

**“ESTRUS SYNCHRONIZATION OF RECIPIENT COWS OF
FARMERS FOR TRANSFER OF *IN VITRO* PRODUCED
SAHIWAL EMBRYOS”**

BY

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ID No. RVM/2019-025**

THESIS SUBMITTED TO

**P.V. NARSIMHA RAO TELANGANA VETERINARY UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS**

**FOR THE AWARD OF THE DEGREE OF
MASTER OF VETERINARY SCIENCE
(VETERINARY GYNAECOLOGY AND OBSTETRICS)
IN THE FACULTY OF VETERINARY SCIENCE**

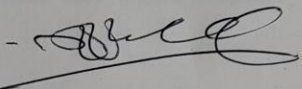


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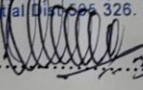
No part of the thesis has been submitted by the student for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of investigations have been duly acknowledged by the author.

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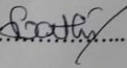
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LIST OF ABBREVIATIONS

Abbreviation	Full form
%	Per cent
@	At the rate of
<	Less than
>	Greater than
±	Plus, or minus
/	Or /Dividing slash
=	is equal to
-	Hyphen
—	Dash
°	Degree (used in longitude and latitude measurement)
'	Minutes of arc (used in longitude and latitude measurement)
"	Seconds of arc (used in longitude and latitude measurement)
AI/A. I	Artificial insemination
ANOVA	Analysis of variance
BCS	Body condition score
B-mode	Brightness mode
CL	Corpus luteum
CIDR	Controlled Internal Drug Release
C°	Degree Celsius
E	East
ET	Embryo Transfer
Et al.,	“and others”
FTAI	Fixed Time Artificial Insemination
gm	gram
GnRH	Gonadotropin Releasing Hormone
hCG	Human Chorionic Gonadotropin
h/hrs	hours
HF	Holstein Friesian

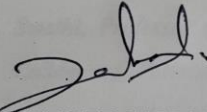
Hz	Hertz
I M/ I/M	Intra muscular
IVEP	<i>In vitro</i> Embryo Production
IVF	<i>In vitro</i> fertilization
IVP	<i>In vitro</i> production
I U	International Unit
Kg	kilogram
LH	Luteinizing Hormone
LN ₂	Liquid Nitrogen
m	meters
µg	Micro gram
ml	Milli litter
Min	Minutes
N	North
OG6	Ovsynch+GnRH on day 6
OG12	Ovsynch+GnRH on day 12
OPU	Ovum pick-up
PGF _{2α}	Prostaglandin F2 alpha
P/ET	Pregnancy Per Embryo transfer
PRID	Progesterone Releasing Intra Vaginal Device
SE	Standard error
SPSS	Statistical Packages for Social Sciences
sec/s	seconds
TAI	Timed Artificial Insemination
TET	Timed Embryo Transfer
TET-F	Timed Embryo Transfer Fresh
TET-V	Timed Embryo Transfer vitrified
vs	Versus

DECLARATION

I, **MOHAMMED SALMAN (I.D. No. RVM/2019-025)**, hereby declare that the thesis entitled **“ESTRUS SYNCHRONIZATION OF RECIPIENT COWS OF FARMERS FOR TRANSFER OF *INVITRO* PRODUCED SAHIWAL EMBRYOS”** submitted to P.V. Narsimha Rao Telangana Veterinary University for 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 submitted for any other degree or diploma.

Date:

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Place: Hyderabad.
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ACKNOWLEDGEMENTS

*It is a great privilege to express my deep sense of gratitude to my major advisor and chairperson of the advisory committee **Dr. K. Ram Chandra Reddy**, Professor and Head, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science, korutla, Hyderabad. for his valuable guidance and help offered during the conduct of research work and presentation of thesis I consider myself truly fortunate to work under his guidance.*

*I express my profound sense of gratitude to my minor guide **Dr. K. Venkata Ramana**, Professor, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science, Rajendranagar, Hyderabad. His cooperation, critical judgement and motivated me throughout the course of this investigation. I sincerely thank him from the inner core of my heart.*

*I express my deepest sense of gratitude to **Dr. B. Swathi**, Professor and University Head, Department of Veterinary Physiology, College of Veterinary Science, Mamnoor. for her timely help, wholehearted support.*

*I feel inadequacy of words to express my deep sense of gratitude to **Dr. K. Chandrashekar Reddy**, Professor and University Head, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science, Rajendranagar, Hyderabad. for his valuable suggestion and kind cooperation during my course of work.*

*I am extremely grateful to **Dr. G. Aruna kumari**, Associate Professor, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science, korutla, Jagyital. for his timely help in carrying out the research work and their constant encouragement and support throughout my research work.*

*I am very much thankful to **Dr. Gopala krishna** and **Dr. Ch. Siva Naga Pavani**, Assistant Professor Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science, Rajendranagar, Hyderabad. Her willingness to render all possible help and her valuable suggestions and timely help and guidance throughout the M.V.Sc. programme.*

*No words can express indebtedness to my mother **Shameem Begum**, Father **Mohammed Ghouse**, sister **Yasmeen Begum** and brothers **Mohammed Imran** and **Mohammed Irfan** for their incomparable love, support and affection which enabled*

Mohammed Irfan for their incomparable love, support and affection which enabled me to reach up to this step. without which I would not have been what I am today. I am always indebted to the Almighty for his fullest grace and provided these relations to me in this birth. I also extend my sincere thanks to my sister-in-law *Afreen Begum* for her help and kindness.

With a special mention, I personally place my deep sense of heart gratitude, satisfaction and pride to the very polite, kind and cultured Associate Dean, *Dr. D. Pramod Kumar*, Professor, College of Veterinary Science, Hyderabad.

I am very much thankful to *Dr. L. Atchamamba* and Library Staff for their help during my PG programme.

I am also grateful to the College of Veterinary Science, Rajendranagar, P.V. Narsimha Rao Telangana Veterinary University for providing the facilities to fulfil my research work.

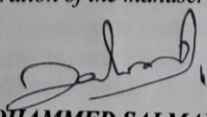
I thank my seniors, *Thota Srinivas Rao, K. Viswanath, V. Sandeep Reddy* and *Sai Prasad* and I thank my colleagues, *K. Urmila, R. Vinay Kumar* and *Anusha* for their constant support.

I thank *Dr. Srinivas Reddy, Dr. Satish, Dr. Nidhi shri, Dr. Athiti lokavya Reddy, Dr. Shaz Murtuza, Dr. Pranay, Dr. Vivek, Isla Uddin* and my juniors, *Sachin, Vinay, Abhijeet, Rakesh, Talib* and *karan* for their willing help during my research.

I am delighted to have friends *Abdul Aziz, Abdul Rouf, khadeer, Sami, Altaf, Fasi, syed Jawad Ali, Osman, Mohammed Afroz, Dr. Ayesha, Dr. Abdulla, Aijaz, D r. Zayeem, Dr. Azeem, Dr. Mohi uddin, Dr. Rajshekar, Dr. Shahriyar, Dr. Sravani* and *Dr. Anjana* who stood by me throughout my M.V.Sc programme.

I would like to extend my thanks to *Mogulamma, Anjaneyulu, Krishna* and *Ramesh* Non-teaching staff, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science, Hyderabad and korutla.

I place on record my apology and sincere thankfulness to the unmentioned personalities who have played a role in this study and preparation of the manuscript.


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Degree to which it is submitted	:	MASTER OF VETERINARY SCIENCE
Faculty	:	VETERINARY SCIENCE
Department	:	VETERINARY GYNAECOLOGY AND OBSTETRICS
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Year	:	2022

ABSTRACT

India is a home to 11.45 percent of the total world livestock population. India has a total livestock population of 535.78 million, out of which 192.49 million are cattle (Livestock census, 2019). India has total 37 registered pure indigenous cattle breeds including Sahiwal, Gir, Red Sindhi, Tharparkar, Rathie etc. (Swaroop, 2020). The Sahiwal is one of the best cattle breeds in India and Pakistan due to its unique traits like heat-tolerant, tick-resistant, high milk production and resistance to parasites (both internal and external). Indigenous and nondescript cattle make up 139.82 million of the 192.49 million cattle in India. The entire Indigenous (both descript and nondescript) cattle population has decreased by 6% since the previous census (Livestock census, 2019). This decrease emphasises the need of putting in place long-term conservation measures.

Embryo transfer technology is the most widely applied reproductive technique to rapidly multiply animals with desirable genetics (Bo *et al.*, 2014). Bovine *in vitro* embryo production (IVEP) is now a well-established and efficient procedure (Brackett and Zuelke, 1993). Moreover, ovum pick-up (OPU) at frequent intervals in

combination with *in vitro* fertilization (IVF), has improved and increased the yield of embryos from designated donors (Garcia and Salaheddine, 1998). IVEP in combination with synchronization and ET can play important role in faster multiplication of superior germplasm to ensure conservation of cattle breeds and increase in milk production.

In this study the selected recipient cows were randomly distributed into 3 groups. Group 1 (ovsynch) cows of this group were estrus synchronized by using ovsynch protocol 10 μ g of GnRH (Buserelin acetate-Receptal on day 0, 500 μ g of cloprostenol (Estrumate) on day 7 and second dose of GnRH on day 9 given IM). Group 2 (CIDR) cows of this group estrous synchronized by using CIDR protocol (day 0 insert CIDR, day 7 CIDR remove and PGF₂ α 500 μ g of cloprostenol (Estrumate) given IM). Group 3 (control) cows in natural estrus were included in control group.

Embryo transfers were done into these recipient cows by using nonsurgical method after day 6-7 of estrus by depositing the embryos in uterine horn (cranial or middle third) on ipsilateral side of ovary having CL. After 30 to 40 days of the transfer, pregnancy was confirmed by rectal palpation and transrectal ultrasonography. The estrus synchronization efficacy was recorded based on estrus response, onset of estrus, duration of estrus, intensity of estrus and conception rates.

The estrus response rates were (13/15) 86.66, (10/10) 100.00 per cent, in treated groups (Ovsynch, CIDR) and (15/27) 55.55 per cent in control group. Statistical analysis revealed that there was significant difference ($P < 0.05$) between treated groups (ovsynch, CIDR) and control group in the present experiment.

The mean duration of onset of estrus was recorded as 53.69 \pm 1.90, 49.1 \pm 0.48 and 109.73 \pm 18.74 in Ovsynch, CIDR, and control groups, respectively in the present experiment. The onset of estrus has no significant ($P > 0.05$) difference between ovsynch and CIDR treated group, but both CIDR and ovsynch group were significantly ($P < 0.05$) different with control group.

The overall intensity of estrus was 15.79, 44.74 and 39.47 per cent, as weak, normal or intermediate and intense, respectively. The Ovsynch study group cows exhibited 7.69, 61.53 and 30.76 percent as weak, normal and intense estrus, respectively. The CIDR study group cows exhibited 0, 30, and 70 per cent as weak, normal and intense estrus, respectively.

The mean score for estrus intensity were observed as 13.38 \pm 0.94, 16.30 \pm 1.59 and 11.07 \pm 1.05 in ovsynch, CIDR, and control groups, respectively. The statistical analysis revealed that there was significant difference ($P < 0.05$) in mean estrus score between CIDR and control group, but CIDR and ovsynch group has no significant difference ($P > 0.05$). Similarly, there was no significant difference ($P > 0.05$) between ovsynch and control group.

The mean duration of estrus recorded was 21.61 \pm 0.77, 20.30 \pm 0.72, and 20.47 \pm 0.70 hrs in ovsynch, CIDR and control groups, respectively in the present experiment. Statistical analysis revealed that there was no significance difference ($P > 0.05$) observed in mean duration of estrus in Ovsynch, CIDR and control groups.

The overall conception rate in therapeutic cows were observed as 0, 14.28 and 13.3 percent in Ovsynch, CIDR and control groups, respectively. There was no

significant ($P>0.05$) difference found among the treated groups and control group. The overall conception rate was more in CIDR group followed by control group, respectively.

The average mean BCS was 2.80 ± 0.48 , 3.04 ± 0.08 , and 2.89 ± 0.37 in Ovsynch, CIDR and control groups, respectively. In the present study there was positive correlation ($P<0.05$) found between BCS and conception rate. The mean BCS was significantly higher in cows that conceived (3.10 ± 0.21) than in those failed to conceive (2.87 ± 0.28) in non-pregnant cows.

In present study, there was significance ($P<0.05$) difference found between site of uterine horn where embryo was deposited and conception rate. The higher conception rate is found in recipients where embryos were deposited at cranial third of the uterine horn when compared to embryo deposited at middle third of uterine horn ($P<0.05$).

The average mean diameter of CL in recipient cows was 14.11 ± 0.39 , 15.63 ± 1.80 , and 13.99 ± 0.95 in Ovsynch, CIDR, and control group of study group respectively. In the present study group, there was no correlation ($P>0.05$) found between CL diameter and conception rate of recipient cows. The mean CL diameter was 16.18 ± 1.60 (14.45-19.35) mm in pregnant cows and 14.19 ± 0.54 (10-23) mm in non-pregnant animals

In the present study it was determined that cervix transfer scores had no statistical ($P>0.05$) effect on conception rates. However, it was also found that the probability of pregnancy was higher when the cervix transfer score was easy and moderate when compared to the difficult.

From the present study it was concluded that among all treated group, CIDR protocol was most effective and efficient synchronization protocol for improving the efficacy of synchronization and conception rates in embryo transfer programme. Further, we have to standardized the estrus synchronization protocol and time of embryo transfer (in days) from onset of estrus to improve the overall portion of suitable recipients and conception rate through ET.

INTRODUCTION

CHAPTER I

INTRODUCTION

India is a home to 11.45 percent of the total world livestock population. India has a total livestock population of 535.78 million, out of which 192.49 million are cattle (Livestock census, 2019). India has total 37 registered pure indigenous cattle breeds including Sahiwal, Gir, Red Sindhi, Tharparkar, Rathie etc. (Swaroop, 2020). The Sahiwal is one of the best cattle breeds in India and Pakistan due to its unique traits like heat-tolerant, tick-resistant, high milk production and resistance to parasites (both internal and external). Indigenous and nondescript cattle make up 139.82 million of the 192.49 million cattle in India. The entire Indigenous (both descript and nondescript) cattle population has decreased by 6% since the previous census (Livestock census, 2019). This decrease emphasises the need of putting in place long-term conservation measures.

Embryo transfer technology is the most widely applied reproductive technique to rapidly multiply animals with desirable genetics (Bo and Mapletoft, 2014). It is also used for genetic testing of bulls, disease control, shortening generation interval, improving fertility in repeat breeder cows (Seidel and Seidel, 1991 and Mapletoft and Bo, 2016), heat stress cows (Putney *et al.*, 1989; Ambrose *et al.*, 1999; Rodrigues *et al.*, 2004, 2007a, 2007b, 2011 and Baruselli *et al.*, 2010) and those diagnosed as repeat breeders (Rodrigues *et al.*, 2007b; 2010; Dochi *et al.*, 2008; Ferreira *et al.*, 2011 and Stewart *et al.*, 2011). In Embryo transfer (ET) programme female of superior genetic merit are super ovulated with gonadotropic hormone, their eggs were fertilized *in vivo* or oocyte fertilization *in vitro*, then resultant embryo will be transferred to recipients (Hafez and Hafez, 2013). Basic requirement for any ET

programme is a) source of embryo b) reliable method of transferring the embryo c) suitable synchronized recipients (prolonging / reducing luteal phase or modifying ovarian follicular wave with GnRH) (Hafez and Hafez, 2013). The embryo for transfer is either produced *in vivo* or *in vitro* produced (IVP) are either transferred fresh or cryopreserved.

Bovine *in vitro* embryo production (IVEP) is now a well-established and efficient procedure (Brackett and Zuelke, 1993). Moreover, ovum pick-up (OPU) at frequent intervals in combination with *in vitro* fertilization (IVF), has improved and increased the yield of embryos from designated donors (Garcia and Salaheddine, 1998). In embryo transfer programs, there are many factors which has been shown to influence pregnancies per embryo transfer (P/ET) (Hasler, 2014 and Bo *et al.*, 2018). Although many factors influence the outcome of embryo transfer, estrus synchronization of recipient females is a vital component of a successful programme, as recipient who express estrus have greater pregnancy rates after embryo transfer (Baruselli *et al.*, 2003 and Bo *et al.*, 2018). Moreover, the number of receivers eligible to receive an embryo is affected by estrus expression following a synchronization process, as those who do not express estrus can be removed from the pool of possible candidates (Bonaker *et al.*, 2020). To achieve estrus synchrony, various hormonal protocols were used to increase the proportion of suitable recipients to receive embryos and improve the efficiency of IVEP programs (Marinho *et al.*, 2012). Moreover, the recipient synchrony with respect to the day of IVEP has been related to the success of pregnancy in cattle (Hasler., 2004 and Chebel *et al.*, 2008).

IVEP in combination with synchronization and ET can play important role in faster multiplication of superior germplasm to ensure conservation of cattle breeds and increase in milk production. The treatment protocol that uses GnRH

(gonadotropin-releasing hormone) and Prostaglandin F₂ alpha (PGF₂α) for fixed-time AI in cattle is called as Ovsynch (Pursley *et al.*, 1995) which consist of an injection of GnRH followed by PGF₂α 7 days later and second injection of GnRH 48 to 56 hours(hrs) after PGF₂α treatment with fixed time A.I (artificial insemination) 16 hrs later. Ovsynch protocol has been used to synchronize ovulation in recipients for embryo transfer which received *in vivo* (Hinshaw, 1999 and Baruselli *et al.*, 2000) or *in vitro* (Ambrose *et al.*, 1999) produce embryos. The overall proportion of recipients pregnant over those synchronized was higher in GnRH based protocol. Synchronization of estrus with progestogens maintains high levels of progesterone in the female system, even after the regression of the corpus luteum. Synchrony of estrus occurs 2 to 5 days following progestin removal. Commercial products that fall into this category are melengesterol acetate (oral feeding), Syncro-Mate-B (Ear Implant) and CIDR (Controlled Internal Drug Release) Intra-vaginal device. The CIDR (contains 1.38 g progesterone) Cattle Insert provides an exogenous source of the hormone progesterone during the 7-day administration period. Removal of the CIDR results in a rapid fall in plasma progesterone levels and sudden surge of gonadotropins causing development of graafian follicle, occurrence of estrus and ovulation. This CIDR used in combination of PGF₂α where in CIDR mimic as source of progesterone and on sudden withdrawal induces the estrus, PGF₂α given on day 7 along with CIDR removal causes regression of CL (corpus luteum). The advantage of CIDR plus PGF₂α treatment when compared to single PGF₂α injection was that CIDR plus PGF₂α could increase the synchronization rate by approximately 30% and pregnancy rate by approximately 20% in both the anestrous and cyclic females (Bo *et al.*, 2012). CIDR protocol has been used to synchronize ovulation in recipients which received *in vitro* (Sala *et al.*, 2016 and Do *et al.*, 2018) produce embryos. After estrus

synchronization, suitability of recipients is assessed by the presence of functional CL on ovaries on the day of embryo transfer and ET will be done on the ipsilateral side of uterine horn of the ovary containing functional CL (Looney *et al.*, 2006). Transfer of embryos to estrus-synchronized recipient cows was most effective when embryos were transferred 6–8 days after the observed estrus (Bo *et al.*, 2002), in order to provide the same conditions such as uterine environment and proper plasma progesterone levels for the embryo to assure pregnancy (Looney *et al.*, 2006). Before the ET, embryo was assigned a developmental stage and quality grade in accordance with the guidelines of the International Embryo Technology Society (Stingfellow and Seidel, 1998) standards. For better conception, IVF Embryo quality should have stage ranged in 4 to 7 and they should belong to grades of 1 or 2 (Bo and Mapletoft, 2018).

Most of the IVEP work done in exotic cattle breeds like Holstein Friesian (HF), Jersey and other breeds in foreign countries. There is no literature available on *in vitro* embryo transfer work particularly in indigenous breeds and estrus synchronization protocols used for IVF embryo transfer in India. Hence the work was taken up to the estrus synchronization of recipient cows for the IVF embryo transfer by using Ovsynch and CIDR plus PGF₂ α protocols with the following objective:

1. To monitor the estrus characters and CL quality in both natural estrus and synchronized estrus cows.
2. To study the conception rates of *in vitro* produced embryos in cows subjected to different estrus synchronization protocols.
3. To study the conception rate of *in vitro* produced embryos in natural estrus cows.

***REVIEW OF
LITERATURE***

CHAPTER II

REVIEW OF LITERATURE

2.1 HISTORY OF EMBRYO TRANSFER

The first successful embryo transfer (ET) of mammalian embryos was done by Walter Heape in 1890 (Betteridge, 2003), later Warwick and colleagues did considerable work on ET in sheep and goats in the 1930s and 1940s (Betteridge, 1981; Betteridge, 2003). In 1949, Umbaugh reported first successful ET in cattle but all pregnancies were terminated before full term (Betteridge, 1981). In 1951 first ET calf was born in Wisconsin, United States of America (U.S.A.) after surgical transfer of abattoir derived day 5 embryos (Willett *et al.*, 1951 and Betteridge, 1981) and in 1981 first IVF calf “VIRGIL” was born in USA (Bracket *et al.*, 1982).

In India, first ET was done at National Institute of Immunology, New Delhi in 1987 and later in 1990, first buffalo calf “PRATHAM” produced by using this technology in National Dairy Research Institute, Karnal (Madan *et al.*, 1991 and Nain *et al.*, 2006). In 2007, Dr. Zavar produced the first calf of Tharparker breed (Purohit, 2018). In India, Ovum Pick-up Unit (OPU) followed by *in vitro* Fertilization (IVF) and development of embryo up to Blastocyst was reported by Verma (2005) in Bovine. The first OPU- IVF in aged Sahiwal cattle done at National Dairy Research Institute, Karnal, 2012 and produced Sahiwal calf “HOLI” (Moore and Hasler, 2017 and Purohit, 2018).

2.2 HISTORY OF ESTRUS SYNCHRONIZATION

The initial estrus synchronization treatment regimen for ET included administration of PGF₂ α to induce luteolysis, however, the lack of efficiency in synchronizing the time of estrus and large cost of recipient maintenance limited widespread application and success of ET (Bo *et al.*, 2002). Subsequently, gonadotropin-releasing hormone (GnRH), estradiol, progestogen-releasing devices

and $\text{PGF}_2\alpha$ have been used to synchronize timing of ovarian follicular and luteal dynamics as well as ovulation among females, enabling transfer of embryos at a fixed time without estrus detection (Bo *et al.*, 2002 and Baruselli *et al.*, 2010, 2011).

2.3 GnRH-BASED SYNCHRONIZATION SYSTEM

Synchrony of estrus and fertility with a combination of GnRH and prostaglandin are good for cyclic females as this combination induces cyclicity in cows experiencing postpartum anoestrus (Pursley *et al.*, 1995). The most recently developed synchronization treatments combine traditional methods of controlling cycle length with the manipulation of follicular development in order to "program" or "select" the ovulatory follicle. The Ovsynch program is comprised of an injection of GnRH on day 1, an injection of prostaglandin on day 8, a second injection of GnRH on day 10 and then timed insemination on day 11 (Pursley *et al.*, 1995). Therefore, a new group of follicles appears in the ovaries within 1 to 2 days after the first injection of GnRH (Vasconcelos *et al.*, 1999). From that new group of follicles, a newly developed dominant follicle emerges, matures, and can ovulate after estrus is induced by $\text{PGF}_2\alpha$ or it can be induced to ovulate after a second injection of GnRH. The GnRH injections release pituitary luteinizing hormone (LH) which will cause ovulation and occurrence of estrus in cows.

2.4 PROGESTERONE + $\text{PGF}_2\alpha$ SYSTEM

A recent generally available and successful estrus synchronization protocol is a combination of the hormone progesterone in the form of a controlled internal drug release (CIDR) device along with $\text{PGF}_2\alpha$ because it is effective in inducing estrus in suckling beef cows, heifers at a younger age (Kajaysri *et al.*, 2017). The exogenous intravaginal impregnated CIDR for 7 to 12 days will induce negative feedback in the hypothalamus and pituitary and inhibit the release of gonadotropin hormone

(Macmillan and Burke, 1996). After CIDR removal, larger quantities of gonadotropin are released to stimulate development of follicles, release the hormone estradiol, resume estrus and synchronize ovulation or to luteinize large dominant follicles (Cerra *et al.*, 2009). The PGF₂ α administration at the time of CIDR withdrawal can regress the functional corpus luteum (CL), reduce progesterone concentration levels and successfully induce the growth of follicles and estrus (Odde, 1990 and Larson and Ball, 1992). The T-shaped CIDR device is inserted intravaginally with an applicator and releases progesterone, which is absorbed into the blood circulation quickly (Mapletoft *et al.*, 2003). On the end opposite the CIDR wing there is an attached tail which hangs outside the animal making it possible to easily remove the inserted device after treatment (Mapletoft *et al.*, 2003). Thus, estrus synchronization with the CIDR is simpler than other progesterone techniques such as the ear implantation method.

2.5 ESTRUS RESPONSE RATE

Eighty-eight HF heifer were randomly designated for 7, 12, 14 days of CIDR insertion period, the overall estrus response rate recorded was 90.7 per cent, specifically the estrus incidence rate observed was 86.8 per cent and 93.3 per cent in 7 and 12 days CIDR protocol while studying efficacy of CIDR device in inducing estrus synchronization in heifer, respectively by Vargas *et al.* (1994).

Ambrose *et al.* (1999) reported estrus induction rate of 76.20 per cent using Ovsynch protocol in dairy cattle.

Lucy *et al.* (2001) observed the estrus response rate of 59 and 84 per cent in post-partum beef cow and dairy heifer respectively, using CIDR and PGF₂ α protocol.

Al katani *et al.* (2002) observed 67.70 per cent of estrus response rate using Ovsynch protocol in lactating dairy cows.

Cavalieri *et al.* (2003) reported 91.67 per cent of estrus response rate using intravaginal progesterone releasing insert in Holstein cows.

Wilson *et al.* (2010) observed the estrus response rate of 85 per cent in Cosynch+CIDR protocol in post-partum beef cows, respectively.

Murugavel *et al.* (2010) observed 81.82 per cent estrus response rate using progesterone-based estrus synchronization protocol in buffaloes, respectively.

Navrange *et al.* (2012) reported 100 per cent display of estrus in buffalos synchronized with Ovsynch protocol with injection of GnRH (10 micro gram(μ g), Intramuscular (I/M) on day 0 and 9 with injection of PGF₂ α (2ml I/M) on day 7 of Ovsynch protocol.

Jyothi *et al.* (2012) reported 97.30 per cent of estrus response rate using CIDR+PGF₂ α protocol in postpartum crossbred cows.

Caraba and Velicevici (2013) observed estrus response rate in dairy cows synchronized with Ovsynch and Co-synch protocol was 63 and 57 per cent.

Velladurai *et al.* (2014) selected sixteen normally calved cows at 35 to 50 days post-partum and divided into two groups as group I and II. Group I were treated with Ovsynch protocol between 50 and 65 post-partum and group II as control, the estrus response rate in group I and II recorded was 100 and 62.5 per cent, respectively.

Dhami *et al.* (2015) observed 100 per cent estrus induction rate by using CIDR, Ovsynch, and Norgestomet ear implant protocols in anestrus crossbred cows and 30 per cent estrus response rate in untreated (natural estrus crossbred cows).

Buhecha *et al.* (2015) reported all cows (100 per cent) exhibited estrus when treated with Triu-B or PRID (Progesterone releasing intra vaginal device), Ovsynch and Heat-synch estrus synchronization protocol.

Ahmed *et al.* (2016, a) observed 100 per cent estrus response rate in cross bred cows treated with Ovsynch and Ovsynch based GnRH treatment.

Linga Swamy (2017) reported estrus response rate in cross bred cows as 90 per cent (Ovsynch), 100 per cent (Ovsynch+GnRH), 100 per cent (Ovsynch+hCG (human chorionic gonadotropin)) and 30 per cent in control groups (natural estrus, respectively).

Doodram (2018) observed estrus response rate in crossbred cows treated with Ovsynch protocol was 100 per cent and control group was 40 per cent, respectively.

Ahmed *et al.* (2016, b) reported 100 per cent cows come to estrus after treatment with Ovsynch and Ovsynch based GnRH treatment.

Borakhatariya *et al.* (2017) observed estrus response rate of 80 and 100 per cent, respectively in summer and winter using Ovsynch protocol in anoestrus crossbred cows.

Haider *et al.* (2017) reported estrus response rate of 82.5 per cent using CIDR+ PGF₂ α protocol in non- descript cows of the Punjab.

Hirole *et al.* (2018) observed the estrus response rate using PGF₂ α and Ovsynch protocol as 100 and 83.33 per cent while studying the efficacy of double synch PGF₂ α and Ovsynch in post-partum dairy cows.

Amin *et al.* (2019) reported estrus synchronization rate with CIDR+PGF₂ α protocol as 80 per cent while studying influence on oxidant/antioxidant biomarker & mineral profile in summer stress anoestrous buffalo.

Ratnaparki *et al.* (2020) reported 100 per cent of estrus induction rate in Ovsynch in postpartum cross bred cows.

Raut *et al.* (2020) noted the estrus induction rate of 91.33 and 83.33 per cent in 7d CIDR+PGF₂ α and 9d CIDR+PGF₂ α protocol while studying comparative efficacy cyclicity induction in post-partum anestrous indigenous cows.

Devi *et al.* (2021) observed the estrus synchronization rate of 100 per cent in crossbred cows using CIDR plus PGF₂ α synchronization protocol.

2.6 ONSET OF ESTRUS

The onset of estrus was measured in hours (hrs) from the time of PGF₂ α treatment to the first appearance of estrus signs in treated cows and for control group of animals it is calculated from the day of selection to exhibition of estrus up to 10-day period.

Larson *et al.* (2006) synchronized the estrus in suckled beef cows using CIDR+PGF₂ α protocol and reported the mean time of onset of estrus as 52.8 \pm 09 hrs.

After PGF₂ α injection, Patil and Pawshe (2011) discovered that the average time required for start of estrus in cows (62.2 \pm 1.56 hrs) was much lesser than in heifers (64.66 \pm 1.79 hrs).

Jyothi (2011) observed 63.25 \pm 1.95 hrs of onset of estrus using CIDR+PGF₂ α protocol in postpartum crossbred cows.

Between day 50 and 65 postpartum cows, group I cows treated with the Ovsynch protocol, while group II served as control group (natural estrus). According to Velladurai *et al.* (2014), the mean delay between the commencement of estrus in group I and II was 47.97 \pm 2.65hrs and 23.50 \pm 4.24 days, respectively.

Buhecha *et al.* (2015) conducted a study to evaluate the clinical response and monitor the peripheral plasma progesterone profile in 46 days postpartum anestrous crossbred cows (>90 days) treated with Triu-B/ PRID, Ovsynch, and Heat synch. All

of the cows (100 per cent) in the three protocols showed estrus within mean intervals of 67.00 ± 1.74 , 69.83 ± 0.87 , and 68.17 ± 1.24 hrs after $\text{PGF}_2\alpha$ injection, respectively.

Thirty-two apparently healthy cyclic crossbred cows were randomly allocated into four groups, each consisting of eight animals: control (Group I), $\text{PGF}_2\alpha$ (Group II), $\text{PGF}_2\alpha \pm \text{GnRH}$ (Group III), and $\text{PGF}_2\alpha \pm \text{hCG}$ (Group IV). The duration of onset of estrus recorded were 70.50 ± 3.27 , 68.00 ± 3.17 , and 68.25 ± 2.21 hrs in Groups II, III, and IV, respectively (Senthilkumar and Chandrahasan, 2015).

Dagefa *et al.* (2016) reported onset of estrus 20.4 ± 1.8 hrs using CIDR+ $\text{PGF}_2\alpha$ protocol in cattle, respectively.

According to Ahmed *et al.* (2016), 24 repeat breeding crossbred cows were randomly assigned into four groups. Group I was kept as a control without therapy due to the design of the work groups. On the sixth day after estrus, Group II was given GnRH. The Ovsynch technique was used to treat Group III. GnRH therapy based on Ovsynch was used in Group IV. In the Ovsynch and Ovsynch based GnRH therapy groups, the mean onset of estrus recorded was 48.75 ± 0.71 and 51.47 ± 1.99 hrs, respectively. There was no difference in onset of estrus between Group I and Group II crossbred cows.

Haider *et al.* (2017) observed the mean onset of estrus time of 73.92 ± 1.79 hrs from $\text{PGF}_2\alpha$ injection and 49.92 ± 11.79 hrs from CIDR removal using CIDR+ $\text{PGF}_2\alpha$ protocol in non-descript cows of the Punjab.

Sauls *et al.* (2016) observed the mean onset of estrus time using CIDR (without CL) and CIDR (with CL) protocol as 25 and 27 hrs while studying expression of estrus in lactating dairy cows.

In anestrus crossbred cows, Ahmed and Doley (2017) found that the mean start of estrus was 48.80 ± 5.74 , 53.25 ± 3.02 , and 46.40 ± 4.33 hrs in treatment groups of Ovsynch, Ovsynch plus CIDR, and G6G ($\text{PGF}_{2\alpha}$, GnRH Day 2, Ovsynch from day 6), respectively.

According to Linga Swamy (2017), the mean onset of estrus in the Ovsynch, Ovsynch+GnRH, Ovsynch+hCG and control groups was 51.80 ± 1.07 , 50.90 ± 1.20 , 52.40 ± 1.32 and 96.00 ± 13.86 hrs, respectively.

Doodram (2018) recorded the mean onset of estrus as 49.7 ± 1.45 , 50.7 ± 1.15 , 52.4 ± 1.27 and 84.0 ± 15.49 hrs in Ovsynch, Ovsynch+GnRH on day 6 (OG6), Ovsynch + GnRH on day 12 (OG 12) and control groups, respectively in the present study.

Hirole *et al.* (2018) reported duration of onset of estrus as 50.67 ± 1.5 and 53.20 ± 1.8 hrs using $\text{PGF}_{2\alpha}$ and Ovsynch protocol while studying the efficacy of double synch $\text{PGF}_{2\alpha}$ and Ovsynch in post-partum dairy cows.

Shahid *et al.* (2019) observed the mean time for onset of estrus in heifer indigenous cattle synchronized using Ovsynch protocol as 46.56 ± 0.24 and 47.00 ± 0.51 hrs in natural estrus group.

Amin *et al.* (2019) observed estrus mean onset of estrus time with CIDR+ $\text{PGF}_{2\alpha}$ protocol as 3 ± 1.27 days while studying influence on oxidant/antioxidant biomarker & mineral profile in summer stress anestrus buffalo.

Ratnaparki *et al.* (2020) observed 54.60 ± 2.44 hrs mean onset of estrus using Ovsynch synchronization protocol in postpartum cross bred cows.

2.7 DURATION OF ESTRUS

Gustaffson *et al.* (1986) discovered that repeat breeding cows (31.50 ± 3.60 hrs) had a longer duration of estrus than virgin heifers (23.84 ± 2.00 hrs).

Albinn (1991) also discovered that repeat breeding heifers had a longer standing estrus period and more ovulatory disturbances than virgin heifers.

Tanabe *et al.* (1994) discovered that the length of estrus differed significantly between first breeding (18.2 hrs) and repeat breeding (20.2 hrs) cows. Estrus lasted anywhere between 8 and 32 hrs.

Portaluppi *et al.* (2006) and Krishna Kumar *et al.* (2008) reported that the duration of estrus in repeat breeding cows suffering from ovulatory disturbances was 28.90 ± 0.41 hrs.

In repeat breeding cows, Selvaraju *et al.* (2009) found that the length of estrus was 29.25 ± 0.70 hrs. However, Das *et al.* (2009) found that the average length of estrus in anovulatory, delayed ovulatory, and normal ovulatory repeat breeding cows was 25.20 ± 1.72 , 33.27 ± 1.56 , and 18.90 ± 1.05 hrs, respectively.

In crossbred cows, Anjum *et al.* (2009) investigated the duration of estrus in Group A (control) and Group B (Treated with GnRH analogue at the time of AI). In group A, the average estrus length was 15 hrs, ranging from 7 to 24 hrs. The average estrus length in group B was 15 hrs, with a range of 8 to 24 hrs.

Mengchao *et al.* (2010) reported the mean duration of estrus as 15.13 ± 3.52 , 15.44 ± 5.41 , and 18.30 ± 6.71 using Ovsynch, CIDR based Ovsynch and naturally estrus group in Japanese black cow.

According to Bhat and Battacharya (2012), the duration of the estrus cycle in repeat breeding cows with anovulation, delayed ovulation, normal ovulation with induction, and control groups was 26.33 ± 2.20 , 34.43 ± 3.67 , 18.43 ± 0.48 , and 18.71 ± 1.40 hrs, respectively.

Similarly, Velladurai *et al.* (2014) also stated that, the mean duration of estrus in cows when synchronized with Ovsynch was 29.84 ± 0.67 hrs.

The cyclic crossbred cows were separated into four groups, each with eight animals: control (Group 1), $\text{PGF}_2\alpha$ (Group II), $\text{PGF}_2\alpha + \text{GnRH}$ (Group III), and $\text{PGF}_2\alpha + \text{hCG}$ (Group IV). In the four groups, the duration of estrus was 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) found that the length of estrus in the control, GnRH therapy on day 6 post estrus, Ovsynch, and Ovsynch based GnRH treatment groups was 22.04 ± 0.94 , 21.45 ± 1.10 , 21.08 ± 0.78 , and 20.07 ± 0.86 hrs, respectively. The duration of estrus did not differ significantly across the groups.

On the other hand, Degefa *et al.* (2016) found that the average duration of estrus in crossbred cows was 21.9 ± 1.1 hrs using CIDR protocol.

Senthilkumar *et al.* (2017) found that utilising different hormonal treatment regimens, the length of estrus synchronised cows was considerably smaller than the control group in repeat breeding dairy cattle.

Sauls *et al.* (2016) observed the mean duration of estrus time using CIDR (Without CL) and CIDR (with CL) protocol as 11.8 ± 1 and 11.9 ± 1.3 hrs in lactating dairy cows.

According to Linga Swamy (2017), the mean duration of estrus in crossbred cows was 21.90 ± 0.69 , 22.30 ± 0.79 , 21.80 ± 0.73 and 21.40 ± 0.60 hrs in the Ovsynch, Ovsynch+GnRH, Ovsynch+hCG and control group, respectively.

Doodram (2018) observed the mean duration of estrus as 23.0 ± 0.70 , 22.7 ± 0.6 , 23.1 ± 0.64 and 21.0 ± 1.22 hrs Ovsynch, OG6, OG12 and control groups, respectively in crossbred cows.

Hirola *et al.* (2018) reported mean duration of estrus as 11.33 ± 0.49 and 21.2 ± 0.58 hrs using $\text{PGF}_2\alpha$ and Ovsynch protocol while studying the efficacy of double synch $\text{PGF}_2\alpha$ and Ovsynch in post-partum dairy cows.

Shahid *et al.* (2019) observed that the mean duration of estrus in heifer indigenous cattle synchronized using Ovsynch protocol was 18.63 ± 1.03 and 18.00 ± 1.58 hrs in natural estrus group

Ratnaparki *et al.* (2020) reported the mean duration of estrus in Ovsynch treated group as 22.80 ± 0.44 in post-partum crossbred cows. Reshma *et al.* (2020) observed the mean duration of estrus as 27.49 ± 0.36 hrs in 9d CIDR + $\text{PGF}_2\alpha$ treated group in repeated breeder cows, respectively.

Devi *et al.* (2021) reported the mean duration of estrus 26.30 ± 0.47 hrs in CIDR+ $\text{PGF}_2\alpha$ crossbred cows, respectively.

2.8 INTENSITY OF ESTRUS

Rao and kodagli (1983) and Singh *et al.* (1984) reported the prominent estrus symptoms such as 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 25 per cent (weak), 50 per cent (intermediate) and 25 per cent (intense) intensity of estrus, respectively in non-descriptive cows.

while the intensity of estrus in crossbred cows were recorded as 41 per cent (intense) and 58.33 per cent (intermediate).

Anjum *et al.* (2009) observed the intensity of estrus of 12 per cent, 41 per cent, and 47 per cent as weak, moderate and intense in GnRH treated crossbred cows, respectively. Whereas in untreated crossbred group intensity of estrus recorded was 16, 44 and 40 per cent as weak, moderate and intense, respectively.

Lu Meng Chao *et al.* (2010) recorded that intensity of estrus in synchronized group (GnRH and CIDR+GnRH) was higher in percentage than natural estrus cows while studying the difference in estrus intensity between natural oestrus and induced estrus.

Marugavel *et al.* (2009) reported intensity of estrus as 18.18 (weak), 18.18 (intermediate) and 63.64 (intense) per cent, respectively using CIDR+PGF₂ α protocol in buffaloes.

Jyothi (2011) observed the intensity of estrus as 0 (weak), 33.33 (intermediate) and 66.67 (intense) per cent using CIDR+PGF₂ α protocol in crossbred cows.

Velladurai *et al.* (2014) observed the intensity of estrus was higher in synchronized group of cows than natural estrus cows while studying pattern of induced oestrus and conception rate following ovsynch programme in post-partum dairy cows.

Senthilkumar and Chandrahasan (2015) observed that synchronized cows showed weak, normal and intense estrus as 8.33 per cent, 58.33per cent, and 33.33 per cent respectively, while studying estrus behaviour in natural and induced estrus using PGF₂ α with GnRH and hCG in dairy cattle.

Ahmed *et al.* (2016) reported weak, intermediate and intense estrus as 33.33, 33.3 and 33.3 per cent in GnRH treatment on day 6 post-estrus group, 16.67, 66.67, and 16.67 in Ovsynch group, 16.67, 50 and 33.33 per cent in Ovsynch based GnRH treatment group, 33.3, 50 and 16.67 per cent in control group, respectively in crossbred cows.

Linga swamy (2017) observed mean intensity of estrus in 50 post-partum crossbred cows were 8 (intense), 66 (normal), and 26 (weak) per cent, respectively using Ovsynch protocol.

Doodram (2018) observed the intensity of estrus of 17.5 per cent (intense), 55 per cent (normal) and 27.5 per cent (weak), while studying the effect of GnRH during early and mid-luteal phase in 40 post-partum crossbred cows.

Ratnaparki *et al.* (2020) observed intensity of estrus of 40 (intense), 40 (intermediate) and 20 (weak) per cent in post-partum cross bred cows treated using Ovsynch protocol.

Reshma *et al.* (2020) observed intensity of estrus as 0 (weak), 33.33 (intermediate), 66.66 (intense) per cent and 0 (weak), 38.46 (intermediate), 61.53 (intense) per cent in control and 9d CIDR + PGF₂ α group in repeated breeder cows.

Devi *et al.* (2021) reported the intensity of estrus as 10 (weak), 30 (intermediate) and 60 (intense) per cent using CIDR+PGF₂ α protocol, respectively.

2.9 SYNCHRONIZATION AND CONCEPTION RATE

2.9.1 Ovsynch

Ambrose *et al.* (1999) observed pregnancy rate in synchronized (Ovsynch +timed embryo transfer (TET) animals were slightly higher in fresh TET (TET-F)

(17.5±3.0), frozen TET (6.7±3.2), when compare to pregnancy rate in TET-F (14.3±2.3), TET-frozen (4.9±2.3) in normal cyclic animals.

Al katanani *et al.* (2002) reported pregnancy rate was higher for TET-F (26.7±6.4) than for the timed artificial insemination (TAI) (5.0±4.3) and TET-V (vitrified) (7.4±4.7) treatment using Ovsynch protocol.

Block *et al.* (2010) recorded that the percentage of recipient cows synchronized with Ovsynch protocol cows pregnant with fresh embryos were 56.3 (d 32), 52.6 (d 46), and 50.5 (d 76) per cent, also concluded that with fresh embryos pregnancy rate was higher when compared with TAI cows or cows that received vitrified embryos.

Stewart *et al.* (2011) reported that percentage of cows pregnant greater with synchronized recipient (ovsynch-protocol) for ET fresh (*in vivo*) (45.5 per cent), ET-V (31.6 per cent) and A.I (24.6 per cent) when compared to non-synchronized animals ET fresh (42.1 per cent), ET-V (29.3 per cent) and A.I (18.3 per cent), respectively

Erdem *et al.* (2020) reported that higher pregnancy rate with fresh embryo (*in vivo*) (code I embryo 44.15 and code II 32.58 per cent) transfer obtained by synchronization of recipients with Ovsynch protocol.

2.9.2 CIDR

Sala *et al.* (2016) recorded pregnancy after ET was 43.9 and 42.8 per cent with 1st use CIDR and 2nd use CIDR, respectively.

Sanches *et al.* (2016) reported that the conception rates at day 30 vs day 60 as 51.35±1.87 vs 43.24±1.23 per cent for the fresh embryos, 35.89±3.87 vs 31.19±4.01 per cent for the vitrified embryos and 40.19±4.65 vs 34.72±4.15 per cent for the frozen embryo transfer using 9-day CIDR+estrogen (E₂) protocol.

Do *et al.* (2018) observed pregnancy rate at day 35 vs day 60 as 41.3 vs 37.6 per cent for fresh embryo, 40.0 vs 40.0 per cent for vitrified embryo using 7day CIDR+PGF₂ α protocol.

Bonacker *et al.* (2020) reported conception of 34 per cent among recipient beef cow using 7day co-synch + CIDR protocol while studying the 7 and 7 synch (PGF₂ α coincident with CIDR insertion on Day 0, GnRH on Day 7, and PGF₂ α coincident with CIDR removal on Day 14) and 7d Co synch + CIDR protocol effect on ET.

Steichen and Larson (2019) reported overall pregnancy as 27.5 per cent and confirm pregnancy as 23.3 per cent in recipient animal synchronized with 7day Co-Synch+CIDR protocol for transfer of invitro produce frozen thaw embryo in dairy heifer cows.

2.10 EFFECT OF BODY CONDITION SCORE (BCS) ON CONCEPTION RATE IN IVF-EMBRYO TRANSFER (ET).

Mapletoft *et al.* (1986) reported body condition influence pregnancy rate whether embryo transfer surgically or non-surgically. The pregnancy rate higher was seen for BCS 3 (55 per cent) and 2 (53 per cent) when compare to recipient with BCS 1 or lower (44 per cent) and female with BCS 4 or higher (47 per cent) has intermediate effect on pregnancy.

Ambrose *et al.* (1999) observed there was an association between body condition and pregnancy rate ($P < 0.01$), with approximately a 37 per cent increase in pregnancy projected for a whole unit increase in BCS.

Al katanani *et al.* (2002) observed that pregnancy rate was higher ($P < 0.05$) in cows with $BCS \leq 2.5$ when compare to cow with $BCS \geq 2.5$. The least square mean \pm S.E.M for cows with $BCS \leq 2.5$ were 8.2 ± 6.4 , 20.3 ± 10.2 , and 10.7 ± 7.5 for TAI

(timed artificial insemination), TET-F and TET-V. the least square means \pm S.E.M for cows with BCS \geq 2.5 were 5.5 ± 7.2 , 6.2 ± 8.0 and 3.9 ± 6.5 in TAI, TET-F, AND TET-V.

Wallace *et al.* (2011) reported that recipients having BCS >5 on scale of 1(emaciated) to 9(obese) at time of ET (fresh or frozen) have greater pregnancy rate than recipients having BCS ≤ 5 .

Pereira *et al.* (2016) observed the effect of BCS (P = 0.05) on P/ET (Pregnancy per ET). The recipient having BCS < 2.75 has lesser pregnancy rate (34.6 per cent) when compared to the recipient with BCS > 2.75 having greater pregnancy rate (39.6 per cent).

Steichen and Larson (2019) observed there was no effect (P <0.05) of BCS on P/ET of dairy heifer recipients after transfer of frozen thawed embryos.

Reshma *et al.* (2020) reported the mean BCS was significantly (P <0.01) higher in cows that were conceived (2.96 ± 0.06) than in those fails to conceive (2.68 ± 0.06).

2.11 EFFECT OF EMRBYO DEPOSITION SITE IN UTERUS ON CONCEPTION RATE

Beal *et al.* (1998) reported higher pregnancy rate with deeper transfer than with shallow transfer regardless of embryo grade.

Previous studies reported that the embryo transfer to the cranial third of the embryo during the ET increased the pregnancy rate (Beal *et al.*, 1998; Scenna *et al.*, 2005).

There was increased in pregnancy rate seen when embryo deposited at cranial third of uterine horn by Bo *et al.* (2018) and Roper *et al* (2018), respectively.

Alkan *et al.* (2020) reported that there was significance difference ($P < 0.05$). observed in embryo deposition site in uterus (cranial or middle third of the uterine horn) and conception rates. It was observed that the region where the embryo was placed in the uterus had an effect on the pregnancy rates (cranial 41.06 and middle 29.67 per cent).

2.12 EFFECT OF CL SIZE AND QUALITY ON CONCEPTION RATE IN ET

Spell *et al.* (2001) reported there was no significant ($P > 0.05$) difference in mean CL diameter among recipient animals that did or did not become pregnant.

Looney *et al.* (2006) observed there was no differences in pregnancy results as the CL increases in size above 10 milli meter (mm) in diameter and earlier reports fail to indicate any correlations between CL size and pregnancy rates (Bo *et al.*, 2001; Hasler *et al.*, 1987; Remsen *et al.*, 1982 and Coleman *et al.*, 1987).

There was no difference in pregnancy rate observed when embryo transfer done to recipients with different CL size by Bo *et al.* (2001), Hasler *et al.* (1987), Remsen and Roussel (1982) and Coleman *et al.* (1987), respectively.

Alkan *et al.* (2020) determined that diameter and type of CL had an effect on the pregnancy rates after embryo transfer. It was observed that the pregnancy rate increased as CL diameter increased.

Many studies have also reported that recipient cows with larger CL have higher pregnancy rates after ET (Ambrose *et al.*, 1999; Baruselli *et al.*, 2010 and Vieira *et al.*, 2014).

Yoshida *et al.* (2012) reported that cows with a functional and larger CL after A.I are more likely to become pregnant.

However, that CL diameter does not have any effect on pregnancy rates as recorded by Spell *et al.* (2001) and Benyei *et al.* (2006).

Erkan *et al.* (2021) observed that the size of the CL has no effect on the conception of cows utilized as recipient in ET. The recipient cows were divided into three groups (CL2+ = >15-20 <mm, CL3+ =>20-25<mm, CL4+ =>25mm) based on CL size. There was no significance difference observed between groups ($P>0.05$) on conception.

2.13 EFFECT OF DURATION TAKEN IN FEMALE GENITALIA FOR ET ON CONCEPTION RATE

Jaskowski *et al.* (2010) observed that if the time needed to insert embryo into the uterus was 10-60 second (sec), the conception rate was 54.3 per cent whereas if the time needed to insert embryo into the uterus was longer than 60 sec, the conception rate was 20.4 per cent.

Aguiar *et al.* (2013) and Lopes *et al.* (2015) reported that the pregnancy rates in cows with difficult cervical passage (>80 sec) during embryo transfer was lower compared to those with easy cervical passage (< 60 sec).

In a previous study with Angus cross beef cows, it was reported that pregnancy rates were lower after ET when it was difficult to perform (transfer with maximum difficulty, with extreme manipulation of the genital tract and with embryo deposited in the upper half of the uterine horn) (Kasimanickam *et al.*, 2018).

Roper *et al.* (2018) reported that longer duration of the transfer had a negative effect on pregnancy rate and that the pregnancy rate decreased especially when the transfer took longer than 14 minutes.

Alkan *et al.* (2020) observed no statistical effect ($P<0.05$) on conception rate, but it was found that probability of pregnancy higher when cervix transfer score was easy (41 per cent) and moderate (34 per cent) when compare to difficult (30.3 per cent).

***MATERIALS AND
METHOD***

Chapter III

MATERIALS AND METHODS

3.1 EXPERIMENTAL LOCATION

The current study was carried in the livestock farm complex, College of Veterinary Science, Korutla, Jagtial district, Telangana (latitude: 18° 49' 36.71"N; longitude: 78° 42' 50.39"E; altitude: 295.99 m above sea level) between August, 2021 to March, 2022. The climate in the region was semi-arid to sub humid, with hot summer and moderate winter. The average annual rainfall was 1375 mm with temperatures ranging from 13 degree Celsius (°C) in January to 45 °C in May/June.

3.2 EXPERIMENTAL ANIMALS

3.2.1 Donor

Sahiwal cows (*Bos indicus*) aged 3 to 6 years and weighing between 250 and 450-kilogram (kg) body weight were selected as oocyte donors through Ovum Pick-Up (OPU). The daily ration of each animal consists of 2 to 3Kg concentrate mixture of high protein feed containing 20% digestible crude protein (DCP) and 70% total digestible nutrient (TDN), 8 to 12 kg chopped green fodder and 3 to 5 Kg paddy straw. Ad libitum water was given throughout the study period. Animals were maintained under hygienic and optimum management conditions in loose housing system with a large, open paddock for free movement. Vaccination protocols were followed as per standard schedule.

3.2.2 Recipient

The selected cows were dewormed using bolus Fenbendazole (Panacur 3.0 Intervet India Pvt. Ltd. Bhiwandi, each bolus contain 3.0 gram (g) of fenbendazole). They were also treated once initially with I/M injection of inorganic phosphorous

(Tonoricin, Vetoquinol India Animal Health Pvt Ltd, Thane. Each ml contains 0.2 g of sodium salt of 4-dimethylphenyl- phosphinic acid) and vitamin inj.AD₃E (VITADE , Zydus Animal Health and Investment Ltd; each ml contains Vitamin A: 250,00 I.U, Vitamin, D₃:25,000 I.U, Vitamin, Vitamin E:100 I.U), and supplemented with mineral mixture for 15 to 20 days before initiating the treatments. Treatment protocol (Ovsynch and 7day CIDR+PGF₂α) were initiated regardless of the stage of the estrous cycle of the selected cows for estrus synchronization.

3.3 BLOOD SAMPLES

Blood samples from recipient cows were collected aseptically from left jugular vein into vacutainer tubes (4ml, L-TUBE, clot activator, LEVRAM LIFE SCI ENCES, SILVASSA, INDIA). Blood samples were stored at 4 °C for ~6 h and centrifuged at 1500 × g for 20 min and separated serum was sent for laboratory test (RBPT/ELISA) for brucella and IBR test at VBRI (Veterinary Biological Research Institute, Shantinagar).

3.4 TECHNICAL PROGRAM OF WORK

In vitro embryo production (IVEP) was done by using standard procedures in ET and IVF lab of the Department of Veterinary Gynaecology and Obstetrics, C.V. Sc, Korutla by aspiration of oocytes from non-pregnant Sahiwal donor cows aged 3-6 years which were maintained at ILFC, C.V. Sc, Korutla. These *in vitro* produced embryos were transferred into recipient cows of farmers in villages. A total of 52 animal (heifer and post-partum cows completed 2-3 months post-partum period) free from uterine infections, belonging to farmers in combined Karimnagar district and some of the recipient animals maintained at ET and IVF project CVSC, Korutla, Jagtial (Dist.) were selected and used for transfer of embryo (fresh or frozen) after estrus synchronization or natural estrus by randomly distributing into 3 groups.

Embryo transfers were done into these recipient cows by using nonsurgical method after day 6-7 of estrus by depositing the embryos in uterine horn (cranial or middle third) ipsilateral to ovary having CL.

3.5 REGIMEN FOR TREATMENT

For the current therapeutic trial, a total of 52 cows with no palpable abnormalities, no uterine infection with Body Condition Score (BCS) of 2 to 3.5 on the scale of 1 (emaciated) to 5 (obese) (Wildman *et al.*, 1982) were selected and randomly assigned to one of the three groups. For the current investigation heifers and postpartum cows after 60 days of postpartum time were chosen. Those animals which were not having CL allotted to CIDR group and those with CL were allotted to Ovsynch and control group. The treatment groups were created in the following manner.

Group 1 (Ovsynch): In this group, a total of 15 cows were selected. Cows of this group were estrus synchronized using Ovsynch protocol 10 µg of buserelin (Receptal, 10ml, MSD, Germany, mark: by Intervet India Pvt. Ltd. Bhiwandi. Each ml contains 0.0042 mg of Buserelin acetate (GnRH analogue) (fig.3.1) intramuscular (I/M) on day 0, 500 µg of cloprostenol (Estrumate, 20ml, MSD, Germany, mark: by Intervet India Pvt. Ltd, Bhiwandi. Each ml contains 250 µg of cloprostenol) (fig.3.1) I/M on day 7 and second dose of GnRH given I/M on day 9 and embryo transfer was done on 6-7 days after observed estrus.

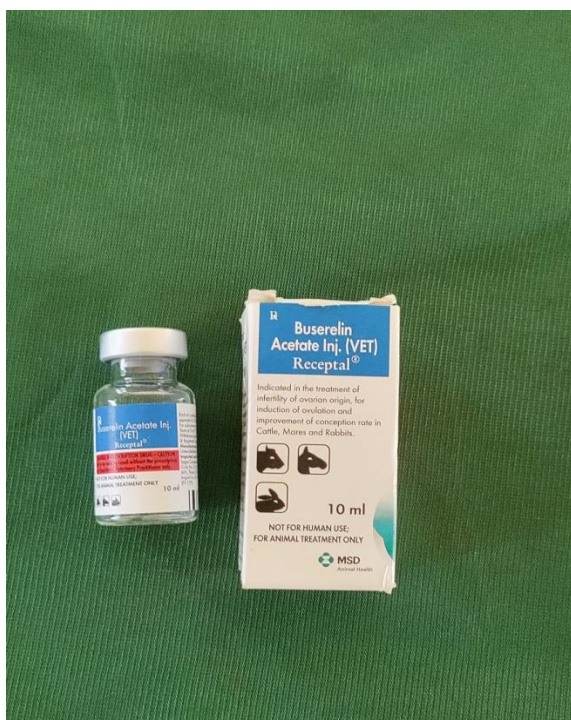
Group 2 (CIDR): In this group totally 10 cows were selected. Cows of this group were estrus synchronized by using CIDR (Eazi-Breed™ CIDR®, Cattle Insert, Zoetis Animal Health, each CIDR contains 1.38g of progesterone) (fig.3.2) protocol day 0 insertion of CIDR, day 7 CIDR removal and PGF_{2α} 500 µg of cloprostenol

(Estrumate) given I/M and embryo transfers were done on 6-7 days after observed estrus.

Group 3 (control): In this group total 27 cows which were in natural estrus were selected and embryo transfers were done on 6-7 days after observed estrus.

3.5.1 Procedure for CIDR insertion (fig.3.3)

1. The cows were restrained in travis appropriately prior to administration.
2. CIDR insert applicator gun was thoroughly cleaned with spirit before loading.
3. Flexible tail end of CIDR was pushed into the applicator gun through the slot and was pointed towards the handle.
4. The wings of the CIDR insert were folded to make straight and continue to advance into the applicator until it was fully seated and only the tips of wings protrude from the end of the applicators gun.
5. The front portion of applicator was lubricated with liquid paraffin.
6. Base of tail, exterior of vulva and perineum were thoroughly cleaned.
7. The CIDR insert loaded applicator gun was inserted into the vagina and squeeze the finger grips with in the handle of the applicator to deposit the CIDR insert into the anterior vagina and then pulled the applicator gun backwards to remove the applicator gun from vagina.
8. The tail of the CIDR insert that protruded from the vulva was clipped to minimize the length.

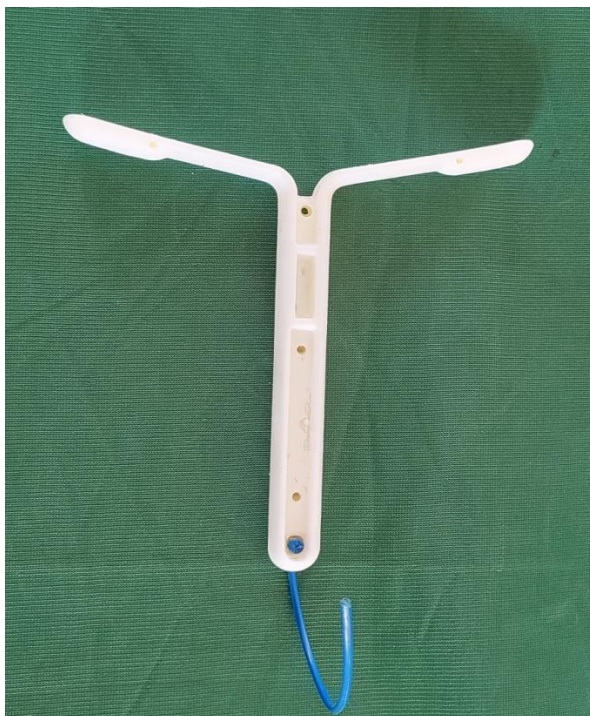


Receptal



Estrumate

Fig. 3.1 Hormones used for Ovsynch Protocol.



CIDR



CIDR applicator Gun

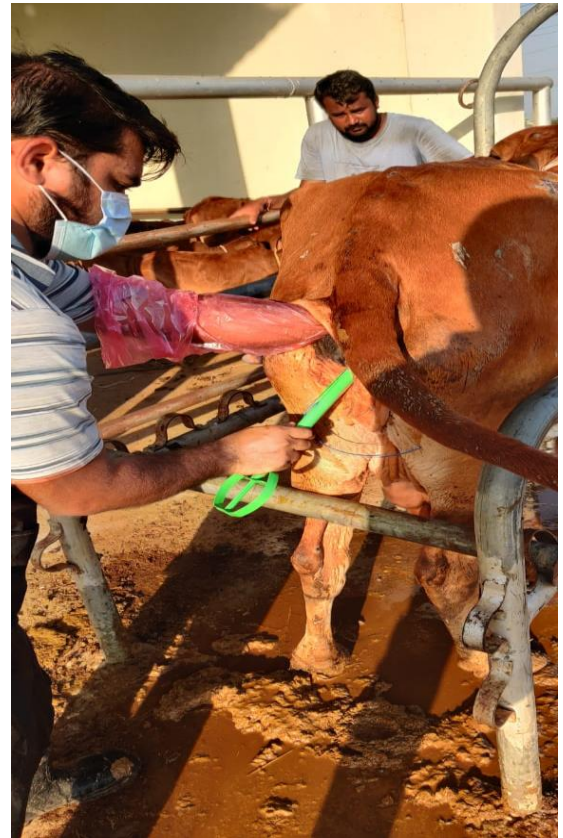


Applicator gun loaded with CIDR

Fig. 3.2 Equipment used for CIDR Protocol.



Loading of CIDR into applicator gun



Introducing CIDR with applicator gun into vagina.



Placement of CIDR in vagina by pushing plunger of CIDR applicator gun.



Removal of CIDR by holding tail of CIDR on 7th day.

Fig. 3.3 Showing images of procedure for CIDR insertion

3.6 ESTRUS DETECTION

The detection of estrus was based on visual observation. The animals were checked for estrus twice daily at least for 30 min each time. The estrus signs (standing to be mounted, bellowing, redness of vulval mucus membrane, swelling of vulva and clear mucous discharge) were recorded.

3.7 THERAPEUTIC EFFICACY

In this study, treatment efficacy was measured in terms of estrus response rate, onset of estrus, intensity of estrus, duration of estrus and conception rate.

3.7.1 Estrus response rate

The estrus response rate was calculated as the percentage of treated cows which exhibited estrus. Estrus was detected on the basis of behavioural, physical and gynaecological examination. The physical examination was carried out 24 hours (hrs) after the PGF₂ α (Estrumate) injection. At every 12 hrs to confirm the estrus, indication signs such as mucus discharges, edema and congestion of vulva, mounting behaviour and stand to be mounted in Ovsynch, CIDR and Control groups of cows were observed. Estrus response rate was expressed in percentage.

3.7.2 Onset of estrus

The onset of estrus was measured in hours (hrs) from the time of PGF₂ α treatment to the first appearance of estrus signs in treated cows and for control group animals it was calculated from the day of selection to exhibition of estrus up to 10-day period.

3.7.3 Duration of estrus

The interval in hours (hrs) between the beginning and cessation of estrus was recorded as the duration of estrus. The length of estrus in cows was established by a history acquired from the dairy farms technical personnel based on visual inspection of external genital organs and rectal examination of internal genital organs for the emergence (or disappearance) of estrus symptoms and structures on the ovaries.

3.7.4 Intensity of estrus

The degree of estrus in cows were determined using the Rao and Rao (1981) estrus intensity score card, which included a visual and rectal inspection of the external and internal genitalia for demonstrated estrus symptoms and certain physiological changes in the reproductive organs. According to the modified and score card, the intensity of estrus was evaluated as weak, normal or intermediate and intense by scoring less than 10, 10 to 15 and >15 points, respectively (Table 3.1).

3.8 EVALUATION AND CLASSIFICATION OF BOVINE EMBRYO

Evaluation and classification of embryo before loading in embryo straw was done based on International Embryo Transfer Society (IETS) guidelines for classification of cattle embryos (Bo and Mapletoft, 2018) (Table 3.2 (a), table 3.2 (b)).

3.9 LOADING OF EMBRYO

3.9.1 Fresh Embryo

The media IVC-ET* (Embryo Transfer Medium, BO 05, Vitrogen, Brasil) was kept overnight in incubator to equilibrate and stabilize the pH. The blastocyst of day 6 and 7 were washed 2-3 times with pre-equilibrated IVC-ET media to remove the overlaid oil. Then the embryos were loaded in a 0.25 ml sterile embryo straw (ref:006300, IMV technologies, IMV technologies, supplied by IMV India Pvt. Ltd, Gurugram, Haryana) between 2 air bubbles and the straw was sealed with plug (ref:007334, IMV technologies, supplied by IMV India Pvt. Ltd, Gurugram, Haryana). One embryo per straw was loaded with identification mark on straw plug. The embryo straws were kept in TED embryo transporter (TC-39/240 OLED, ref:503008, Brasil, supplied by IMV technologies, Gurugram, Haryana) to maintain at 37.5°C temperature and were transferred to the recipient animal having functional corpus luteum.

Table 3. 1 Score card for assessment of Intensity of estrus in cows (Rao and Rao, 1981).

	Points allotted
Estrus symptoms	
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 tonocity	3
Graafian follicle on the ovary	3
Temporary engorgement of teats	1
Decreased milk yield	1
Decreased feed intake	1
TOTAL	25
GRADING Weak estrus: < 10 points Normal estrus: 10-15 points Intense estrus: >15 points	

Table 3.2 (a) Classification of Bovine embryo based on stage of development.

Stage-1	Unfertilized ovum
Stage-2	2-12 cells
Stage-3	Early morula
Stage-4	Compact morula
Stage-5	Early blastocyst
Stage-6	Blastocyst
Stage-7	Expanded blastocyst
Stage-8	Hatched blastocyst
Stage-9	Expanded hatched blastocyst

3.2 (b) Based on embryo quality – morphological integrity

Code	Characteristic
I	Excellent/Good Embryos have symmetrical and spherical mass with individual blastomeres that are of uniform size, color and density.
II	Fair Moderate irregularities in the overall shape of the embryonic mass or in size, color, density of individual cells.
III	Poor Major irregularities in shape of embryonic mass or in size, color and density of individual cells.
IV	Dead or degenerated with oocyte or dead cell with incomplete division

3.9.2 Slow Freeze Embryo

The embryos were loaded in a 0.25 ml sterile embryo straw with one embryo per straw. The loading of straw was done as following - Wash media - air bubble – EG (ethylene glycol) –air bubble - EG + Embryo – air bubble – EG – air bubble – HDT- sealing plug. Then straws were placed in programmable freezer at -6°C and seeding (the upper part of the ethylene column + embryo) was done by touching the straw with cold forceps or cotton dipped in liquid Nitrogen (LN_2) and kept the straw for 10 min. at -6°C . The straws were then cooled from -6 to -35°C at a rate of $0.5^{\circ}\text{C}/\text{min}$. After reaching the target final temperature of -35°C , straws were finally transferred into LN_2 . On the day of embryo transfer before loading into ET gun, thawing of straw was done by holding the frozen straw for 10 seconds (sec) in air and 30 sec in a 35°C water bath. The embryos were then transferred to the recipient animal having functional corpus luteum.

3.9.3 Vitrified Embryo

After devitrification of embryos using media D1, D2 and D3 (BOV14, Devitrification Media, Vitrogen, Brazil), the embryos were washed 2-3 times with IVC-ET media. Then the labelled sterile 0.25 mL embryo straws were loaded with embryos in following way - IVC-ET media -air bubble- IVC-ET media -air bubble- IVC-ET media+embryo -air bubble- IVC-ET media-Sealing plug.

3.10 TRANSPORT OF EMBRYO

The embryo straws (fresh and vitrified) were kept in portable TED Embryo transporter (TC-39/240 OLED, ref- 503008, Brasil) to maintain at 37.5°C temperature and then transferred to the recipient animal having functional CL.



Fig. 3.4 Keeping straw in Portable TED Embryo Transporter

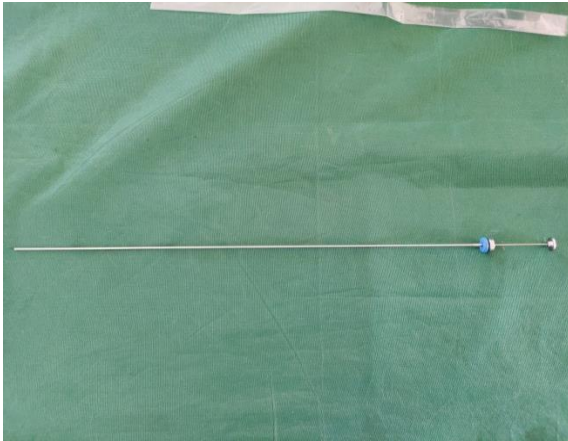
3.11 RECIPIENT SELECTION AND CL MEASUREMENTS FOR EMBRYO TRANSFER

Before embryo transfer (ET) healthy corpus luteum and its side (right or left ovary) was detected. Recipients with at least one CL and normal reproductive tract were separated from non-suitable recipients (without a CL) and used as embryo recipients to deposit a potentially viable embryo in the uterine horn (ipsilateral to CL). The recipients were examined by using ultrasonography (transvaginal probe/rectal probe) at 6 to 7 days after observed estrus. At that time, the presence of a CL was confirmed and measured, taking the average of the CL width and length in mm. Measurements were obtained with the electronic caliper in freeze mode.

3.12 EMBRYO TRANSFER PROCEDURE

3.12.1 Equipment (fig.3.5)

1. ET gun (Ref:007239, IMV technologies, IMV technologies, supplied by IMV India Pvt. Ltd, Gurugram, Haryana)
2. ET sheath (Ref: 00540, IMV ET blue 5, IMV technologies,
3. ET sanitary sheath (Ref: 005563, IMV Chemises, IMV technologies, supplied by IMV India Pvt. Ltd, Gurugram, Haryana)
4. 0.25 ml embryo straw
5. lignocaine hydro chloride 2% injection (NEON laboratories limited, Andheri East)
6. Portable TED Embryo transporter
7. Cloth Tissue paper (Origami, supplied by quality traders, Hyderabad)
8. Surgical spirit spray (HY-CHEM SURGICAL SPIRIT, HY-CHEM laboratories, Hyderabad)



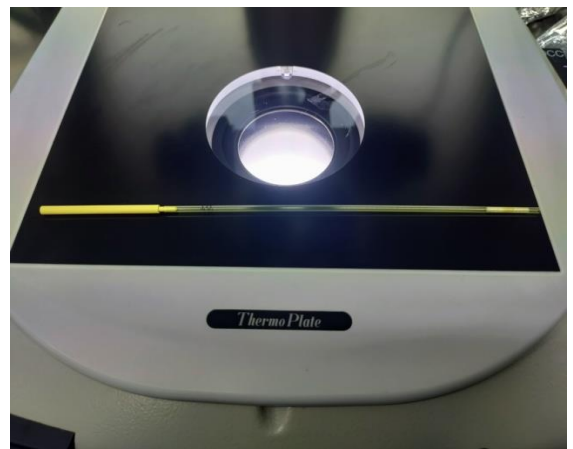
ET gun



ET sheath



ET sanitary sheath



ET straw



TED Embryo, Oocyte Transporter



Lignocaine (2%)

Fig. 3.5 Showing Equipment Used During Embryo Transfer procedure.

All embryo transfers were performed non surgically under epidural anaesthesia with 3 to 5 mL lignocaine hydro chloride 2% injection. ET was performed into the uterine horn ipsilateral to the side where the CL was present. The embryo straw containing *in vitro* produced fresh/vitrified/slow freeze embryos, in developmental stages ranging from morula to blastocyst and quality grade 1 or 2 transferrable embryos (according to the IETS manual) was loaded in the embryo transfer (ET) gun and the laboratory seal was cut. ET gun was then covered with ET sheath, which was then covered with ET sanitary sheath and embryo transfer was performed.

After embryo transfer deposition site (cranial or middle third of uterine horn ipsilateral to the ovary with a CL) of embryo transfer was recorded. During the embryo transfer the easiness of passage of ET gun through cervix was also recorded and three groups were formed based on cervix transfer score (easy < 60 sec, moderate 60-80 sec and difficult > 80 sec).

Note: During the interval between straw loading and ET, the straws were kept in a portable embryo transporter at 37.5° C.

3.12.2 Procedure

1. Before passing gun, the perineum was washed and dried, then passed the gun through the vagina and manipulated to cervical entrance, before entering cervix grasp the chemise with the thumb and index finger of the right hand and pull the chemise backward causing tip of sheath to pass through.

Manipulate the rod through the cervix and stop just before entering the uterine body.



Loading of embryo straw into ET gun



Cutting laboratory seal of ET straw after putting in ET gun



Covering ET gun loaded with embryo straw with ET sheath



Finally Covering ET gun with ET sanitary sheath



Insertion of ET gun into cow genitalia

Fig. 3.7 Showing procedure of Embryo Transfer

2. The cervix is allowed to shield the sheath tip from entering and damaging the uterine wall.
3. The cervix is released and focus on retracting the horn.
4. Then the hand is extended forward and both horns are retracted back toward floor of the pelvis.
5. Both horns are released and then rapidly pass your finger forward, over, down and around the horn that you wish to transfer.
6. When confident that opening of the horn is directly Infront of the sheath tip, the rod is advanced forward gently.
7. When ET gun tip reach deposition site, 4-5 second were allowed to slowly press the plunger and the embryo are expelled into upper third of uterine horn or midpoint of uterine horn between palpable horn bifurcation and end of horn.

Immediately after embryo transfer (ET) all recipient cows were administered inj. flunixin meglumine (Megludyne ® Injectable Solution, each ml contains 50 mg flunixin meglumine, Virbac, India) at the dose rate of 1.1 mg/kg body weight (B/W) I/M.

3.13 PREGNANCY DIAGNOSIS

The cows in all the groups of present study were subjected to pregnancy diagnosis after 30 to 40 days of embryo transfer by using ultrasound scanning or per rectal examination method. A portable transrectal ultrasonography (Easi scan, BCF technology, Scotland, Uk) with a 7.5 MHz linear array transducer was used. A diagnosis of pregnancy was made if there were irregularly shape, non-echogenic dense fluid in the uterine lumen was present and a corpus luteum in the ipsilateral ovary was visible. In the absence of an allanto chorion and /or embryo, the female was considered not pregnant.

3.14 CONCEPTION RATE

The conception rate was calculated for each group by the following equation.

Conception rate = Percent of animals conceived / among total animals received embryos.

3.15 STATISTICAL ANALYSIS

Statistical analysis was done using SPSS software (version 20.0). The data on onset of estrus, intensity of estrus and duration of estrus were analysed using one way analysis of variance (ANOVA) to compare variation between groups. Estrus response rate and conception rates in different groups were compared by Chi-square test. The effect of embryo transfer location and cervix transfer score on conception rate was determined using Chi-square test. The effect of BCS and CL diameter on conception was analysed using t-test.



Fig. 3.7 Pregnancy diagnosis using transrectal ultrasonography

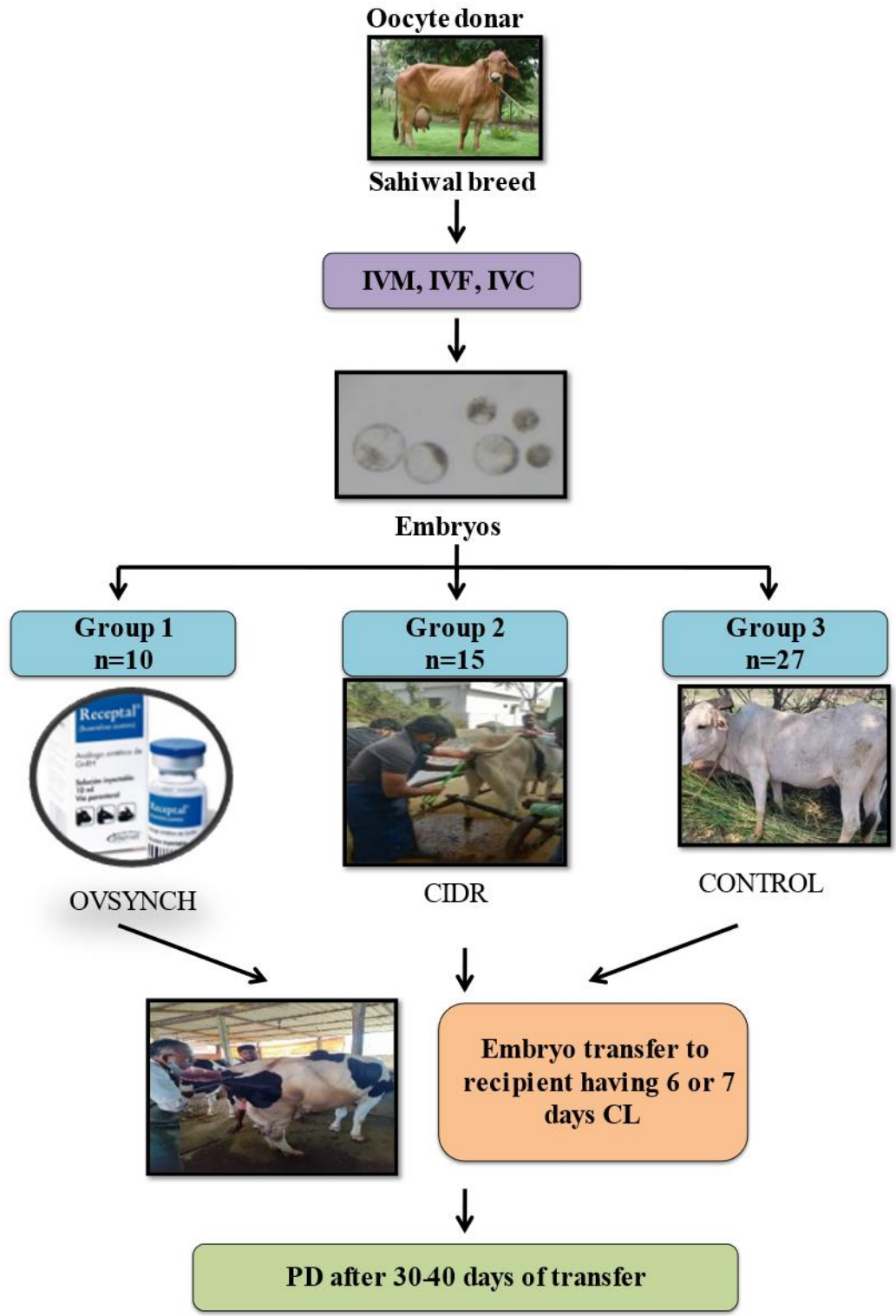


Fig. 3.8 Schematic design of experimental program

RESULTS

CHAPTER IV

RESULTS

The present research work entitled “Estrus synchronization of recipient cows of farmers for transfer of *in vitro* produced Sahiwal embryos” was undertaken in cows of farmers of combined Karimnagar district and also in some of the recipient animals maintained at ET and IVF project at C.V. Sc, Korutla, PVNRTVU, Jagtial (Dist.).

4.1 ESTRUS SYNCHRONIZATION EFFICACY

The estrus synchronization efficacy was recorded based on estrus response, onset of estrus, duration of estrus, intensity of estrus and conception rates illustrated from Table 4.1 to 4.11 and Fig 4.1 to 4.11.

4.1.1 Estrus Response Rate

The Estrus response rates were (13/15) 86.66, (10/10) 100.00 per cent in Ovsynch and CIDR groups respectively, and (15/27) 55.55 per cent in control group. Statistical analysis revealed that there was significant difference ($P < 0.05$) between treatment groups (Ovsynch, CIDR) control group in the present experiment (Table 4.1 and Fig 4.1). The highest estrus induction rate was observed in CIDR group (100.00 per cent) followed by Ovsynch group (86.66 per cent) and lowest in control group (55.55 per cent).

4.1.2 Onset of estrus

The mean duration of onset of estrus was recorded as 53.69 ± 1.90 , 49.1 ± 0.48 and 109.73 ± 18.74 hrs in Ovsynch, CIDR and control groups, respectively. The onset of estrus has no significant ($P > 0.05$) difference between Ovsynch and CIDR treated group, but both CIDR and Ovsynch group were significantly ($P < 0.05$) different with control group.

Table 4.1. Estrus response rate in therapeutic groups of cows

S.NO	Name of the group	No. of cows treated	No. of cows exhibited estrus	Per cent cows responded	Chi Square value
1	OVSYNCH	15	13	86.66	9.31
2	CIDR	10	10	100.00	
3	CONTROL	27	15	55.55	

There was significant difference between Ovsynch, CIDR and Control group ($P < 0.05$)

Table 4.2 Onset of estrus(hrs) in therapeutic groups of cows after PGF_{2α} injection and in control group from beginning of selection.

S.NO	Name of the group	No. of cows treated	No. of cows exhibited estrus	Mean onset of estrus(hrs)
1	OVSYNCH	15	13	53.69±1.90 ^a
2	CIDR	10	10	49.1±0.48 ^a
3	CONTROL	27	15	109.73±18.74 ^b

^{a, b} superscript means with different superscripts in a column differ significantly: ($P < 0.05$)

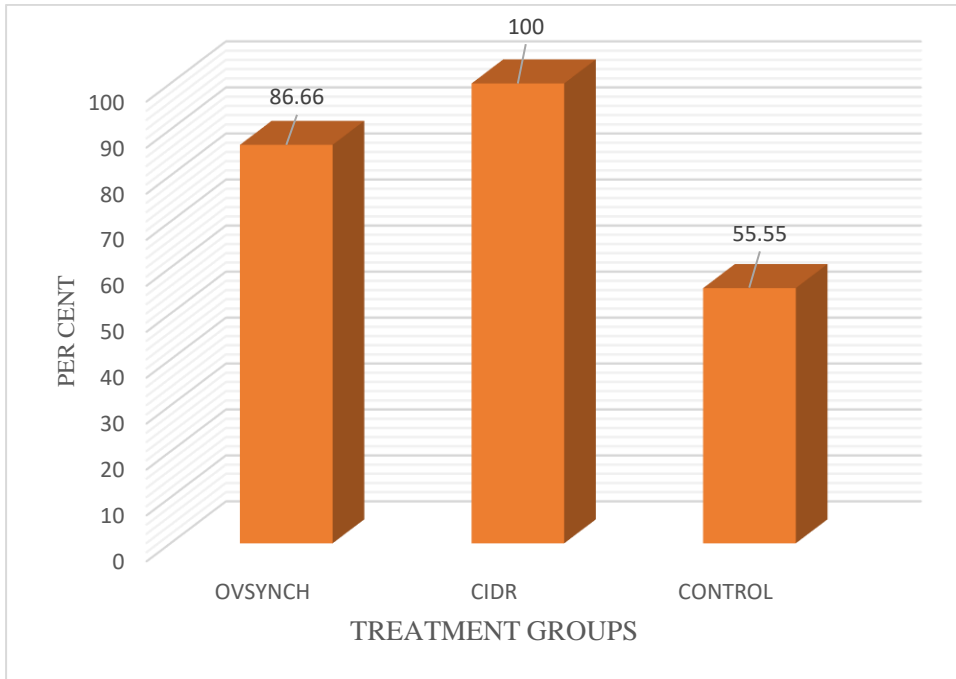


Fig. 4. 1. Showing Estrus Response Rate (per cent) in therapeutic groups of Cows

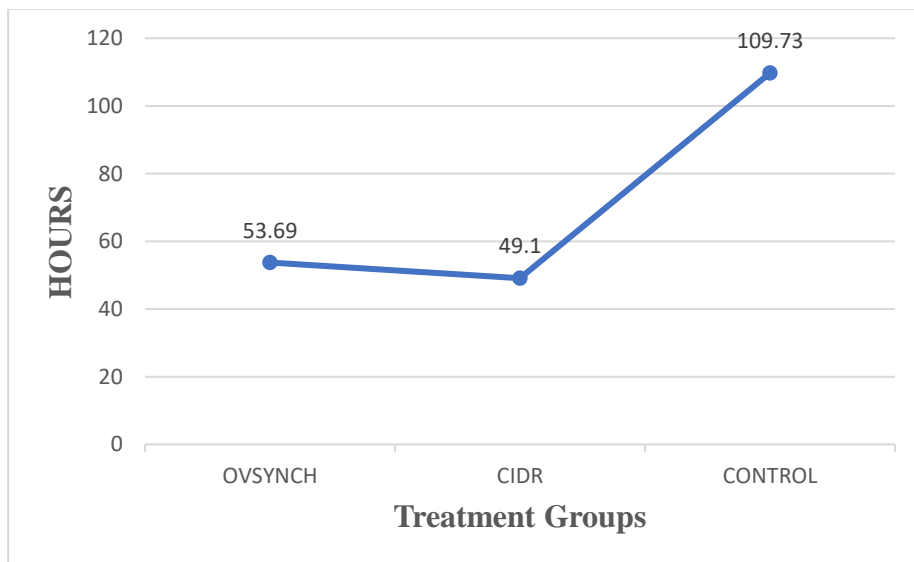


Fig. 4.2 Onset of Estrus (hrs) in therapeutic groups of cows after $\text{PGF}_{2\alpha}$ Injection.

4.1.3 Intensity of estrus

The overall intensity of estrus was 15.79, 44.74 and 39.47 per cent as weak, normal or intermediate and intense, respectively. The experimental cows exhibited the weak estrus intensity was 7.69, 0, and 33.33 per cent in Ovsynch, CIDR, and control groups, respectively. Normal or intermediate estrus intensity was 61.53, 30, and 40 per cent in Ovsynch, CIDR, and control groups, respectively. Similarly, the intense estrus was observed 30.66, 70.00, and 26.66 per cent cows of Ovsynch, CIDR and control groups, respectively. The mean score for estrus intensity were observed as 13.38 ± 0.94 , 16.30 ± 1.59 and 11.07 ± 1.05 in Ovsynch, CIDR and control groups, respectively (Table 4.4, Fig 4.4).

The statistical analysis revealed that there was significant difference ($P < 0.05$) in mean estrus score between CIDR and Control group, but CIDR and Ovsynch group has no significant difference ($P > 0.05$). Similarly, there was no significant difference ($P > 0.05$) between Ovsynch and Control group (table 4.4 and fig.4.4).

4.1.4 Duration of estrus

The mean duration of estrus recorded was 21.61 ± 0.77 , 20.30 ± 0.72 , and 20.47 ± 0.70 hrs in Ovsynch, CIDR and control groups, respectively in the present experiment (Table 4.5 and Fig 4.5). Statistical analysis revealed that there was no significance difference ($P > 0.05$) observed in mean duration of estrus in Ovsynch, CIDR and control groups.

Table 4.3 Showing the intensity of estrus in synchronized groups of cows

S. No	Name of the group	No. of cows exhibited estrus / No. of cows treated	Scale of intensity of estrus					
			<10 points		10-15 points		>15 points	
			(weak)		(Normal)		(Intense)	
			No.	%	No.	%	No.	%
1	OVSYNCH	13/15	1	7.69	8	61.53	4	30.76
2	CIDR	10/10	-	-	3	30.00	7	70.00
3	CONTROL	15/27	5	33.33	6	40.00	4	26.66
TOTAL		38/52	6		17		15	
OVERALL PERCENT				15.79		44.74		39.47

Table 4.4 Mean estrus intensity score in synchronized groups of cows

S. No	Name of the group	No. of cows treated	No. of cows exhibited estrus	Mean estrus intensity score
1	OVSYNCH	15	13	13.38±0.94 ^{ab}
2	CIDR	10	10	16.30±1.59 ^b
3	CONTROL	27	15	11.07±1.05 ^a

*^{a,b} Means with different superscripts in a column differ significantly (P<0.05)

Table 4.5 Duration of estrus (hrs) in synchronized groups of cows

S.NO	Name of the group	No of cows exhibited estrus	Mean duration of Estrus (hrs)
1	OVSYNCH	13/15	21.61±0.77 ^a
2	CIDR	10/10	20.30±0.72 ^a
3	CONTROL	15/27	20.47±0.70 ^a

There was no significant difference in duration of estrus among 3 groups (P>0.05)

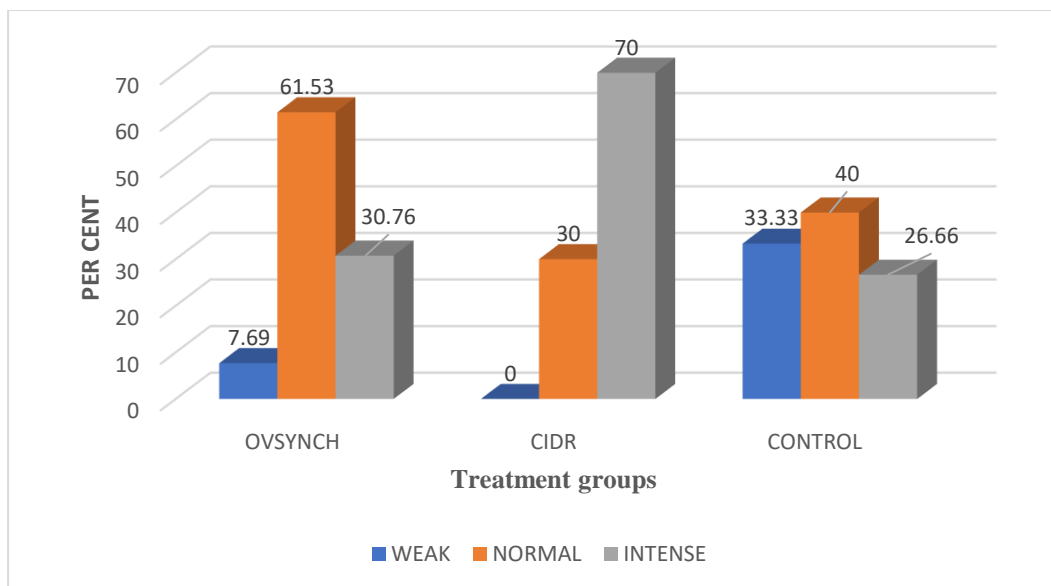


Fig. 4.3 Showing the Intensity of estrus in therapeutic groups of cows

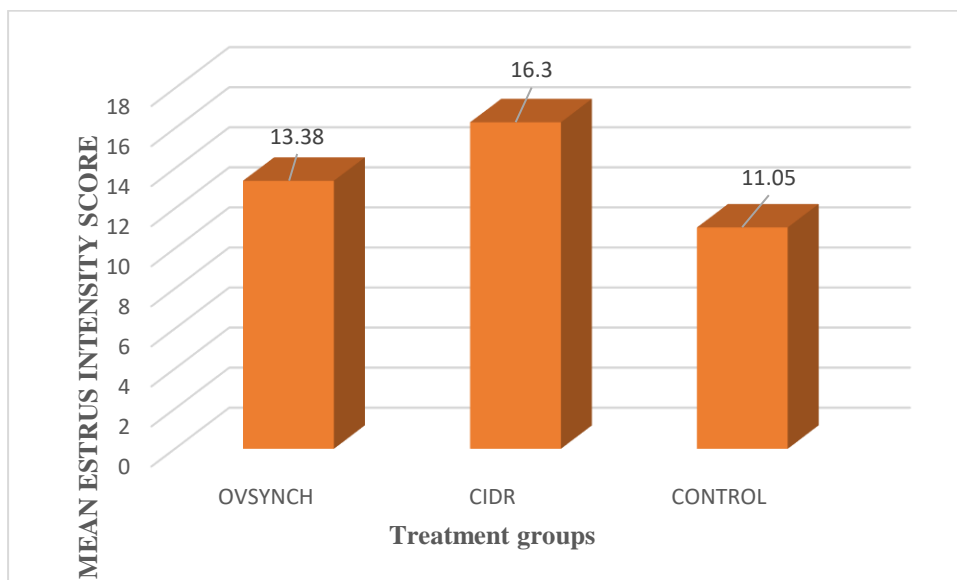


Fig. 4.4 Showing Mean Estrus Intensity Score in synchronization groups of cows

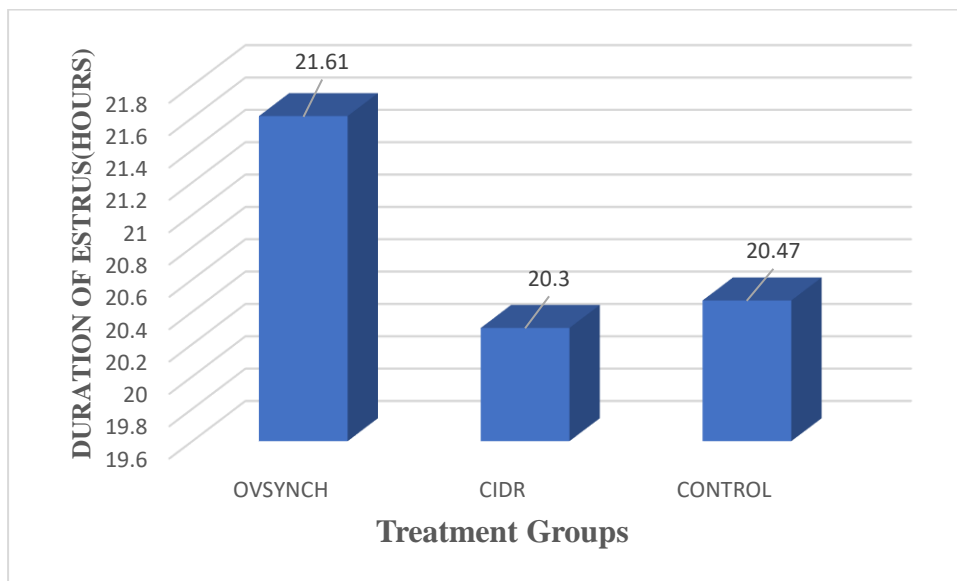


Fig. 4.5 Showing Duration of Estrus in synchronization groups of cows

4.2 CONCEPTION RATE

The overall conception rate in therapeutic cows were observed as 14.28, 0, and 13.3 percent in CIDR, Ovsynch and control groups, respectively.

There was no significant ($P>0.05$) difference found among the treated groups and control group (Table 4.6 and Fig 4.6). The overall conception rate was more in CIDR group followed by control group, respectively.

4.3 EFFECT OF BODY CONDITION SCORE ON IVF EMBRYO TRANSFER

CONCEPTION RATE CONCEPTION RATE

The average mean BCS was 2.80 ± 0.48 , 3.04 ± 0.08 , and 2.89 ± 0.37 in Ovsynch CIDR and control groups, respectively.

In the present study there was positive correlation ($P<0.05$) found between BCS and conception rate. The mean BCS was significantly higher in cows that conceived (3.10 ± 0.21) than in those failed to conceive (2.87 ± 0.28) in non-pregnant cows (table 4.7, Fig 4.7).

4.4 EFFECT OF DEPOSITION SITE IN UTERUS ON CONCEPTION RATES

In the present study, there was significance ($P<0.05$) difference found between site of uterine horn where embryo was deposited (cranial or middle third) and conception rates (Table 4.8, Fig 4.9).

The higher conception rate was found in recipients where embryos were deposited at cranial third of the uterine horn when compared to embryo deposited at middle third of uterine horn ($P<0.05$).

Table 4.6 Conception rates in Synchronized groups of cows after IVF-embryo transfer

S. No	Name of the group	No. of cows Embryo transfer	No. of cows pregnant	Per cent of conception	Chi - Square value
1	OVSYNCH	11	-	-	1.949
2	CIDR	7	1	14.28	
3	CONTROL	15	2	13.33	

There was no significant difference in conception rate among 3 groups ($P>0.05$)

Table 4.7 Effect of BCS on IVF-Embryo Transfer conception rates

S. No	Mean BCS of Pregnant	Mean BCS of Non- Pregnant
1	3.10±0.21	2.87±0.28

There was significant difference between BCS and Conception rate ($P<0.05$).

Table 4.8 Relationship between embryo transfer location and conception rates

S. No	Deposition site in Uterus	No. of Animals	No. Animal became pregnant	Conception rate among total pregnant animal (%)	P Value
1	Cranial	10/33	2	75	<0.05
2	Middle third	23/33	1	25	

There was significant difference between ET location and Conception rate ($P<0.05$).

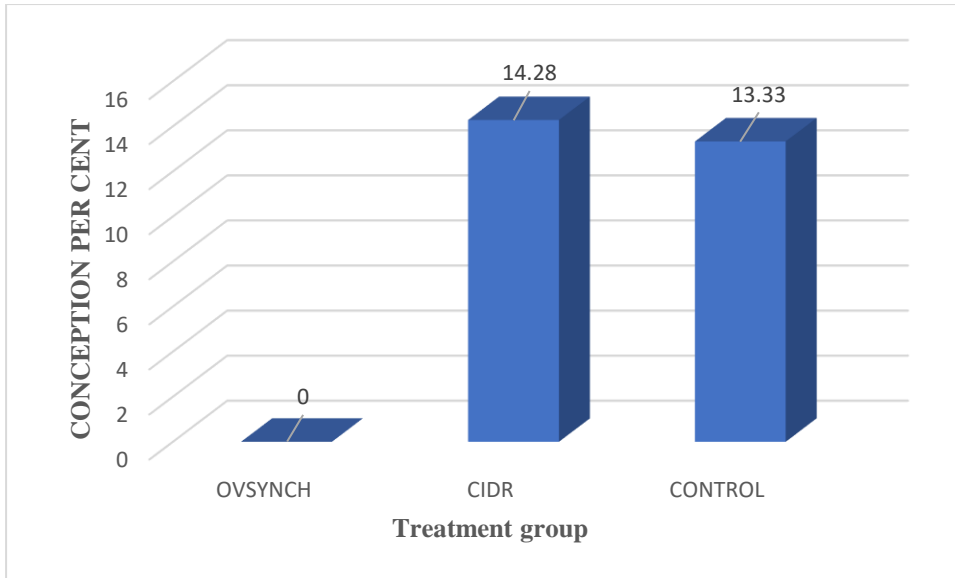


Fig. 4.6 Showing conception rate in IVF- Embryo transfer in synchronized group of cows.

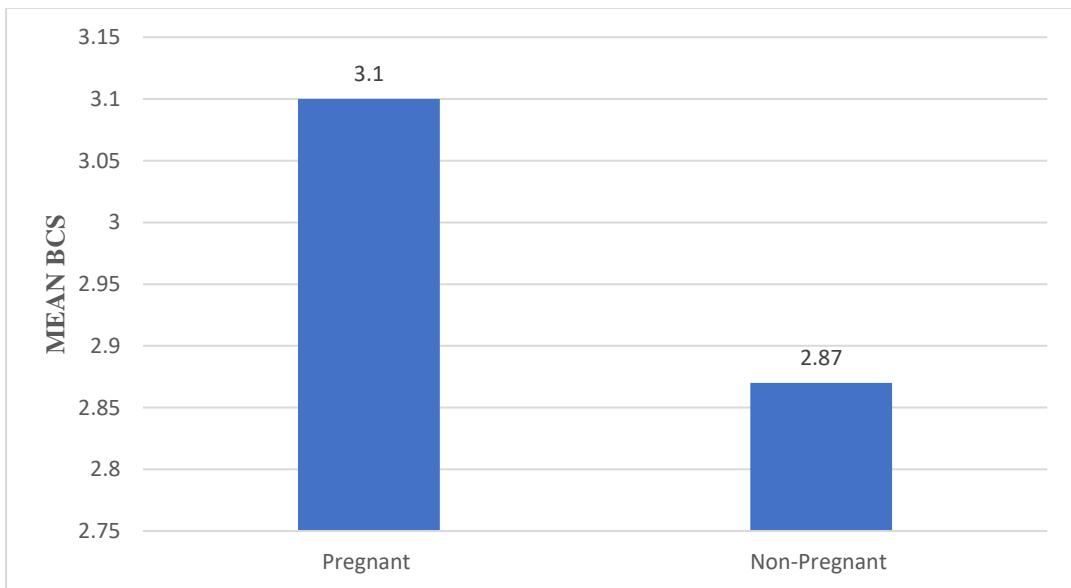


Fig. 4.7 Relationship between BCS on IVF-embryo transfer conception rate

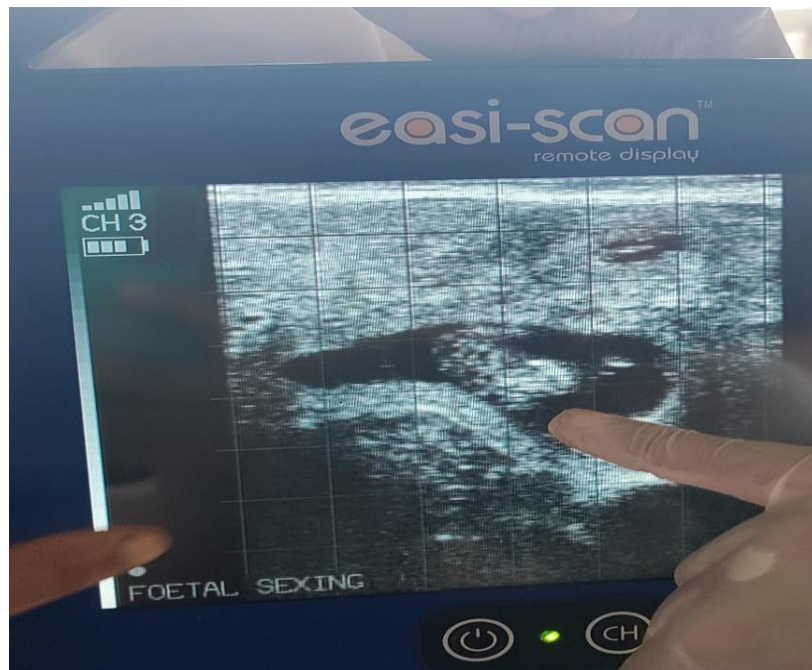


Fig. 4.8 (a) Pregnancy diagnosis in CIDR group cow at 30th day

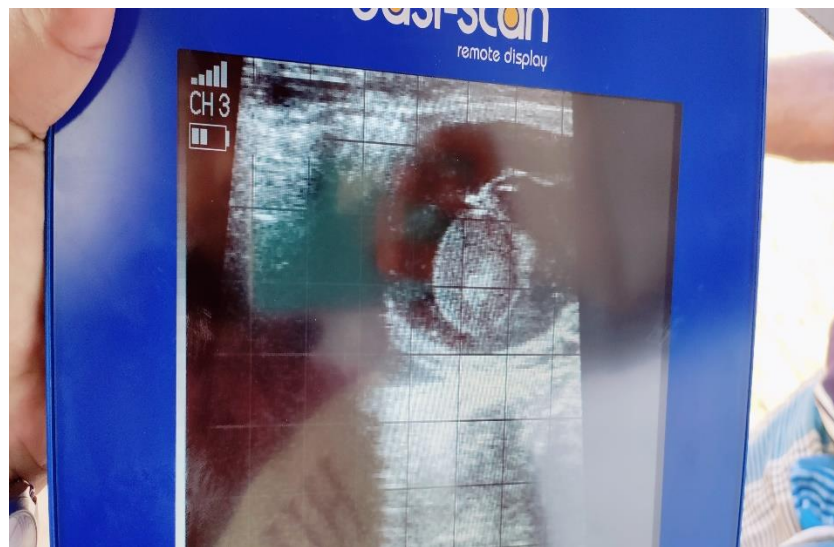


Fig. 4.8 (b) Pregnancy diagnosis in control group cow at 40th day

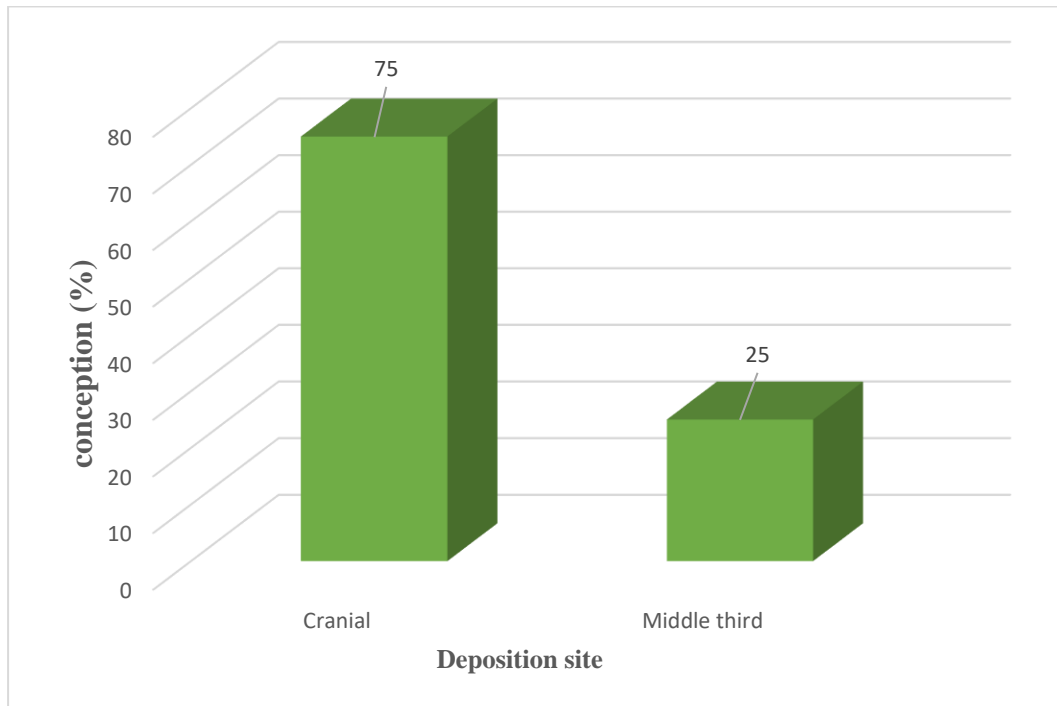


Fig. 4.9 Relationship between Embryo transfer location and Conception

4.5 MEASUREMENT OF CL DIAMETER (MM) AND ITS EFFECT ON CONCEPTION RATE

The average mean diameter of CL in recipient cows was 14.11 ± 0.39 , 15.63 ± 1.80 , and 13.99 ± 0.95 mm in Ovsynch, CIDR and control group of study group, respectively.

In the present study group, there was no correlation ($P > 0.05$) found between CL diameter and conception rate (Table 4.9) of recipient cows. The mean CL diameter was 16.18 ± 1.60 (14.45-19.35) mm in pregnant cows and 14.19 ± 0.54 (10-23) mm in non-pregnant animals (Table 4.9, Fig 4.10).

4.6 EFFECT OF EASINESS OF PASSAGE OF ET GUN THROUGH CERVIX ON CONCEPTION RATE

The easiness of passage of ET gun through cervix was measured using cervix transfer score (easy < 60 sec, moderate: 60-80 sec and hard > 80 sec).

It was found that cervix transfer scores had no statistical ($P > 0.05$) effect on conception rates. However, it was also found that the probability of pregnancy was higher when the cervix transfer score was easy (<60 sec) and moderate (60-80 sec) when compared to the difficult (>80 sec) (Table 4.10, Fig 4.11).

conception rates according to the interaction between cervix transfer score and transfer location are given in Table 4.10.

Table 4.9 Relationship between mean CL diameter and Conception rates

S.no	Mean CL (mm) of Non-Pregnant Cows	Mean CL (mm) of Pregnant Cows
1	14.19±0.54	16.18±1.60

There was no significant difference between CL diameter and conception rate ($P>0.05$).

Table 4.10: Relationship between Cervix Transfer Score and among the animals became pregnant after embryo transfer

S.no	Cervix Transfer Score	No of Animals	No of animal become pregnant	Pregnancy percentage among total pregnant animals (%)	P
1	Easy	10/33	2	66.7	>0.05
2	Moderate	13/33	1	33.3	
3	Hard	10/33	0	-	

There was no significant difference between cervix transfer score and pregnancy ($P>0.05$).

Table 4.11: No. of animal suitable for embryo transfer based on the presence of CL and cervical patency/cervical transferability of ET gun

S.NO	Group name	No of animal exhibited estrus	No of animal having CL /No animal exhibited estrus	No of animal having cervical patency/No animal exhibited estrus	No of animal fit for embryo transfer
1	Ovsynch	13/15	13/13	11/13	11/13
2	CIDR	10/10	9/10	8/10	7/10
3	Control	15/27	15/15	15/15	15/15

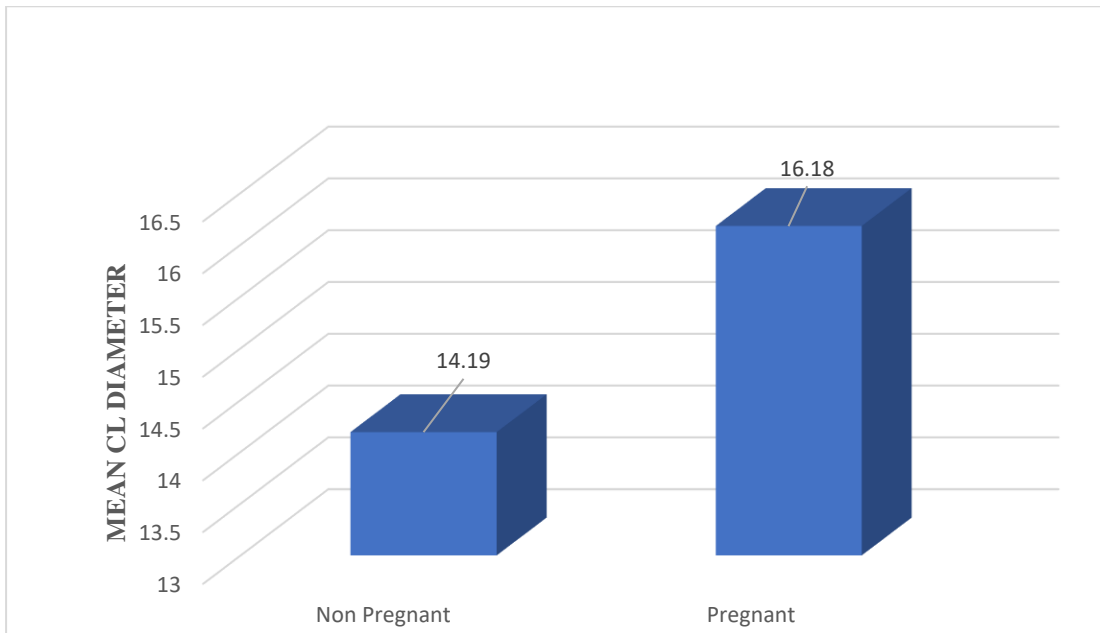


Fig. 4.10 Relationship between CL diameter and conception rates.

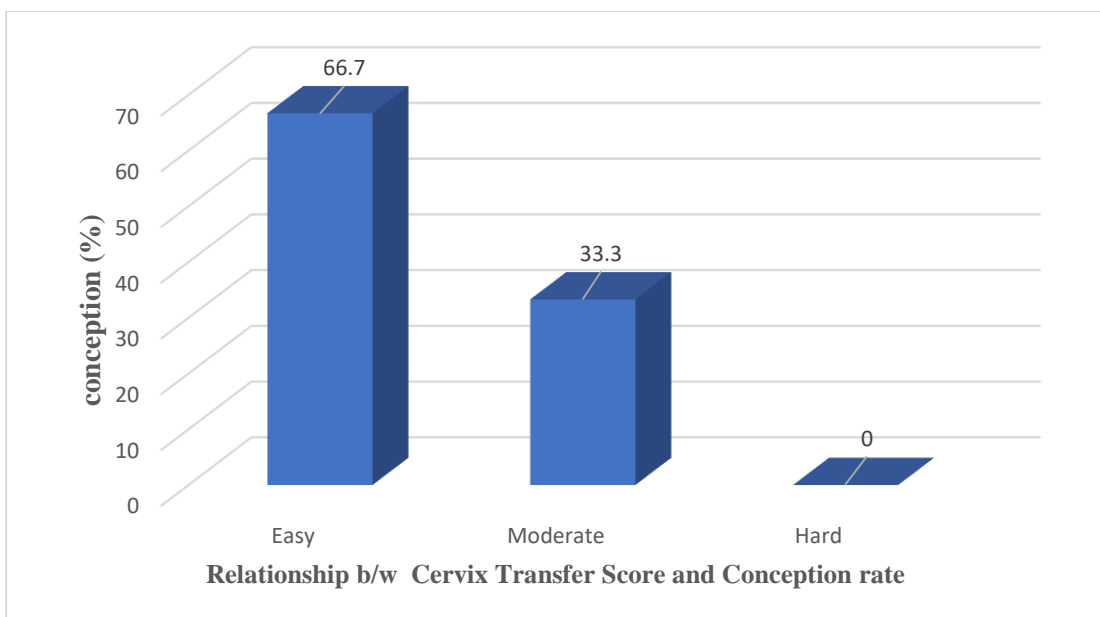


Fig. 4.11 Relationship Between Cervix transfer score and conception rates

DISCUSSION

CHAPTER V

DISCUSSION

A total of 52 cows (heifer and post-partum cows completed 2-3 months post-partum period) free from uterine infections, belonging to farmers in combined Karimnagar district and some of the recipient animals maintained at ET and IVF project C.V.SC, Korutla, PVNRTVU, Jagtial (Dist.) were selected and used for embryo transfer after estrus synchronization or natural estrus by randomly distributing into the following 3 groups: Group 1 (Ovsynch), Group 2 (CIDR) and Group 3 (control). Embryo transfers were done into these recipient cows by using non-surgical method after day 6-7 of estrus by depositing the embryos in uterine horn (cranial or middle third) on ipsilateral side of ovary having CL. After 30 to 40 days the transfer, pregnancy was confirmed by rectal palpation and transrectal ultrasonography. The results of estrus synchronization and conception rate obtained in this experiment with different groups are discussed in this chapter.

5.1 ESTRUS SYNCHRONIZATION EFFICACY

5.1.1 Estrus Response Rate

In the present experiment, the estrus response was 86.66 and 100.00 per cent in treated groups (Ovsynch and CIDR, respectively) whereas, 55.55 per cent in control group. In statistical analysis there was no significant difference ($P < 0.05$) observed among the treatment groups, but significant difference was observed between treated groups (CIDR, Ovsynch) and control group (Table 4.1 and Fig. 4.1).

Similar result of estrus response rate with Ovsynch group was reported by Hirole *et al.* (2018). Contrary to this, the greater estrus response rate was reported by Velludurai *et al.* (2014), Buchecha *et al.* (2015), Ahmed *et al.* (2016) and Linga swamy (2017) and Doodram (2018). But the lesser estrus response was observed by Al katani (2002) and Ambrose *et al.* (1999).

The similar results of estrus response rates were reported with CIDR synchronization protocol by Dhami *et al.* (2015) and Devi *et al.* (2021). While Vargas *et al.* (1994), Lucy *et al.* (2001), Cavalieri *et al.* (2003), Murugavel *et al.* (2010), Jyothi *et al.* (2012), Haider *et al.* (2017), Amin *et al.* (2019) and Raut *et al.* (2020) reported lower estrus response rate than the CIDR group. The reason for getting higher estrus response rate in present study in CIDR protocol could be attributed to season of the experiment, feeding, breed and managerial differences.

5.1.2 Onset of Estrus

The duration of onset of estrus is calculated in hours (hrs) from the time of administration of PGF₂ α to the time of first appearance of estrus signs in treated cows and for control group of animals it is calculated from the day of selection to exhibition of estrus up to 10 days period.

The mean onset of estrus was observed as 53.69 \pm 1.90, 49.1 \pm 0.48, and 109.73 \pm 18.74 hrs in Ovsynch, CIDR, and control groups, respectively in experiment cows. The onset of estrus has no significant ($P>0.05$) difference between Ovsynch and CIDR treated group, but both CIDR and Ovsynch group were significantly ($P<0.05$) different with control group. (Table 4.2 and Fig. 4.2).

The mean duration of onset of estrus in Ovsynch study group has similarity to results of Linga Swamy (2017), Hirole *et al.* (2018), Doodram (2018) and Ratnaparki *et al.* (2020). Contrary to this, longer onset of estrus was observed by Buhecha *et al.* (2015) and Senthilkumar and Chandrahasan (2015).

The mean duration of onset of estrus in CIDR group was in accordance with observation of Jyothi *et al.* (2012), Haider *et al.* (2017) and Amin *et al.* (2019). Contrary to this longer time for onset of estrus as observed by Larson *et al.* (2006), Jyothi *et al.* (2011)

and Murugavel *et al.* (2010). shorter time for onset of estrus was observed by Lucy *et al.* (2001), Dagefa *et al.* (2016) and Devi *et al.* (2021). The CIDR group significantly ($P < 0.05$) differ with control group, as CIDR group required shorter time for estrus induction. The shorter induction time of estrus in CIDR group was due to the application of short-term progesterone treatment for 7 days and administration of $PGF_{2\alpha}$ on day of CIDR removal which results in close synchrony between estrus and ovulation.

5.1.3 Intensity of Estrus

The overall intensity of estrus was 15.79, 44.74 and 39.47 per cent as weak, normal or intermediate and intense, respectively in present study groups. (Table 4.3).

The mean scores for estrus intensity were observed as 13.38 ± 0.94 , 16.30 ± 1.59 , and 11.07 ± 1.05 in Ovsynch and CIDR and control groups, respectively. The statistical analysis revealed that there was significant difference ($P < 0.05$) in mean estrus scores between CIDR and Control group, but CIDR and Ovsynch group has no significant difference ($P > 0.05$). Similarly, there was no significant difference ($P > 0.05$) between Ovsynch and Control group (table 4.4 and fig.4.4).

The Ovsynch study group cows exhibited 7.69, 61.53 and 30.76 per cent as weak, normal and intense estrus respectively (Table 4.3, Fig.4.3). Similar results were reported by Ahmed *et al.* (2016), Doodram (2018), and Senthilkumar and Chandrahasan (2015) where incidence of normal or intermediate and intense estrus was higher in Ovsynch treated animals.

The CIDR study group cows exhibited 0, 30, and 70 per cent as weak, normal and intense estrus, respectively (Table 4.3, Fig 4.3). The result was in accordance with observation of Jyothi *et al.* (2011), Reshma *et al.* (2020), Marugavel *et al.* (2009) and Devi *et al.* (2021) where incidence of intense estrus is higher followed by normal and weak estrus.

The mean scores for estrus intensity were observed as 13.38 ± 0.94 , 16.30 ± 1.59 and 11.07 ± 1.05 in Ovsynch, CIDR, and control groups, respectively. The higher mean score of intensity of estrus in CIDR group was because of higher incidence of intense estrus among all study group. The variation in intensity of estrus was attributed to season, climatic conditions, nutritional status and managerial condition of cows.

5.1.4 Duration of Estrus

The mean duration of estrus observed in experiment animals was 21.62 ± 0.77 , 20.30 ± 0.72 , and 20.47 ± 0.70 hrs in Ovsynch, CIDR and control groups, respectively.

In present study there was no significance ($P>0.05$) difference observed between treated and control groups.

The present study results for duration of estrus in Ovsynch group was in accordance with the studies of Ahmed *et al.* (2016) and Hirole *et al.* (2018). Contrary to this result, the longer duration of estrus in Ovsynch group was reported by Velladurai *et al.* (2014), Linga Swamy (2017) and Doodram (2018) and shorter duration of estrus was reported by Shahid *et al.* (2019), Anjum *et al.* (2009) and Senthilkumar and Chandrahasan (2015).

The duration of estrus in CIDR group were is in accordance with the observation of Dagefa *et al.* (2016). Contrary to these results, the shorter duration of estrus in CIDR protocol was observed by Sauls *et al.* (2016) and Mengchao *et al.* (2010) and longer duration of estrus was reported by Reshma *et al.* (2020) and Devi *et al.* (2021). The shortening in the duration of estrus might be because use of progesterone device might have increased the ability of the hypothalamus-pituitary gonadal axis to generate estrus and LH surge in response to an increase in endogenous estradiol response to ovulation.

5.2 TREATMENT EFFICACY ON CONCEPTION RATE

The overall conception rate in therapeutic cows were observed as 0, 14.28 and 13.33 per cent in Ovsynch, CIDR and control groups, respectively. However, there was no significant ($P>0.05$) difference in conception rate observed among the treated groups and control group (Table 4.6 and Fig.4.6).

5.2.1 Conception Rate in Ovsynch Group

In present study out of 13 synchronized recipient animals, 2 cows were not found suitable for embryo transfer because of genital defect. In the present study, there was no conception rate seen in Ovsynch treated group, but the number of animals responded to estrus was higher compared to control group. Contrary to these results, the higher conception rate in recipient animals using Ovsynch protocol was observed by Ambrose *et al.* (1999), where synchronized (Ovsynch+TET) animals pregnancy rate was slightly higher (fresh TET 17.5 ± 3.0 , frozen TET 6.7 ± 3.2), when compared to pregnancy rates of fresh-TET (14.3 ± 2.3), TET-frozen (4.9 ± 2.3) in normal cyclic animals. Al-katanani *et al.* (2002) reported higher pregnancy rate for TET-F (26.7 ± 6.4) than for the TAI (5.0 ± 4.3) and TET-V (7.4 ± 4.7).

Block *et al.* (2010) recorded that the percentage of recipient cows synchronized with Ovsynch protocol cows pregnant with fresh embryos were 56.3% (d 32), 52.6% (d 46), and 50.5% (d 76). Stewart *et al.* (2011) reported that, percentage of cows became pregnant was greater with synchronized recipient (Ovsynch protocol) for ET fresh (*in-vivo*) (45.5%), ET-V (31.6%), A.I (24.6%) when compared to non-synchronized animals ET fresh (42.1%), ET-V (29.3%), A.I (18.3%).

The possible reason why there was no conception in Ovsynch group might be because of smaller size of treatment group, nutritional status, environmental stress and managerial condition of recipient cows. However, the number of available recipients at a time for embryo

transfer was seen higher as estrus induction rate was higher (86.66 per cent) in Ovsynch treated group. The possible reason for higher recipient animals during embryo transfers due to GnRH injection which causes release of enough LH, to ovulate the largest follicle on the ovary and formation of CL. A new wave of follicle will start to grow since GnRH removes the dominant follicle, on seven-day injection of PGF₂α regress the CL to synchronize final follicular growth and exhibition of estrus.

5.2.2 Conception Rate in CIDR Group

In CIDR group, out of 10 synchronized recipient animals, 3 cows were not found suitable for embryo transfer because of genital defect and absence of CL. In present study, the conception rate in CIDR group was observed as 14.28 per cent. Contrary to these, the greater conception rate was reported by Sala *et al.* (2015), Sanches *et al.* (2016), Do *et al.* (2018), Bonacker *et al.* (2020) and Steichen and Larson (2019).

Sanches *et al.* (2016) reported that the conception rates at day 30 vs day 60 as 51.35±1.87% vs 43.24± 1.23% for the fresh embryos, 35.89±3.87% vs 31.19±4.01% for the vitrified embryos and 40.19±4.65% vs 34.72 ±4.15% for the frozen embryo transfer using 9day CIDR + E2 protocol.

Do *et al.* (2018) observed pregnancy rate at day 35 vs day 60 as 41.3% vs 37.6 for fresh embryo transfer and 40.0% for vitrified embryo transfer at 30-day pregnancy diagnosis using 7 d CIDR+PGF₂α protocol. Bonacker *et al.* (2020) reported conception of 34 % among recipient beef cow using 7d co synch + CIDR protocol for embryo transfer.

Steichen and Larson (2019) reported overall pregnancy as 27.5% and confirmed pregnancy as 23.3 % in recipient animal synchronized with 7day CO-Synch+ CIDR protocol for transfer of invitro produce frozen thaw embryo in dairy heifer cows.

However, the overall conception rate in present study was higher in CIDR group (14.28 per cent) when compared to Ovsynch (0 per cent) and control group (13.30 per cent). The CIDR plus $\text{PGF}_{2\alpha}$ could have increase the synchronization and pregnancy rate (Bo *et al.*, 2012). It is possible that, providing exogenous progesterone by the inclusion of CIDR device might have improved the conception rate in CIDR group. Furthermore, the plasma progesterone (P4) concentration in CIDR treated recipient cows remains elevated for 7 days upto the time of removal of CIDR, which might have contributed to increased conception rate in CIDR group.

The exogenous intravaginal impregnated CIDR for 7 days will induce negative feedback in the hypothalamus and pituitary and inhibits the release of gonadotropin hormone. After CIDR removal, larger quantities of gonadotropin were released to stimulate development of follicles, release the hormone estradiol, resume estrus and synchronize ovulation or to luteinize large dominant follicles. The $\text{PGF}_{2\alpha}$ administration at the time of CIDR withdrawal can regress the functional corpus luteum (CL), reduce progesterone concentration levels and successfully induce the growth of follicles and estrus.

In the current study, overall pregnancy rates were lower than those of other researchers on bovine embryo transfer. This may have been because, as embryo transfer work was still in its early stage of standardization. Moreover, the small sample size might be one of the causes of lesser pregnancy rate. The use of frozen thaw embryo might have effect on conception because of poor survival of IVP (*in-vitro* produced embryo) after cryopreservation (Al katani *et al.*, 2002 and Ambrose *et al.*, 1999).

However, based on present study, we may conclude that estrus synchronization of selected animals increases the number of recipients available for embryo transfer.

5.2.3 Conception Rate in Control Group

The overall conception rate in naturally cyclic animal was 13.33 per cent. The present study result was in accordance with Ambrose *et al.* (1999) finding where the pregnancy rate was recorded as 14.3 ± 2.3 in recipient animal exhibiting natural estrus.

Contrary to these results, higher conception rate was observed in naturally cyclic animals by Stewart *et al.* (2011) where the percentage of cows pregnant in non-synchronized animals was noted in as ET fresh (42.1%), ET-V (29.3%), A.I (18.3%), respectively.

5.3 EFFECT OF BODY CODITION SCORE (BCS) ON CONCEPTION RATE

In the present study, the average mean BCS of recipient cows was 2.80 ± 0.48 , 3.04 ± 0.08 , and 2.89 ± 0.37 in Ovsynch, CIDR and control groups, respectively. There is positive correlation ($P < 0.05$) found between BCS and conception rates. The mean BCS was significantly ($P < 0.05$) higher in cows that conceived (3.10 ± 0.21) than in those failed to conceive (2.87 ± 0.28) in non-pregnant cows (Table 4.7, Fig.4.7).

Similar results by Mapletoft *et al.* (1986), Ambrose *et al.* (1999), Wallace *et al.* (2011), Pereira *et al.* (2016) and Reshma *et al.* (2020) confirming the positive correlation between BCS and conception rates. Contrary to these Steichen and Larson (2019) observed that there was no effect of BCS on pregnancy.

In the present study it was found out that $BCS > 2.5$ was observed in conceived recipient cows, whereas $BCS < 2.5$ was observed in recipient cows which failed to conceive. These results are similar to Block *et al.* (2003), Wallace *et al.* (2011) and Pereira *et al.* (2016) where pregnancy rate was found to be higher when recipient animal have $BCS > 2.75$ (1-5 scale) and > 5 (1-9 scale).

The reason for better conception in recipient animals whose BCS > 2.5 was there is relationship between BCS and progesterone (P₄) plasma concentrations. Ambrose *et al.* (1999) observed increase of 2.9 ng of P₄ for whole unit increase in BCS. Thus, recipient cows with good BCS at the time of ET is important to obtain satisfactory pregnancy rates.

5.4 EFFECT OF DEPOSITION SITE IN UTERUS ON CONCEPTION

In the present study, a significance (P<0.05) difference was found between site of uterine horn where embryo was deposited and conception rate. The higher conception rates was found in recipient where embryos were deposited at cranial third of the uterine horn when compared to embryo deposited at middle third of uterine horn (Table 4.8, Fig.4.8).

Similar result was determined by Beal *et al.* (1998), Scenna *et al.* (2005), Roper *et al.* (2018) and Alkhan *et al.* (2020). This result was contradictory to Bo *et al.* (2018) where no significant difference was observed between embryo deposition site but the conception rates was found to be higher when deposited proximal to ovary.

The embryo reaches from oviduct into the uterus at approximately 6-8 days after fertilization. Therefore, in uterine flushing on day 7, most of the embryo were collected from cranial part of uterine horn (Gordon, 2004; Niemann and Wrenzyeti, 2018 and Sponchado *et al.*, 2019).

This practise of depositing embryo at cranial site might have contributed to increase in conception rate (Hasler, 2010), as uterine physiological synchrony following ovulation is at similar stage of embryo development following fertilization (Pope, 1988 and Barnes, 2000). Moreover, reproduction tract secretion varies within different location in the tract and with the stage of the estrous cycle. The current study data were in accordance with this theory of matching uterine environment with stage of embryo development to improve conception rate.

5.5 EFFECT OF CL SIZE ON CONCEPTION RATE

The average mean diameter of CL (mm) in recipient cows was 14.11 ± 0.39 , 15.63 ± 1.80 , and 13.99 ± 0.95 in Ovsynch, CIDR, and control group of study group, respectively.

In the present study group, the mean CL diameter was found to be higher in pregnant animal (16.18 ± 1.60 mm), when compared to non-pregnant (14.19 ± 0.54 mm), but statistically no difference ($P>0.05$) was observed between CL diameter and conception rates (Table 4.9) of recipient cows.

Similar results were found by Spell *et al.* (2001), Bo *et al.* (2001), Hasler *et al.* (1987), Ramsen and Rouseel. (1982), Coleman *et al.* (1987), Spell *et al.* (2001), Benyei *et al.* (2006), Looney *et al.* (2006) and Erkan *et al.* (2021) where there was no correlation seen between CL diameter and conception.

This study results are found to be contrary to Ambrose *et al.* (1999), Baruselli *et al.* (2010), Vieira *et al.* (2014) and Alkhan *et al.* (2020) who found CL diameter has an effect on pregnancy after embryo transfer. They informed that as the CL diameter increases, the pregnancy rate in recipient embryo transfer increases.

Though there was no significant ($P>0.05$) difference found between CL diameter and conception rates, but in present study the mean CL diameter for pregnant recipient cows was found to be higher (16.18 ± 1.60 mm) when compared to recipient cows in non-pregnant (14.19 ± 0.54 mm). The reason for higher pregnancy could be attributed to higher circulating progesterone concentration because of larger CL structure (Ambrose *et al.*, 1991 and Baruselli *et al.*, 2010).

5.6 EFFECT OF CERVICAL RESISTANCE WHILE PASSING ET GUN ON CONCEPTION.

In the present study, it was determined that cervix transfer scores (easy, moderate, difficult) had no statistically significant ($P>0.05$) effect on conception rates (Table 4.10). However, the higher pregnancy rate noted when cervix transfer was easy, followed by moderate and zero conception rate when cervix transfer was difficult (Table 4.10, Fig.4.10).

conception rates according to the interaction between cervix transfer score and transfer location are given in Table 4.10. The cervix transfer score is based on difficulty of accessing the site of embryo deposition and amount of time to complete the transfer. In this study the pregnancy rate followed by Embryo transfer was 66.6 (easy), 33.7 (Moderate) and 0 (hard) Per cent based on cervix transfer score.

Similar results were found by Alkan *et al.* (2020), Aguiar *et al.* (2013), Lopes *et al.* (2015), Kasimanickam *et al.* (2018) and Jaskowski *et al.* (2010).

In this study results indicate that when the time taken for conducting embryo transfer was less, there was improvement in conception rates. A lower cervix transfer score was associated with more pregnancy rate in the present study. A lower score is also an indication of less difficulty in accessing the caudal portion of ipsilateral uterine horn.

In the current experiment, as the cervix transfer score increases the pregnancy decreases. The decrease in pregnancy can be attributed to excessive uterine manipulation during embryo transfer because of difficult passage of the cervix and prolonged application leading to the release of chemical inflammatory mediators such as $\text{PGF}_2\alpha$ in response to mechanical manipulation during ET. It was reported that, $\text{PGF}_2\alpha$ may cause pregnancy losses by preventing the survival and development of the embryo (Schrick *et al.*, 1993; Purcell *et al.*, 2005 and Scenna *et al.*, 2005).

In this study, following were the reason for low conception rate 1) Site of embryo deposition in the uterine horn 2) Trauma which might have occurred to genitalia due to less experience in embryo transfer 3) Because of low number of recipients available, they were not thoroughly screened for selecting the best recipient. 4) Use of a greater number of cryopreserved embryos than fresh embryos. 5) More ET were done in field animals, so there was less control over managerial effect of recipients 6) Small sample size 7) Early embryonic losses 8) Exposure to different environment stress, nutritional status and faulty management.

From the present study it was concluded that among all treated group, CIDR protocol was most effective and efficient synchronization protocol for improving the efficacy of synchronization and conception rates in embryo transfer programme. Further, we have to standardize the estrus synchronization protocol and time of embryo transfer (in days) from onset of estrus to improve the overall portion of suitable recipients and conception rate through ET.

SUMMARY

CHAPTER VI

SUMMARY

In current study entitled “Estrus synchronization of recipient cows of farmers for transfer of *in vitro* produced sahiwal embryos” *in vitro* embryo production (IVEP) was done by using standard procedures in embryo transfer (ET) and IVF lab of the Dept of Veterinary Gynaecology & obstetrics, C.V. Sc, Korutla by aspiration of oocytes from non-pregnant Sahiwal donor cows aged 3-6 years which were maintained at, ILFC, C.V. Sc, Korutla. A total of 52 animals (heifers and post-partum cows completed 2-3 months post-partum period) free from uterine infections, belonging to farmers in combined Karimnagar district and some of the recipient animals maintained at ET and IVF project, C.V. Sc, Korutla, Jagtial (Dist.) were selected and used for embryo transfer after estrus synchronization or natural estrus to study estrus synchronization rate, onset of estrus, intensity of estrus, duration of estrus, conception rate and effect of various recipient animal factors on conception.

The selected recipient cows were randomly distributed into the following 3 groups. Group 1 (ovsynch) Cows of this group Estrous synchronized by using Ovsynch protocol (10 µg of buserelin (Receptal) given IM on day 0, 500 µg of cloprostenol (Estrumate*) given IM on day 7 and second dose of GnRH (10 µg) IM on day 9). Group 2 (CIDR) Cows of this group were estrus synchronized by using CIDR protocol (day 0 insert CIDR, day 7 CIDR remove and PGF₂α 500 µg of cloprostenol (Estrumate) given IM. Group 3 (control) cows were natural estrus were in this group as a control group. Embryo transfers will be done into these recipient cows by using nonsurgical method after day 6-7 of estrus by depositing the embryos in uterine horn (cranial or middle third) on ipsilateral side of ovary having CL.

The estrus response rates were (13/15) 86.66, (10/10) 100.00 per cent, in treated groups (ovsynch, CIDR) and (15/27) 55.55 per cent in control group. Statistical analysis revealed that there was significant difference ($P < 0.05$) between treated groups (ovsynch, CIDR) and control group in the present experiment.

The mean onset of estrus was recorded as 53.69 ± 1.90 , 49.1 ± 0.48 and 109.73 ± 18.74 in ovsynch, CIDR, and control groups, respectively in the present experiment. The onset of estrus has no significant ($P > 0.05$) difference between ovsynch and CIDR treated group, but both CIDR and ovsynch group were significantly ($P < 0.05$) different with control group.

The overall intensity of estrus was 15.79, 44.74 and 39.47 as weak, normal or intermediate and intense respectively. The ovsynch study group cows exhibited 7.69, 61.53 and 30.76 percent as weak, normal and intense estrus respectively. The CIDR study group cows exhibited 0, 30, and 70 per cent as weak, normal and intense estrus, respectively.

The mean score for estrus intensity were observed as 13.38 ± 0.94 , 16.30 ± 1.59 and 11.07 ± 1.05 in ovsynch, CIDR, and control groups, respectively. The statistical analysis revealed that there was significant difference ($P < 0.05$) in mean estrus score between CIDR and Control group, but CIDR and Ovsynch group has no significant difference ($P > 0.05$). Similarly, there was no significant difference ($P > 0.05$) between ovsynch and control group.

The mean duration of estrus recorded was 21.61 ± 0.77 , 20.30 ± 0.72 , and 20.47 ± 0.70 hrs in ovsynch, CIDR and control groups, respectively in the present experiment. Statistical analysis revealed that there was no significance difference ($P > 0.05$) observed in mean duration of estrus in ovsynch, CIDR and control groups.

The overall conception rate in therapeutic cows were observed as 14.28, 0, and 13.3 percent in CIDR, Ovsynch and control groups, respectively. There was no significant ($P>0.05$) difference found among the treated groups and control group. The overall conception rate was more in CIDR group followed by control group.

The average mean BCS was 2.80 ± 0.48 , 3.04 ± 0.08 , and 2.89 ± 0.37 in Ovsynch, CIDR and control groups, respectively. In the present study there was positive correlation ($P<0.05$) found between BCS and conception rates. The mean BCS was significantly higher in cows that conceived (3.10 ± 0.21) than in those failed to conceive (2.87 ± 0.28).

In present study, there was significance ($P<0.05$) difference found between site of uterine horn where embryo is deposited and conception rate. The higher conception rates was found in recipients where embryos were deposited at cranial third of the uterine horn when compared to embryo deposited at middle third of uterine horn ($P<0.05$).

The average mean diameter of CL in recipient cows was 14.11 ± 0.39 , 15.63 ± 1.80 , and 13.99 ± 0.95 in Ovsynch, CIDR, and control group of study group respectively. In the present study group, there was no correlation ($P>0.05$) found between CL diameter and conception rates of recipient cows. The mean CL diameter was 16.18 ± 1.60 (14.45-19.35) mm in pregnant and 14.19 ± 0.54 (10-23) mm in non-pregnant cows.

In the present study it was determined that cervix transfer scores had no significant ($P>0.05$) effect on conception rates. However, it was also found that the probability of pregnancy was higher when the cervix transfer score was easy and moderate when compared to the difficult.

From the present study it was concluded that among all treated group, CIDR protocol was most effective and efficient synchronization protocol for improving the efficacy of synchronization and conception rates in embryo transfer programme. Further, we have to standardize the estrus synchronization protocol and time of embryo transfer (in days) from onset of estrus to improve the overall portion of suitable recipients and conception rate through ET.

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