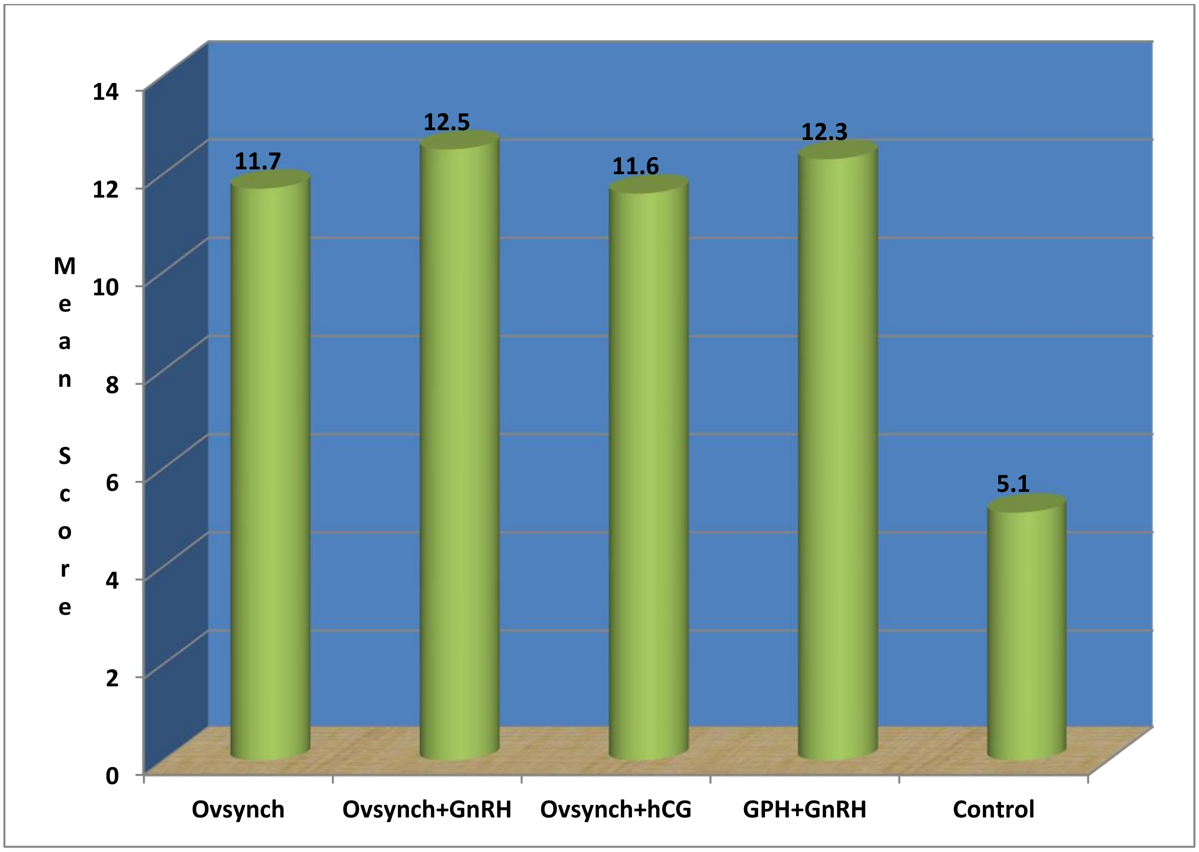


Table.8: Analysis of variance (ANOVA) for estrous intensityand duration of estrous in therapeutic groups of crossbred cows.

Parameter	Variance	Sum of Squares	df	Mean Squares	F
Onset of Estrous	Between Groups	5470.26	4	1367.56	2.62*
Estrous Intensity	Between Groups	59.85	4	14.96	2.72*
Estrous Duration	Between Groups	28.60	4	7.15	1.34*

Means bearing different superscripts differed significantly ($p<0.05$)

Table 9: Mean duration of oestrous (hrs) in therapeutic crossbred cows.

Sl.No	Name of the group	Cows treated (n)	Mean duration of oestrous (hrs)
1	**Ovsynch**	10	21.90± 0.69
2	**Ovsynch+GnRH**	10	22.30± 0.79
3	**Ovsynch+hCG**	10	21.80 ± 0.73
4	**GPH+GnRH**	10	23.60 ± 0.82
5	**Control**	10	21.40± 0.60

Fig.9.Showing mean duration of oestrous (hrs) in therapeutic crossbred cows.

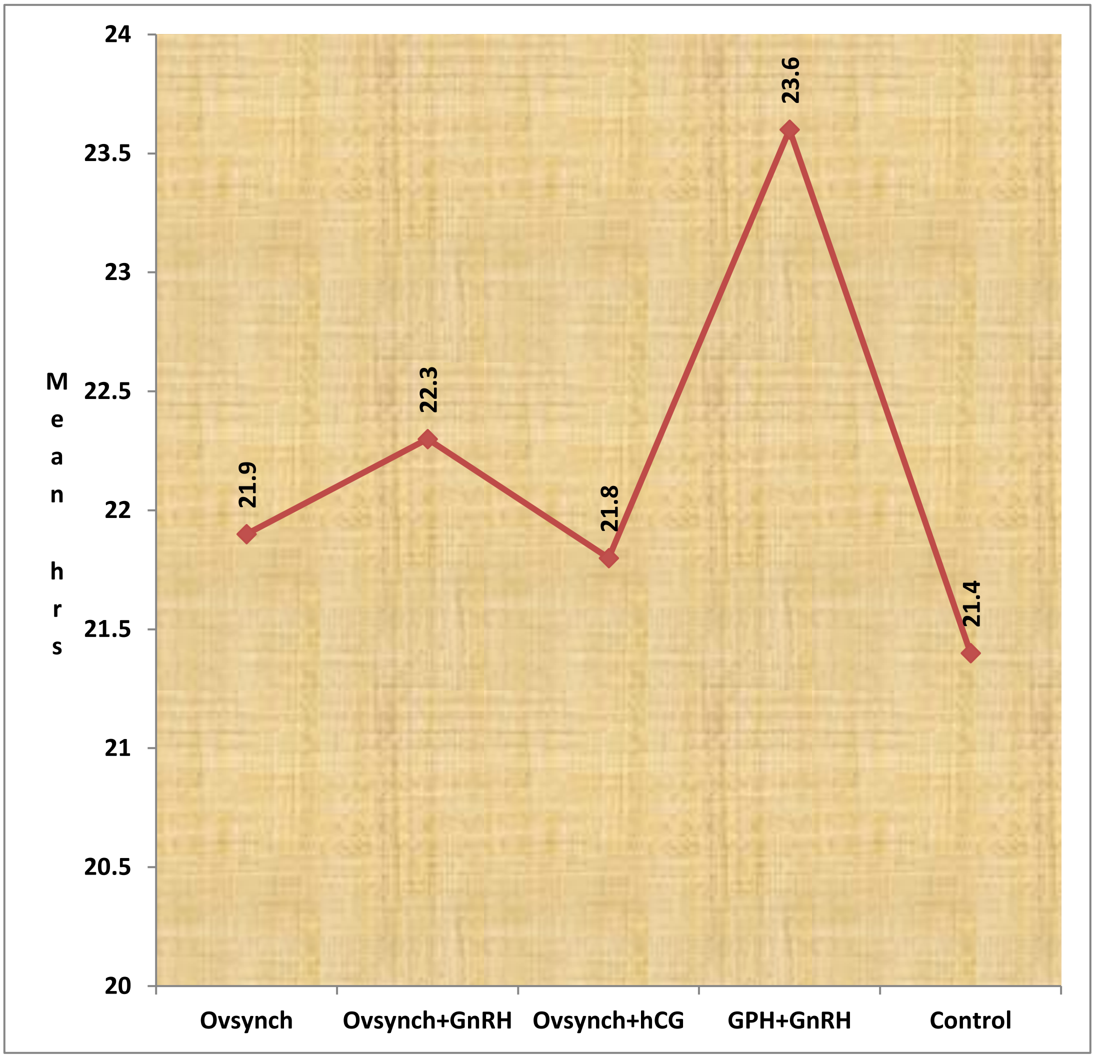


Table.10: Conception rate in therapeutic groups of crossbred cows.

Sl.No	Name of the group	Cows inseminated	Cows conceived	Conception rate	Chi-square test
1	**Ovsynch**	10	6	60.00	**2.825** Chi Square Value**
2	**Ovsynch+GnRH**	10	5	50.00	
3	**Ovsynch+ hCG**	10	4	40.00	
4	**GPH+GnRH**	10	6	60.00	
5	**Control**	10	2	20.00	
	TOTAL	**50**	**22**	**51.16**	

****p<0.01**

Fig.10.Showingconception rate in therapeutic groups of crossbred cows.

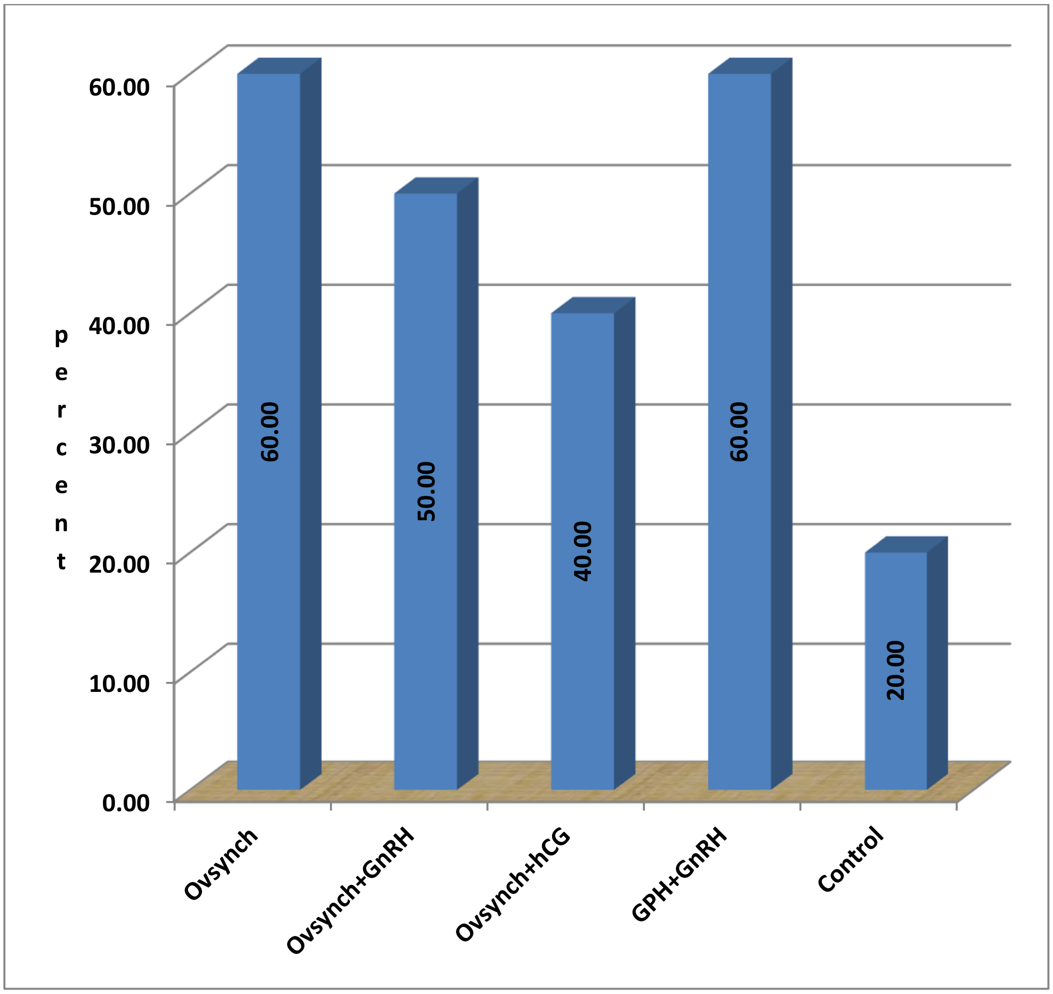


Table.11: Services wise conception rate in therapeutic groups of crossbred cows.

Sl.No	Name of the group	Cows inseminated (n)	Services wise conception rate						Per cent of Conception
			1st	Per cent	2nd	Per cent	3rd	Per cent	
1	**Ovsynch**	9	4	44.44	2	40.00	0	0	60.00
2	**Ovsynch+ GnRH**	10	3	30.00	2	28.57	0	0	50.00
3	**Ovsynch+ hCG**	10	3	30.00	1	14.29	0	0	40.00
4	**GPH+ GnRH**	10	5	50.00	1	20.00	0	0	60.00
5	**Control**	10	1	33.33	1	50.00	0	0	20.00

Fig.11.ShowingServices wise conception rate in therapeutic groups of crossbred cows.

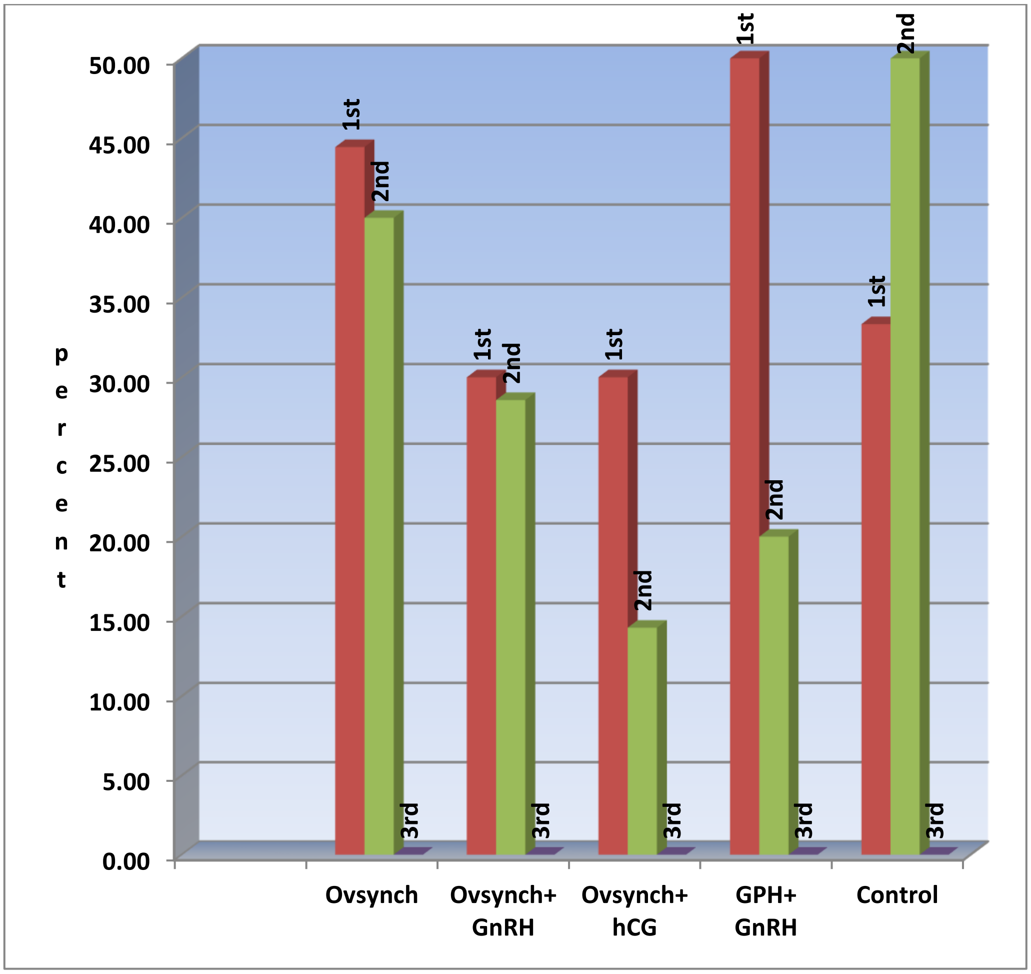
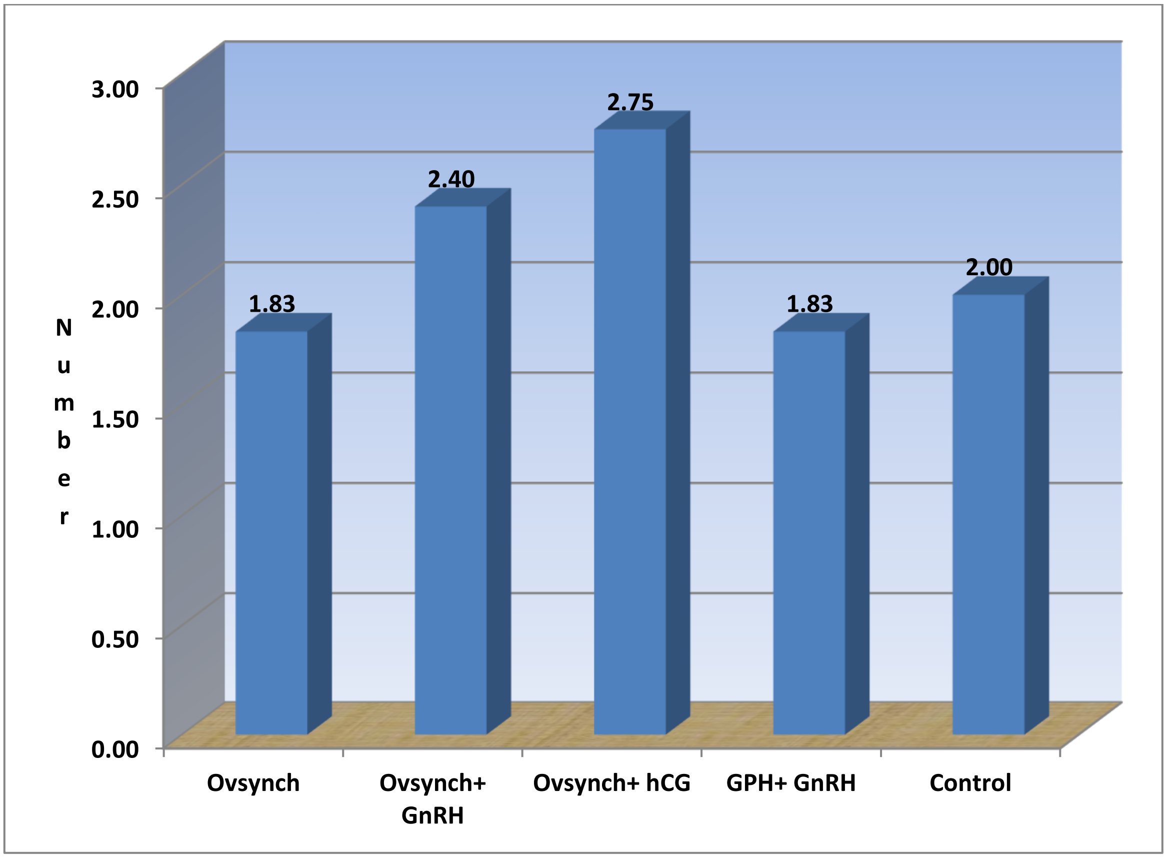


Table.12: No.of services taken per conception in therapeutic groups of crossbred cows.

Sl.No	Name of the group	Cows inseminated 1 st service (n)	Cows inseminated 2nd service (n)	Cows inseminated 3rd service (n)	Total inseminations done(n)	cows pregnant (n)	No.of services per Conception
1	**Ovsynch**	9	2	0	11	6	1.83
2	**Ovsynch+ GnRH**	10	2	0	12	5	2.40
3	**Ovsynch+ hCG**	10	1	0	11	4	2.75
4	**GPH+ GnRH**	10	1	0	11	6	1.83
5	**Control**	3	1	0	4	2	2.00

Fig.12.Showing number of services per conception in therapeutic groups of crossbred cows.



CHAPTER-V

DISCUSSION

Post insemination treatment with Gonadotrophin releasing hormone (GnRH) or human chorionic gonadotrophin (hCG) have been widely used for the improvement of conception rate in post partum crossbred cows, to stimulate luteal tissues and to control follicular waves in cows. Moreover, most of the studies are focused on obtaining higher pregnancy rates with fixed time insemination.

In the present study, total of 50 post partum crossbred cows without any palpable abnormalities were selected. Post partum crossbred cows were randomly divided into five equal groups so that each group consists of10 crossbred cows.

5.1 INCIDENCE OF REPRODUCTIVE DISORDERS IN CROSSBRED COWS

In the present study, 9217 post partum anoestrous crossbred cows were included to investigate the reproductive disorders and the major incidences were classified into functional causes (68.02 per cent), infectious causes (29.65 per cent) and anatomical causes (2.32 per cent).

The incidence of postpartum anestrum was found to be 21.82 per cent,which was analysed from the records available at District Veterinary and Animal Husbandry Office, Ranga Reddy.

The incidence of anatomical causes include hypoplasia of ovaries and genital infantilism was 1.47 and 0.86 per cent, respectively in the crossbred cows. The incidence of functional causes includequiescent ovaries, silent ovulations, anovulatiory heat, luteal persistency, luteal cysts, follicular cysts and repeat breeder was 45.26, 3.98, 3.13, 2.1, 3.21, 3.42 and 6.92 per cent, respectively. Among the quiescent ovaries, the incidence of postpartum anoestrous, smooth ovaries and post service anestrous were recorded as 21.82, 15.62 and 7.82 per cent, respectively. The incidence of infectious causes include vaginitis, cervicitis, endometritis, metritis, pyometra, salphingitis and bursal adhesions was recorded as 4.31, 8.06, 15.24, 0.71, 1.33, 0 and 0 per cent, respectively.

In the present study, the incidence of postpartum anestrum was recorded to be 21.82 per cent. The above findings were comparable to the reports of Shiferaw *et al*. (2005), Muneer *et al*. (2010), Hadush *et al.* (2013), Adane Haile *et al*. (2014), Barua *et al.* (2016), Khan*et al.* (2016), Sujit Das *et al.* (2016) and Siyoum *et al.*(2016), who observed 38.60, 19.97, 12.90, 12.06, 2.50, 31.79, 2.52 and 26.48 per cent respectively, in crossbred cows.

In contrast, Shiferaw *et al*. (2005), Khan*et al.* (2016) and Siyoum *et al.*(2016) reported the higher incidence of anestrum as 38.60, 31.79 and 26.48 per cent respectively. These were not correlating the observations of the present study. Whereas, Hadush *et al.* (2013), Adane Haile *et al*. (2014), Barua *et al.* (2016), and Sujit Das *et al.* (2016) reported the lower incidence of anestrum as 12.90, 12.06,2.50 and 2.52 respectively, in crossbred cows.

The incidence of postpartum anestrum (19.97 per cent) was in close agreement with the reports of Muneer *et al*. (2010) in crossbred cows.

Prolonged postpartum anestrum due to sub-functional ovaries in the crossbred cows in high lactation may be due to insufficient ovulation surge of LH which may result from inefficient synergistic action of progesterone and estrogen. In these high yielding crossbred cows, high level of prolactin concentration in the circulating blood suppressed the action of FSH and LH affecting growth and ovulation of the standing ovarian follicle (Heranjal et al., 1979). The milking and suckling stimulates bursts of prolactin secretion (Bawa et al., 1978) and ovarian sensitivity to LH is reduced (Bartosik et al., 1967) which may leads to smooth ovarian condition

The variation among findings of above mentioned scientists and the present findings might be due to factors like difference in age, parity, body weight, milk yield, breed, nutrition of dam during late gestation, season of calving, gestation length, sex and birth weight of the calf and weight of its placental membrane as well as the postpartum husbandry practices including feeding, milking or suckling practice and hygiene as well as time of treatment in postpartum period.

5.2 ESTROUS RESPONSE.

Cattle are polyestrous animals and display estrous behavior approximately every 21 days. The estrous cycle is regulated by the hormones of the hypothalamus (gonadotropinreleasing hormone; GnRH) and the anterior pituitary (follicle-stimulating hormone; FSH and luteinizing hormone; LH).

The pulsatile secretion of basal levels of GnRH from the tonic center of the hypothalamus and the pre-ovulatory surge of GnRH from the surge center of the hypothalamus prevents the desensitization of the GnRH receptor on the gonadotroph cells of the anterior pituitary. After transportation of GnRH from the hypothalamus to the pituitary gland via the hypophyseal portal blood system (Moenter *et al.,* 1992). GnRH binds to its G-protein coupled receptor on the cell surface of the gonadotroph cells (Kakar *et al.,* 1993). This binding releases intracellular calcium which activates intermediaries in the mitogen activated protein kinases (MAPK) signaling pathway culminating in the release of FSH and LH from storage compartments in the cytoplasm (Weck *et al*., 1998). FSH is only stored in secretory granules in the cytoplasm for short periods of time, whereas LH is stored for longer periods during the estrous cycle (Farnworth, 1995). During the follicular phase of the estrous cycle there is a hormonal environment of basal progesterone due to the regression of the corpus luteum. The increased E2 concentrations, derived from the rapid proliferation of the pre-ovulatory dominant follicle, concomitant with the decrease in circulating concentrations of progesterone, induces a surge in GnRH and allows the display of behavioral estrous during which heifers/cows are sexually receptive and will stand to be mounted (Fradson *et al*.,2003). This pre-ovulatory GnRH surge induces a coincidental LH and FSH surge (Sunderland et al., 1994). Only when serum progesterone concentrations are basal and LH pulse frequency increases to one per hour for 2–3 days does the dominant follicle ovulate (Roche, 1996).

In the present study, the estrous response was observed to be 90.00, 100.00, 100.00, 100.00 and 30.00 per cent in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups in postpartum crossbred cows, respectively.

Similar findings of estrous response were reported 100 per cent in crossbred cows treated with Ovsynch protocol by Naidu (2004) Selvaraju *et al*.(2008**)**, Ramakrishnan *et al*. (2012), Navrange *et al.* (2012) ,Mohd Alyas *et al*. (2013) , Buhecha *et al.* (2015), Dhami *et al*. (2015) and Ahmed *et al*. (2016) .

In contrast the estrous response in Ovsynch group and control group was 100 and 62.5 per cent, respectively in crossbred cows recorded by Velladurai *et al*. (2014). But, Ion Caraba *et al*. (2013) who recorded the lower estrous response in cows treated with Ovsynch protocol was of 63 per cent.

John *et al*. (2009) illustrated the GnRH hormone which is synchronize follicular growth and ovulation so all cows ovulate within a few hrs of one another. Another advantage of the GnRH systems is that they induce ovulation and estrous cycles in non-cycling cows. If cows are given an injection of GnRH, then enough LH is released to cause the largest follicle on the ovary to ovulate and form a CL. A new wave of follicles will start to grow since GnRH "removed" the dominant follicle. Now, the follicular growth of the cows is synchronized. Seven days later an injection of an analog of prostaglandin PGF2α is given which regressesthe CL to synchronize final follicular growth and exhibition of estrous.

5.2.1 Onset of Estrous

The onset of estrous was calculated in hrs (h) from the time of PGF2α administration to the time of the first appearance of estrous signs in therapeutic crossbred cows. The mean onset of estrous was recorded as 51.80±1.07, 50.90±1.20, 52.40 ±1.32, 52.40±1.32 and 96.00±13.86 hrs in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively in crossbred cows in the present study.

Similar findings recorded the mean onset of estrous as 48.75±0.71 and 51.47±1.99 hrs in Ovsynch and Ovsynch based GnRH treatment groupsrespectively,in crossbred cows by Ahmed *et al*. (2016) and 48.80±5.74 hrs by Ahmed *et al*. (2017) in anestrous crossbred cows. Likewise Velladurai *et al*. (2014)reported the similar mean onset of estrous in Ovsynch group was 47.97±2.65 hrs in crossbred cows. While, Yendraliza *et al.* (2011) recorded the lower onset of estrous range 37.4 to 38.4 hrs in post partum swamp buffalo by treating with ovsynch protocol.

In contrary, Selvaraju *et al*. (2008**)** and Patil *et al*. (2011) reported the higher side meanonset of estrous in crossbred cows in Ovsynch protocol was 59.38±0.81 and 62.2±1.56 hrs, respectively.

However, Buhecha *et al.* (2015) treated with Triu-B/ PRID, Ovsynch and Heatsynch, and recorded the mean intervals of estrous were 67.00±1.74, 69.83±0.87 and 68.17±1.24 h, respectively, from PGF2α injection in crossbred cows. Senthilkumar and Chandrahasan (2015) reported the time taken for the onset of oestrous was 70.50 ± 3.27, 68.00 ± 3.17 and 68.25±2.21 hrs in PGF2α group, PGF2α+GnRH group and PGF2α+hCG group, respectively in cyclic crossbred cows.

5.3ESTROUS PATTERN

The estrous pattern includes types of estrous, intensity of estrous and duration of estrous will discussed.

5.3.1 Type of Estrous

It was found that 95.30 per cent of crossbred cows exhibited normal estrous whereas lower incidence than the present study was reported by Srinivasreddy (2014)was 90.48 per cent. But this observation was contrary with the studies of Agarwal and Purbay (1983) and Kavani *et al* (1984) who reported still lower incidence of normal estrous in repeat breeder animals. The higher per cent of normal estrous might be due to the fact that all selected crossbred cows were had normal genitalia without any palpable abnormalities in the present study.

In the present study the split estrous was found that 4.70 per cent whereas Kumar *et al.* (1988) observed the high incidence of split estrous was 62.02, 40.98, 20.03, and 10.97 per cent in crossbred cows, indigenous cows, crossbred heifers and indigenous heifers, respectively. On contrary Suryaprakasam and Rao (1998) reported very low incidence of split estrous was 0.77, 1.33 and 0.81 per cent in zebu, crossbred cows and buffaloes, respectively under village conditions.

5.3.2 Intensity of Estrous

Among 50 post partumcrossbred cows, mean normal intensity of estrous was exhibited by 66.00 per cent of crossbred cows whereas mean weak and mean intense estrous was exhibited by 26.00 and 8.00 per cent, respectively in the present study.

The present results of normal intensity of estrous were in accordance with the observation of Senthilkumar and Chandrahasan (2015) and Ahmed *et al*. (2016) who recorded as 58.33 and 66.67 per cent, respectively in crossbred cows. The incidence of intermediate estrous intensity was found to be highest. Senthilkumar and Chandrahasan (2015) recorded the higher per cent (33.33) of intense estrous and lower per cent (8.33) of week estrous than the present study in crossbred cows.

However, these findings varied with findings of Sahatpure *et al*. (2008) where the intensity of estrous in non-descriptive cows was intense in 25.00 per cent, intermediate in 50.00 per cent and weak in 25.00 per cent cows. While in crossbred cows, it was intense in 41.67 per cent and intermediate in 58.33 per cent cows. The variation in intensity of estrous was attributed to season, climate and nutrition status and managemental conditions of crossbred cows.

5.3.3 Duration of Estrous.

The mean duration of estrous was recorded as 21.90±0.69, 22.30±0.79, 21.80±0.73, 23.60±0.82 and 21.40±0.60 hrs in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively in the present study.

These findings were not in corroboration with the findings of Selvaraju *et al*. (2008), Honparkhe *et al*. (2010) and Velladurai *et al*. (2014) reports where the duration of estrous was longer (28.50±0.70, 24-36 and 28.78±0.67 hrs, respectively) in crossbred cows. On otherhand, Das *et al*. (2009) and Bhat *et al*. (2012) recorded that the mean duration were variable in unovulation, delayed ovulation and normal ovulation in repeat breeder crossbred cows.

The present results of mean duration of estrous were in accordance with the observation of Senthilkumar and Chandrahasan (2015) in the cyclic crossbred cows that were treated with PGF2α (Group-II), PGF2α+GnRH (Group-III), and PGF2α+hCG (Group-IV). The duration of estrous in Group (I), Group (II), Group (III) and Group (IV) were 20.50 ± 0.41, 21.00 ± 0.47, 19.50 ± 0.33 and 19.25 ± 0.56 hrs, respectively.

However, Ahmed *et al*. (2016) findings also supported the present investigation and theyrecorded the duration of estrouswas 22.04±0.94, 21.45±1.10, 21.08±0.78 and 20.07±0.86 hrs, respectively, in control, GnRH treatment on day 6 post-estrous, Ovsynch and Ovsynch based GnRH treatment groups in crossbred cows. There was no significant difference in duration of estrous among the groups.

5.4 THERAPEUTIC EFFICACY ON CONCEPTION RATE IN CROSSBRED COWS.

The overall conception rate in therapeutic crossbred cows was recorded as 60.00, 50.00, 40.00, 60.00, and 20.00 per cent in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively in the present study.

Various studies reported that gronadotropin-releasing hormone (GnRH) (Mehni *et al*., 2011) injection or hCG (Stevenson *et al*., 2007) between day 5 to 7 after FTAI have potential to increase progesterone concentration in plasma of cattle at different stages of pregnancy (Dahlen *et al*., 2014). Previous study suggested that exogenous GnRH administered 5 days after FTAI causes ovulation and (or) luteinization of an antral follicle resulting in the formation of an accessory CL (Howard *et al*., 2006). Similarly, the administration of hCG in the early luteal stage induces the formation of accessory CL, increases the surface area and the volume of the CL and may increase the diameter of CL (Santos *et al*., 2001). Both GnRH and hCG effectively induced ovulation and increased CL number, but circulating progesterone concentrations were only increased in hCG-treated cows (Lonergan, 2011). Finally, the comparison of post-FTAI administration of different progesterone sources in early lactating crossbred cows needs to conduct more investigation (Mehni *et al*., 2011).

5.4.1 Post Insemination Treatment Efficacy Of GnRH.

Mechanism of Action:Gonadotrophin-releasing hormone (GnRH)is a key integrator between the neural and endocrine systems. This decapeptide is synthesized in hypothalamic neurosecretory cells then released in a pulsatile pattern into the hypothalamo-hypophyseal portal circulation. This pulsatile pattern provokes the secretion of the gonadotropins, LH and FSH, from the anterior pituitary. Gonadotropins, in turn, control the function of the gonads; LH stimulates ovulation and corpus luteum formation in females and androgen secretion in males, whereas FSH stimulates the growth and maturation of ovarian follicles in females and spermatogenesis in males (Hazum and Conn, 1988).

By administering GnRH followed by PGF2α 6 to 7 days later and a second dose of GnRH 24 h later, cows can be subjected to FTAI without the need for estrous detection (Pursley *et al*., 1997). However, 40 to 60 per cent of treated cows do not become pregnant at the time of FTAI. Several factors may lead to the failure of FTAI. One such cause could be related to the use of GnRH to induce ovulation (De Rensis *et al*., 2002). In effect, it has been observed that a single dose of a GnRH-analogue induces an LH surge that lasts for about 5 h (Chenault *et al*., 1990), which is approximately half the duration of a naturally occurring LH surge (Rahe *et al*., 1980). This short-lived LH surge may determine the incomplete development of the ovulatory follicle, giving rise to a less functional CL (De Rensis *et al*., 2008). The effectiveness of hCG in inducing ovulation and the formation of a functional CL has been described by several authors (Longergan, 2011). In the classic Ovsynch protocol, hCG is given to synchronize, induce ovulation (De Rensis *et al*., 2002), and subsequent plasma progesterone concentrations (De Rensis *et al*., 2008).

In the present study, Ovsynch+GnRH and GPH+GnRH group cows received GnRH @10µg on day 6 of mid-luteal stage (post AI). The conception rate in therapeutic crossbred cows was recorded as 50.00 and 60.00 per cent in Ovsynch+GnRH and GPH+GnRH groups, respectively.Which was significantly ($p<0.01$) higher than that of control group (20 per cent).

The present study was in accordance with the findings of Dirandeh *et al*. (2014) who studied the effects of two different time of GnRH injection after AI on reproductive performances of Holstein cows during the warm season. Cows randomly assigned to treatments 1) No GnRH injection (control), 2) GnRH injection at day 6 after AI and 3) GnRH injection at day 12 after A.I and reported that administration of GnRH on day 6 after AI improved reproductive performance in dairy cows. Ahmed *et al.* (2016)reported a conception rate of50.0 per cent in crossbred cows. The present conception rate was also in accordance with Beltran *et al.* (2008) whose findings should an enhanced conception rate in treatments with GnRH or hCG on day 5 after AI in cows.

This was corroborated with the findings of Sterry *et al.* (2006) whostudied on Lactating Holstein cows assigned randomly to treatments to improve fertility after first postpartum fixed timed artificial insemination (FTAI) and concluded that the treatment with CIDR inserts after FTAI had no effect on pregnancy per AI, whereas treatment with GnRH 5 d after FTAI improved pregnancy per AI for noncycling, but not for cycling cows. Peters (2005) suggested the use of GnRH in cattle as an effective means in increasing pregnancy rates when given either at the time of insemination (first or repeat) or between days 11 and 14 after insemination,and an overall increase of 18.0 per cent in pregnancy rate was reported. Dadarwal *et al.* (2007) reported an increase of 18 and 35 per cent pregnancy rate over those observed in cows given single injection of buserelin (20µg) and control groups, respectively than second injection was given on day 12 after post insemination in crossbred cows.

Whereas, Carvalho *et al*. (2007) concluded the use of GnRH six days after the insemination in treatment sequence of GnRH/PGF2α/GnRH can induce an accessory CL in buffaloes and increased the conception and birth rates. Beltran *et al. (*2008) revealed that increased concentrations of progesterone between days 7 and 12 in cows treated with GnRH and hCG; but not in control cows, suggesting that a new CL was formed and treatments with GnRH or hCG on day 5 after AI increased conception rates in cows.

On other side, Mahesh *et al.* (2010) reported that GnRH therapy irrespective of day of administration during the estrous cycle resulted in an overall enhancement of conception rate of 83.33 per cent as against 33.33 per cent in control group of cows.

In agreement with the present study, Gordan *et al.* (2009) examined two stratagies to improve pregnancy rate following ovsynch timed artificial insemination in lactating dairy cows. The cows were assigned randomly to receive one of three treatments: Ovsynch protocol (GnRH 7 d before and 48 h after one PGF2a treatment), Presynch-Ovsynch (two treatmentsof PGF2a 14 d apart followed by Ovsynch 14 d later), or Ovsynch-Post-AI GnRH (GnRH 6 d after Ovsynch FTAI) for first service breeding. Pregnancy rates among treatments were not different in lactating cows (42.5, 48.0, and 44.9 per cent) for Ovsynch, Presynch-Ovsynch, and Ovsynch-Post-AI, respectively.

In contrary to the present study, Abdurraouf Omar Gaja*et al.* (2008) designed a study to evaluate the reproductive performance of Japanese black cows following 3rd injection of gonadotropin releasing hormone (GnRH) analogue administered concurrently with Ovsynch-based treatment on day 6. However,the pregnancy rates on day 45 did not differ among groups. In conclusion, administration of GnRH analogue on day 6 following

Ovsynch-based treatment did not improve the reproductive performance of Japanese black cows, even though the progesterone concentration was higher in groups that received the GnRH. According to Zahid Paksoy andCahit Kalkan (2010), the GnRH and hCG administration during and at the 12th day after AI failed in increasing pregnancy rate.

Vasconcelos *et al.* (2011) studied the effects of treatments with human chorionic gonadotropin (hCG) or GnRH 7 d after induced ovulation on reproductive performance of lactating dairy cows submitted to fixed timed artificial insemination (FTAI) or timed embryo transfer (TET) and concluded that the treatment with GnRH or hCG 7 days after induced ovulation increased conception rates in lactating dairy cows submitted to TET, but not in cows submitted to FTAI. Ergene (2012) concluded the application of PRID and GnRH after artificial insemination did not significantly improve pregnancy rates, despite the fact that serum progesterone concentrations were higher in treatment groups.

In contrary to the present study, Howard *et al*. (2006) summarized the results of his study that GnRH administered 5 days after AI increased serum progesterone by developing an accessory CL but did not improve conception rates in dairy cattle. And Jae Kwan Jeong *et al*. (2016) reported that the GnRH treatment on post AI had no beneficial effects on pregnancy outcomes, although the percentage of cows with an accessory CL after FTAI was higher among GnRH-treated cows than non-treated cows.

In agreement with the present study, Krishnakumar and Chandrahasan (2012) reported that the conception rate was higher in group treated with 20µg of GnRH agonist, intramuscularly at 48 and 72 hrs after PGF2α administration and day 5 post insemination than the control and concluded that PGF2α synchronized estrous combined with GnRH 72h after PGF2α administration and on day 5 post-insemination were found to have achieved fairly a good overall conception rate in repeat breeder cows. Jaswal *et al*. (2013) concluded that the Buserelin acetate improved conception in cows if administered either on day 0 along with AI or on Day 5 or 12 post insemination. Mendonça *et al*. (2017) studied in lactating holstein cows and reported increased pregnancy rate after treatment with GnRH 5 day after AI or the combined treatment of GnRH at AI and 5 days after AI because of reduced early embryonic loss.

From the present study, it could be concluded that the Ovsynch+GnRH and GPH+GnRH group animals which were given GnRH @10µg on day 6 of post insemination resulted considerable conception rates. Further studies should be carried out to ascertain the effect of GnRH on day 6 of post insemination by undertaking large number of clinical cases.

5.4.2 Post Insemination Treatment Efficacy of hCG.

Mechanism of Action: This glycoprotein is composed of two polypeptide chains with carbohydrates attached to each.Human chorionic gonadotropin is produced by the trophoblast of the blastocyst and can be detected in the peripheral circulation as soon as day 8 to 10 of gestation. The hormone maintains the CL of pregnancy and is used to detect early pregnancy (Jameson and Hollenberg, 1993). Once hCG binds to the LH-CG receptor it directs the CL to produce different hormones including progesterone and estrogen. Over time, the CL becomes less sensitive to hCG, but increasing concentrations of the hormone maintain its functional capacity until approximately 7 weeks of pregnancy (Jameson and Hollenberg, 1993).

Human choronic gonadotrophin (hCG) was more effective than GnRH at stimulating ovulation in dairy cattle (Stevenson *et al*., 2006), a proper dose of hCG might be a substitute for GnRH in various FTAI protocols. It was hypothesised that the longer period of LH-like stimulation of the ovulatory follicle due to the extended half-life of hCGin blood (Schmitt *et al*., 1996) should better stimulate the ovulatory follicle at concurrent ovulation induction and FTAI in Ovsynch strategy. Because poor luteal activity after FTAI has been association with deficiency of plasma progesterone concentrations and subsequent infertility in cattle (De Rensis *et al*., 2008; Longergan, 2011), we hypothesized that administering hCG during the final maturation of the preovulatory follicles or administering hCG or GnRH after FTAI would augment CL characteristics such as increased number of accessory CL and increased concentrations of plasma progesterone in crossbred cows.

In the present study, Ovsynch+hCG group cows received hCG @1500IU IM on day 6 of mid-luteal stage (post AI). The conception rate in therapeutic crossbred cows was recorded as 40.00 per cent in the group, which was significantly ($p<0.01$) higher than that of control group (20 per cent).

This is in accordance with the findings of Zahid Paksoy andCahit Kalkan, (2010) who administered1500 IU hCG immediately and on the 12th day after AI in group of cows and recorded thepregnancy rate of 46.7 per cent. Nascimento *et al*. (2013) conducted a field research trial in which lactating Holstein cows from 6 commercial dairy herds were stratified by parity and breeding number and then randomly assigned to one of 2 groups: control (no further treatment) or hCG [Chorulon: 2,000 IU or 3,300 IU] on day 5 after a timed AI (ovulation synchronized with Ovsynch, Presynch-Ovsynch, or Double-Ovsynch). Pregnancyrate were greater in cows treated with hCG (40.8 per cent) than the control (37.3 per cent) cows. Sanchez *et al*. (2015) recorded significantly higher pregnancy rate in hCG2 and hCG5 groups than in the control group (43.1 and 45.3 per cent,υs 27.7 per cent). Gonzalez *et al*. (2017) evaluated in cows which were inseminated after overt estrous or at a fixed-time. Five days post-insemination, cows received 3500 IU of hCG by intramuscular injection and recorded the pregnancy rate as 47.5 per cent.

This is corroborated with the findings of Schmitt *et al*. (1996), who reported thatinduction of an accessory CL with hCG on day 5 or 6 after insemination increased progesterone concentrations, but did not increase pregnancy rate in fertile heifers or lactating dairy cows during summer heat stress. Tefera *et al*. (2001) found no effects on the rate of embryonic mortality with the administration of hCG on day 4 or GnRH on day 12 post-AI. Funston *et al.* (2005) summarized that the administration of hCG 5 to 6 d after AI did not improve conception or pregnancy rates at two out of three locations evaluated, suggesting insufficient progesterone is not a major factor contributing to early pregnancy failure in beef heifers.

The present study was also in agreement with the findings of Hanlon *et al*. (2005) reported that 1500 IU of hCG 5 days after insemination did not improve first service conception rate in anovulator anoestrous dairy cows. Shams *et al.* (2007) observed that the effect of administration of exogenous hCG 5 days after artificial insemination (AI) on serum progesterone concentration and conception rate in lactating dairy cowsand concluded that treatment with hCG (3000 IU)5 days after insemination did not improve pregnancy rate. Beltran *et al.* (2008) revealed that increased concentrations of progesterone between day 7 and 12 in cows treated with GnRH and hCG; but not in control cows, suggesting that a new CL was formed and treatments with GnRH or hCG on day 5 after AI increased conception rates in cows.

Vasconcelos *et al.* (2011) studied the effects of treatments with human chorionic gonadotropin (hCG) or GnRH 7 days after induced ovulation on reproductive performance of lactating dairy cows submitted to fixed timed artificial insemination (FTAI) or timed embryo transfer (TET) and concluded that the treatment with GnRH or hCG 7 days after induced ovulation increased conception rates in lactating dairy cows submitted to TET, but not in cows submitted to FTAI.

On other side, Alnimer and Shamoun (2015) conducted a studyto evaluate the effect of human chorionic gonadotropin (hCG) administration 4 or 6 days after timed AI (FTAI) on progesterone concentration and pregnancy outcome in repeat breeding dairy cows, and summarized thatadministration of hCG 6 days after FTAI was beneficial in improving pregnancy rate either in summer or winter as a result of reducing pregnancy loss and increasing progesterone concentration.

Contrary to the present study, Santos *et al*. (2001) stated that treatment with hCG on day 5 resulted in 86.2 per cent of the cows with more than one CL compared with 23.2 per cent in controls. Plasma progesterone concentration were increased by 5.0ng/mL in hCG treated cows. hCG injection on day 5 after AI induces accessory CL and improves concepetion rate in dairy cows. Selvaraj *et al*. (2003) observed that higher conception rates of 66.66,33.33 and 66.66 per cent after intramuscular injection of hCG 1500 IU on day 0,7 and 14 of estrous cycle when compared to (16.66 per cent) control group of repeat breeder crossbred cows.

Khoramian *et al*. (2011) compared the three different treatments to improve pregnancy per artificial insemination (P/AI) in repeat-breeder (RB) dairy cows. All cows were assigned to one of four groups: (1) gonadotropin-releasing hormone (GnRH); (2) human chorionic gonadotropin (hCG); (3) once-used controlled internal drug release (CIDR) device; and (4) control. All treatments performed 5–6 days after artificial insemination (AI). Pregnancy rate on day 45 in hCG and CIDR groups were significantly higher than GnRH and control groups (60.0 per cent and 56.0 per cent vs. 26.9 per cent and 29.6 per cent, respectively).

Further, Dahlen *et al*. (2014) determined the effects of administering hCG 7 days after fixed-time AI (FTAI) on ovarian response, concentrations of progesterone, and pregnancy rates in postpartum suckled beef cows. They concluded that treatment with hCG increased the volume of luteal tissue on day 14 and concentrations of progesterone on day 14 and 33 after FTAI. Treatment with hCG tended to increase pregnancy rates at 5 of 6 locations from 1.1 to 27 per cent points compared with saline, but cumulative pregnancy rates determined on day 68 after FTAI were similar between treatments.

The present results was not in agreement with the results of Kunde *et al*. (2017) in repeat breeder cattle synchronized using intravaginal progestational device (TRIU-B) based Co-Synch protocol. On day 6 post artificial insemination (AI), all the animals were administered either 10µg GnRH analogue, 1500 IU hCG or normal saline. Pregnancy diagnosis was carried out on day 45 post-AI. In GnRH, hCG and control group, the respective conception rates were40, 46.7 and 33.3 per cent. In brief, they reported thatadministration of hCG on day 6 post AI improves subsequent luteal profile with tendency for better conception rate in repeat breeder cattle.

Several methods have been tried to increase conception rate by increasing plasma progesterone concentrations during the luteal phase. This can be achieved by inducing the formation of accessory corpora lutea, which can be obtained by hCGtreatment on 4 to 6 days after insemination (Binelli *et al.*, 2001). Apart from the initiation of formation of additional corpora lutea, this treatment is believed to provide further LH support to the corpus luteum verum, which resulted from the ovulation of the dominant follicle.

On day 5 of the estrous cycle, granulosa cells of the dominant follicle contain LH receptors so that hCG will induce ovulation and the formation of an accessory corpus luteum. Therefore, administration of hCG5 days after AI has potential to increase progesterone secretion during early pregnancy. The positive effect of hCG on conception rate is mediated by reducing early embryonic lossesandin addition, most of the benefit of hCG treatment was observed in lactating dairy cows that were losing body condition during the breeding period. Since high yielders have a greater metabolism of progesterone they are more likely respond to hCG treatment (Wiltbank *et al*., 2006).

The present study revealed that, administration of hCG on day 6 of post insemination has not improvedt the conception rate (40 per cent) when compared to GnRH injection (50 per cent) administration on day 6 of post insemination. The study of several authors mentioned above supported the findings of present study.

5.4.3 Services taken per conception.

The number of services required per conception was found to be 1.83, 2.40, 2.75, 1.83 and 2.00 in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups respectively, in the present study

Similar findings were also recordedby Naidu, (2004) in postpartum anestrous cows followed Ovsynch protocol.He reported that the number of services per conception was 1.70 ± 0.18. Whereas, Ansari (2007) recorded the lessnumber of services required per conception (1.44) in postpartum anestrous crossbred cows treated with Ovsynch protocol.

Finally, it is concluded that synchronization of postpartum crossbred cows with Ovsynch + post insemination treatment of GnRH on day 6 yeilded better results/conception rates than the Ovsynch + post insemination treatment of hCG on day 6 in the present study.

CHAPTER VI

SUMMARY

The present investigation entitled “Studies on Post Insemination Supplementation of GnRH or hCG to Ovsynch Protocol In Crossbreed Cows” was undertaken on 50 post partum crossbreed cows in private dairy farm at Nagole, Rangaredy District to studythe estrous pattern, estrous response and the efficacy of post AI supplementation of GnRH and hCG on conception rate.

A total of 50 postpartum crossbreed cows without any palpable abnormalities were selected for the present study. The crossbreed cows were randomly divided into five equal therapeutic groups so that each group consist 10 crossbreed cows. Ovsynch Group cows were injected with 10µg of GnRH (Buserelin acetate-Receptal) IM on day 0, 500 µg of cloprostanol (PGF2α)(Pragma) IM on day 7 and 2^{nd} dose of GnRH 10µg IM on day 9. Fixed time AI was done after 16 hrs of the 2^{nd} dose GnRH of injection.Ovsynch+GnRH Group cows were treated as in the Ovsynch Group and these animals were given an supplemental dose of GnRH @ 10µg IMon day 6 of post insemination. Ovsynch+hCG Groupcows were treated as in the Ovsynch Group and these animals were given an supplemental dose of hCG @1500IU IM on day 6 of post insemination. GPH+GnRH Group cows were injected with 10µg of GnRH IM on day 0, 500 µg of cloprostanol(PGF2α) IM on day 7 and hCG @1500IU,IM on day 9. Fixed time AI was done after 16 hrs of hCG injection. On day 6 of post insemination, GnRH@10µg IM was administered. Control Group cows were injected with 2.5 ml of Normal Saline IM on day 0,7and 9,andwhenever the crossbreed cows exhibited estrous then cows was inseminated. On day 6 of post insemination 2.5 ml of Normal Saline IM was administered.

The estrous response was 90.00, 100.00, 100.00, 100.00 and 30.00 per cent in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively. The mean onset of estrous was recorded as 51.80±1.07, 50.90±1.20, 52.40 ±1.32, 52.40 ±1.32 and 96.00 ±13.86 hrs in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively. The onset of estrous in theraputic groups were not significantly ($p< 0.05$) different among the groups. Among 50 post partum crossbred cows, normal intensity of estrous was exhibited by 66.00 per cent of crossbred cows whereas weak and intense estrous was exhibited by 26.00 and 8.00 per cent of crossbred cows. The mean score for estrous intensity was recorded as 11.70±0.79, 12.50±0.83, 11.60±0.53, 12.30±0.77and 05.10±2.07, in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively. Statistical analysis revealed that the intensity of estrous in treatment groups were significantly ($p<0.05$) different with control group. The mean duration of estrous was recorded as 21.90±0.69, 22.30±0.79, 21.80±0.73, 23.60±0.82 and 21.40±0.60 hrs in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively in the present study.

Overall conception rate in therapeutic crossbred cows was recorded as 60.00, 50.00, 40.00, 60.00, and 20.00 per cent in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively. Statistical analysis revealed that the conception rates were higher in Ovsynch group, GPH+GnRH group and followed by group Ovsynch+hCG, Ovsynch+GnRH and control. However, the conception rates were significantly ($P<0.01$) lower in control group of crossbred cows than the treatment groups. The number of services required per conception was foundas 1.83, 2.40, 2.75, 1.83 and 2.00 in Ovsynch, Ovsynch+GnRH, Ovsynch+hCG, GPH+GnRH and control groups, respectively.

The present study concluded that the use of GnRH or hCG 6 days post insemination had no significant effect on the conception rate in crossbred cows over Ovsynch protocol. It was therefore, suggested that administering GnRH or hCG alone post insemination was not an effective method to enhance conception rate in post partum crossbred cows. Further studies warranted to study the effect of GnRH or hCG during mid-luteal stage of estrous cycle on large number of clinical cases.

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