STUDIES ON CERTAIN HORMONAL AND NUTRITIONAL STRATEGIES TO IMPROVE SYNCHRONIZATION OF ESTRUS AND FERTILTIY IN EWES UNDER FARM CONDITIONS DURING NON BREEDING SEASON

By

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B.V.Sc. & A.H

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CERTIFICATE

Dr. T. Srisandhya, ID. No. GVM/2018-025 has satisfactorily prosecuted the course of research and that the thesis entitled ***"studies on certain hormonal and nutritional strategies to improve synchronization of estrus and fertility in ewes under farm conditions during non breeding season"*** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any university.

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CERTIFICATE

This is to certify that the thesis entitled ***"studies on certain hormonal and nutritional strategies to improve synchronization of estrus and fertility in ewes under farm conditions during non -breeding season"*** submitted in partial fulfilment of the requirements for the degree of **Master of Veterinary Science** of the Sri Venkateswara Veterinary University, Tirupati, is a record of the bonafide research work carried out by **Dr. T. Srisandhya** (**GVM/2018-025)** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

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 the investigations have been duly acknowledged by the author of the thesis.

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Dr. T. SRISANDHYA…

DECLARATION

I **Dr. T. Srisandhya** (GVM/2018-025) hereby declare that the thesis entitled ***"studies on certain hormonal and nutritional strategies to improve synchronization of estrus and fertility in ewes under farm conditions during non breeding season"*** submitted to Sri Venkateswara 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 published earlier in any manner.

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Signature of the candidate

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ABSTRACT

The present study was undertaken to determine the efficacy of hormonal and nutritional strategies in postpartum anestrous Vizianagaram ewes during the non-breeding season. A total of 48 healthy postpartum anestrous ewes aged about 2 to 4 years were selected and randomly divided into four groups Selectsynch (Group 1), Progesterone+Ovsynch (Group 2) nutritional supplement (Group 3) and conventional feeding control (Group 4) with each group consisting of twelve ewes.

In Selectsynch group, each ewe was administered with GnRH analogue (4.0 µg) on day 0 and $PGF_2\alpha$ analogue (125 µg) on day 7 intramuscularly. In Progesterone+Ovsynch group, progestrone sponge was inserted intra-vaginal and GnRH analogue @ 4.0 µg was administered along with sponge insertion on day 0. On day 7 $PGF_2\alpha$ analogue cloprostenol (125µg) was administered intramuscularly and the sponge was removed and second injection of 4.0 µg GnRH analogue was administered on day 9. In feed supplement group, each ewe was given 200 gm of additional concentrate feed along with 20 gm of mineral and vitamin mixture for 9 days along with regular concentrate feed. The control group ewes were maintained on conventional feeding and grazing.

The estrus response rate was 66.66, 100.00, 50.00 and 33.33 per cent in group 1, 2, 3 and 4, respectively. Significantly ($P<0.05$) shorter time interval to onset of estrus (hrs) was observed in Progesterone+Ovsynch (38.00±4.63) group followed by Selectsynch (45.00±7.08), nutritional supplement (56.00±8.00) and control group (90.00±18.00) groups. The mean duration of estrus was 24.00±4.82, 26.00±2.88, 24.00±1.64 and 24.00±2.01 hrs in Groups 1, 2, 3 and 4, respectively without significant difference ($P>0.05$) among treatment groups but with significantly intense estrus ($P<0.05$) in Progesterone+Ovsynch (8.60±0.47) group than in Selectsynch (7.50±0.65), nutritional supplements (6.10±0.24) and control (5.00±0.22) groups.

Significantly (P<0.05) higher conception rate was recorded in Progesterone+Ovsynch group (66.66%) when compared to Selectsynch (50.00%), nutritional supplement (33.33%) and control (25.00%) groups. The overall percentage of conception rate by using ultrasonography was 39.58% (19/48) Vs 43.75% (21/48) non-return basis among all treatment groups**.**

In the present study it was observed that Progesterone sponge plus Ovsynch treatment achieved higher estrus response rate with higher conception rate when compared to other treatments during non-breeding season in Vizianagaram anestrous ewes.

List of symbols and abbreviations

"	Inch
%	Per cent
<	Lesser than
>	Greater than
±	Plus or minus
≤	Lesser than or equal
≥	Greater than or equal
µg	Microgram
µl	Microlitre
^{0}C	Degree of Celsius
AI	Artificial insemination
AV	Artificial Vagina
Cm	Centimeter
D	Days
eCG	Equine Chorionic gonadotropin
ELISA	Enzyme Linked Immunosorbent Assay
FGA	Fluorogestone acetate
Fig	Figure
gms	Grams
i/m	Intramuscular
IU	International Units
Kg	Kilogram
Lbs	Pounds
mg	Milligram

Min	Minute
Ml	Millilitre
ng/mL	Nanogram per milliliter
NRR	Non return Rate
$PGF_{2\alpha}$	Prostaglandin F_2 alpha
PMSG	Pregnant Mare Serum Gonadotropin
Rpm	Rotations Per Minute
SE	Standard Error
Sec	Second
TAI	Timed Artificial Insemination
TCFY	Tris Citric acid Fructose Egg Yolk
U/S	Ultrasound
Vs	Versus

CHAPTER-I

1. INTRODUCTION

Sheep hold an important place among the livestock species for livelihood by the farming community, contributing considerably to the Indian economy especially in arid, semi-arid and mountain areas. Large proportion of small farmers and landless labourer's rear sheep for either meat, wool or both. The current population of sheep in the world is 1610.78 million with their numbers in India as 74.26 million and the total sheep population in Andhra Pradesh is 17.6 million (Livestock census, 2012).

About 13.8% of the total livestock is contributed by sheep. India stands 5th among the meat producing countries in the world and 9th in wool production. In Andhra Pradesh, sheep are socio-economically important as they are primarily reared for meat in rural areas. The two main breeds of Andhra Pradesh are Nellore and Deccani. Apart from these, north coastal region consisting of Srikakulam, Vizianagaram and Visakhapatnam is endowed with Vizianagaram lesser known genetic group of sheep which is akin to Nellore sheep (Jodipi), constituting 4.5 per cent of total sheep population of AP, which is yet to be recognized. This sheep is the main source of meat supply in this part of Andhra Pradesh.

Besides, contributing food and inputs for crop production, sheep are important as savings or investments for the poor household and provide security and monetary benefit through various ways in different production systems (Kitalyi *et al.*, 2005). Most of the Indian sheep breeds, however, are less prolific in terms of fecundity rate and have a prolonged postpartum anestrous interval (Perez *et al*., 2002; Rhodes *et al*., 2003 and Millesi *et al*., 2008). Therefore, there is a dire need to identify more prolific sheep breeds suitable for rural conditions of feeding and management in order to improve the economic status of the rural farmers.

The reproductive performance of the sheep is influenced by several extrinsic factors such as social, sexual interactions and nutritional status (Alvarez and Zarco, 2001; Gundogan, *et al*., 2003 and Bearden *et al*., 2004). The reproductive functions are highly challenging with both, quality and quantity of nutrients (Blache *et al*., 2008).

There is paucity of information on the reproductive performance of Vizianagaram sheep as limited studies were made at one Livestock Research Station, Garividi of SVVU. In the existing data, it was observed that most of the ewes were bred during the months of July and August (breeding season), with minimum breeding activity during the months of March, April and May (non-breeding season).

Sheep exhibit their seasonality in reproduction and domestication did not modify their pattern of seasonal reproduction. Indian sheep breeds show their cyclicity throughout the year but majority exhibit estrus symptoms during autumn (August-October) and spring (February-March).

Estrus synchronization is one of the efficient ways to overcome seasonality and manage the reproduction efficiently (Homeida *et al.*, 2009) for enhancement of the productivity, synchrony of breeding programme and reduction in labour cost (Das *et al.*, 1999). The improvement of reproductive efficiencies (Hashemi *et al.*, 2006) and management practices like nutritional and/or hormonal treatments caused higher estrus responses and subsequent fertility rates (Kridli *et al.*, 2003) and thus enhanced the productivity of sheep (Dudhatra *et al.*, 2012).

Estrus synchronization by hormonal treatments improved the reproductive efficiency and fertility rate in indigenous sheep breeds and was useful to design cost effective breeding programmes (Wildeus, 2000). Prostaglandin ($PGF_2\alpha$) was the earliest hormone used in the livestock industry as a management tool to induce estrus by regression of the corpus luteum (CL) and subsequent events with expression of behavioural estrus. The ability of exogenous $PGF_2\alpha$ to cause CL regression depended

on day of administration of drug (day of estrous cycle), dose, frequency of exposure and route of administration (uterine Vs systemic) (Kridli *et al.*, 2003)

Some trials were conducted to improve the response obtained with the standard progesterone impregnated intra-vaginal sponges by incorporating the use of gonadotrophic releasing hormone which is administered either at the beginning of estrus or at the time of artificial insemination. The gonadotropin releasing hormone (GnRH) treatment induced an LH peak, within 1-4 hrs of treatment and reduced the period of events leading to ovulation (Eppleston *et al*., 1991). Many authors have successfully used GnRH treatment in combination with progestagens, gonadotropins and $PGF_2\alpha$ (Rubianes *et al*., 2003 and Reyna *et al.*, 2007) and it is well documented that all the preovulatory events and ovulation could be induced in seasonally anovulatory ewes, if they were treated with multiple injections of GnRH. Besides, it has been demonstrated that exogenous GnRH treatment immediately after artificial insemination increased the rate of multiple births in synchronized ewes (Turk *et al*., 2008).

Fertility improvement programmes in small ruminants were not given much attention with regards to fertility by supplementation of improved nutrition and hormonal treatments during the non-breeding season. Therefore, the present study was aimed to evaluate the use of GnRH, $PGF_2\alpha$ and intra-vaginal progesterone sponges along with improved nutrition and nutritional supplements in Vizianagaram sheep on reproductive parameters like estrus and ovulation synchronization, conception rate during the non-breeding season. Hence, the present study entitled “Studies on certain hormonal and nutritional strategies to improve synchronization of estrus and fertility in ewes under farm conditions during non-breeding season” was undertaken with the following objectives.

Objectives of investigation:

1) To assess the efficacy of Selectsynch protocol for induction of estrus and fertility in Vizianagaram ewes
2) To assess the efficacy of Ovsynch protocol with progesterone sponges for induction of estrus and fertility in Vizianagaram ewes.
3) To study the efficacy of improved nutrition and nutritional supplements on estrus, ovulation and fertility in Vizianagaram ewes.

CHAPTER-II

2. REVIEW OF LITERATURE

The present research work was planned on the topic entitled "Studies on certain hormonal and nutritional strategies to improve synchronization of estrus and fertility in ewes under farm conditions during non-breeding season" and efforts have been made to review available literature of research conducted in sheep in India and abroad on synchronization of estrus, pregnancy diagnosis and fertility.

2.1 ESTRUS SYNCHRONIZATION IN EWES:

Estrus synchronization is a valuable management tool, which has been successfully employed to enhance reproductive efficiency, particularly in ruminants (Kusina *et al.*, 2000). The synchronization of estrus can be defined as predictably altering the time of estrus within the breeding season. The estrous cycle can also be controlled with the aim of inducing estrus and ovulation in pubertal females irrespective of the phase of the cycle (Lewis, 1996).

There are several ways to control the estrous cycle in ewes, such as manipulation of lighting, the ram effect and hormone treatments with progesterone, prostaglandin (PG), equine chorionic gonadotropin (eCG) and gonadotropin releasing hormone (GnRH) (Iida *et al*., 2004). Among these hormonal treatments, the synchrony of estrus had been highlighted as a tool to improve the reproductive efficiency of herds and flocks (Ozyurtlu *et al.,* 2008). In small ruminants, estrus synchronization was achieved either by reducing the length of the luteal phase with prostaglandin$F_2\alpha$ or by extending the cycle artificially with exogenous progesterone or more potent progestagens (Kusina *et al*., 2000).

The most common protocol for estrus synchronization in sheep was based on progestagen/progesterone treatment in the form of intra-vaginal implants (sponges /CIDR) (Abecia *et al*., 2011). This hormonal manipulation that could be used during

the breeding and the seasonal anestrous period, induced a great negative feedback on LH secretion and, in some instances, might have caused spontaneous luteolysis, after the withdrawal of the pre-ovulatory LH surge, ovulation occurred in a controlled manner.

Treatment with progestagens during the luteal phase accelerated follicular development, but at the same time, it reduced the number of large follicles, increased follicular atresia rate and supported the persistence of large estrogenic follicles, while treatment during the follicular phase reduced both the number of large follicles and ovulation rate (Noel *et al*., 1994 and Leyva *et al*., 1998).

These observations were attributed to the follicular status at the time of progestagen treatment or to the fact that progestagen concentrations gradually decreased after the second day of treatment, which altered the physiologic mode of LH secretion (Scaramuzzi *et al*., 1988). To overcome this insufficient progestational support, the use of two consecutive sponges inserted in a 7-day interval has been proposed in conjunction with a luteolytic dose of $PGF_2\alpha$ at the time of second sponge removal (Gonzalez *et al*., 2005). Without a clear physiological reasoning, the duration of progesterone treatment traditionally lasted for 11-14 days.

Short-term exposure to progesterone could sustain acceptable synchronization rates in superovulatory response (Menchaca *et al.,* 2007 and Martemucci and Dalessandro, 2010). Beyond the possible alteration in endocrine status, use of progestagens had also increased the consumers concern on the safety of meat products from the treated animals (Galbraith, 2002).

Prostaglandin $F_2\alpha$ was the most potent luteolytic agent for small ruminants and it could be used during the breeding season as an alternative to progestagens for estrus synchronization. The responsiveness of the corpus luteum to $PGF_2\alpha$ was limited between days 3 and 14 of the estrous cycle and, consequently, the stage of the estrous

cycle when $PGF_2\alpha$ was administered, it affected the timing of preovulatory LH surge and subsequent ovulation (Acritopoulou and Haresign, 1980). Acceptable embryo production could be achieved in previously superovulated sheep by a single cloprostenol injection administered during the mid-luteal phase of the estrous cycle (Mayorga *et al.*, 2011).

During the breeding season, combined administration of GnRH analogue and $PGF_2\alpha$ resulted in desirable estrus synchronization rates, as exemplified by the acceptable conception rate (50%) subsequent to fixed time insemination. The protocol consists of two GnRH injections given 7 days apart, while $PGF_2\alpha$ was administered on the fifth day. Fixed time laparoscopic intrauterine insemination is performed 12-14 hrs after the second GnRH injection (Deligiannis *et al*., 2005).

2.1.1. Use of prostaglandins for the synchronization of estrus in ewes:

Prostaglandin $F_2\alpha$ and its synthetic analogues (PG) had been studied extensively since its discovery in 1970 as a powerful luteolytic agent (McCracken *et al.*, 1970). Administration of PG between days 5 and 14 of the estrous cycle induced luteolysis (rapid luteal regression), followed by estrus and ovulation (Acritopoulo and Haresign, 1980). However, the onset of estrus, the preovulatory LH surge and the interval from the PG treatment to ovulation differed among ewes, depending on the stage of the estrous cycle at treatment (Deaver *et al*., 1986).

The most widely used synthetic analogue was Cloprostenol which was 100-fold more potent than $PGF_2\alpha$, and with more selective biological properties. The luteolytic effect of Cloprostenol administered intramuscularly was identical to that produced by a local ovarian infusion of $PGF_2\alpha$ via the ovarian artery (Baird *et al*., 1976). Its effectiveness was partial because of the most selective action of this compound on the mature CL (Dukes *et al*., 1974) and owing to its long half-life (Baird *et al*., 1976). An injection of 100 µg of Cloprostenol resulted in a high degree of

synchrony to induce estrus and the timing of the LH peak. Other researchers suggested that the appropriate dose of this analogue was 125 mg (Abecia *et al.*, 2011). However, doses as low as 50 mcg were reported to be effective to induce luteolysis in ewes (Baird *et al.*, 1976).

When PG was administered to a group of cycling ewes, luteal regression occurred in 66.0% of the ewes with the subsequent induction of estrus (37.7±1.6 hours). The administration of a second injection of PG induced estrus in most of the ewes when there was no reference to the stage of the estrous cycle at the time of the first injection (Acritopoulou *et al*., 1978).

In a 7 days interval between two PG treatments, Rubianes *et al*. (2003) and Contreras *et al.* (2009) recorded the onset of estrus as 40.6±0.5 hrs, whereas in a 9 day interval Acritopoulou *et al*. (1978) reported the onset of estrus as 38.3±1.3 hrs and in 11 day interval, Oyediji *et al*. (1990) obtained the onset of estrus as 41.2±2.2 hrs after second PG injection.

Similarly, when PG was administered during the mid-luteal phase, plasma progesterone concentrations slowly declined to sub-luteal values; therefore, estrus behaviour and ovulation were delayed (Houghton *et al.*, 1995).

Oliveira *et al.* (2011) observed that the conception rate in Corriedale ewes synchronized by 2 injections of delprostenate 7 and 8 days apart with a dose of 160 and 80 µg and timed insemination with fresh semen at 42 hrs after 2nd $PGF_2\alpha$ was 42.0 and 24.0%, respectively and 33.0 and 29.0%, respectively. While the same authors synchronized ewes with a dose of 160 µg given 7 or 8 days apart with two TAI times 42 or 48 h after 2nd $PGF_2\alpha$ and observed that the conception rates were 44.83 and 51.03%, respectively and the same given 8 days apart with two TAI was 32.65 and 28.77%, respectively.

2.1.2 Use of gonadotropins for the synchronization of estrus in ewes:

2.1.2.1 Estrus response rate:

Ali *et al.* (2009) observed that the estrus response rate (ERR) was 68.80% in Farafra ewes treated with two IM injections of 25 µg of GnRH on day 0 and 7 along with an IM injection of 15 mg of $PGF_2\alpha$ on day 6.

Martinez *et al.* (2011) reported that the ERR was 25.50% in Dorper ewes treated with two IM injections of 100 µg of GnRH on day 0 and 6.5 along with an IM injection of 7.5 mg of Luprostinol on day 7.

Ashmawy (2012) studied the effect of Ovsynch protocol on the reproductive performance of Rahmani ewes. They were divided into three treatment groups. All ewes in the treatment groups were intramuscularly injected on day 0 with 1 ml of GnRH analogue, followed by injection of 0.7 ml of $PGF_2\alpha$ analogue by 5 days (G1), 6 days (G2) or 7days (G3). A second dose of 1ml of GnRH analogue was administered on day7 (G1), day8 (G2) or day9 (G3). The ERR was 10.00, 30.00 and 30.00% in G1, G2 and G3 groups, respectively.

Kulaksiz *et al.* (2013) observed that the estrus response rate was 82.3±9.5% in fat tailed ewes treated with two IM injections of 0.004 mg of GnRH on day 0 and 9 along with an IM injection of 125 µg of $PGF_2\alpha$ on day 7.

Hashem *et al.* (2015) studied effects of two estrus synchronization protocols (Ovsynch Vs. double PGF2α injection) on estrus activity and recorded ERR in GPG and $PGF_2\alpha$ group as 42.85 and 78.57%, respectively.

Lone *et al.* (2016) observed that the ERR was 28.57% in infertile Corriedale sheep treated with two I/M injections of 5 mg of Buserelin acetate on day 0 and 7 along with an IM injection 263 mg of Cloprostenol on day 5.

Rekik *et al.* (2016) reported that the ERR was 73.3% in Menz breed ewes and were received two IM injections of 50 µg of GnRH analogue (Gonadorelin) on day 0 and 9 along with an IM injection of 1 ml Enzaprost on day 7.

Abu *et al.* (2016) reported that the ERR was 93.33% in Rahmani ewes treated with two IM injections of GnRH, 1 ml on the first day (day 0) and 1.5 ml on day 11 along with a single IM injection of 125µg of $PGF_2\alpha$ administered on day 9 prior to the second injection of GnRH.

Daghash *et al.* (2017) observed that the ERR was 100.00% in Egyptian ewe lambs treated with two IM injections of 1.25 ml of Receptal (0.5 µg of GnRH) on day 0 and 9 along with a single IM injection of 0.5 ml of Estromate (125 µg of $PGF_2\alpha$) on day 7.

Waheeb *et al.* (2017) reported that the ERR was 50.00% in ewes treated with two IM injections of 4 µg of Buserelin on day 0 and 7 along with an IM injection of 250 µg of Cloprostenol on day 5.

2.1.2.2 Onset of estrus:

Ali *et al.* (2009) observed that the onset of estrus was 41.0±9.6 hrs after $PGF_2\alpha$ injection in Farafra ewes treated with two IM injections of 25µg of GnRH on day 0 and 7 along with an IM injection of 15 mg of $PGF_2\alpha$ on day 6.

Oliveira *et al.* (2009) reported that the onset of estrus was 13.37±8.42 hrs after $PGF_2\alpha$ injection in Santa Ines ewes treated with two IM injections of 25µg of GnRH on day 0 and 9 along with an IM injection of 37.5 µg of $PGF_2\alpha$ on day 7.

Ashmawy (2012) studied the effect of Ovsynch protocol on the reproductive performance of Rahmani ewes. The ewes were equally divided into three treatment groups. All ewes in the treated groups were intramuscularly injected on day 0 with 1 ml of GnRH analogue, followed by intramuscular injection of 0.7 ml of $PGF_2\alpha$

analogue (Estromate) by either 5 days (G1), 6 days (G2) or 7 days (G3). A second dose of GnRH analogue (1 ml) was given on day 7 (G1), day 8 (G2) or day 9 (G3). The onset of estrus observed in groups G1, G2 and G3 after injection of $PGF_2\alpha$ analogue was 42.0±0.00, 46.0±1.63 and 49.3±1.08 hrs, respectively.

Kulaksiz *et al.* (2013) observed that the interval to onset of estrus was 50.5±1.3 hrs in fat tailed ewes treated with two IM injections of 0.004 mg of GnRH on day 0 and 9 along with an IM injection of 125 µg of $PGF_2\alpha$ on day 7.

Hashem *et al.* (2015) studied the effects of two estrus synchronization protocols (Ovsynch Vs double $PGF_2\alpha$ injection 11 days apart) on estrus activity and recorded the interval to onset of estrus in GPG and $PGF_2\alpha$ group as 34.0±2.1 and 50.4±7.3 hrs, respectively.

Abu *et al.* (2016) treated 15 Rahmani ewes with two IM injections of GnRH, 1 ml on the first day (day 0) and 1.5 ml on day 11^{th}, with a single IM injection of 125 µg of $PGF_2\alpha$ given on day 9 prior to the second injection of GnRH and observed that the mean interval from treatment to onset of estrus was 5.40±0.60 days.

Rekik *et al.* (2016) reported that the mean onset of estrus was 11.0±2.84 hrs after second injection of 50 µg of Gonadorelin in ewes treated with two IM injections of 50 µg of GnRH analogue Gonadorelin on day 0 and 9 along with an IM injection of 1ml of Enzaprost on day 7.

Daghash *et al.* (2017) observed that the average time for onset of estrus was 74.00±3.49 hrs in Egyptian ewe lambs treated with two IM injections of 1.25 ml of Receptal (0.5 µg, GnRH) on day 0 and 9 along with an IM injection of 0.5 ml of Estromate (125 µg, $PGF_2\alpha$) on day 7.

2.1.2.3 Estrus intensity:

Perusal literature revealed that no reports were available with regards to intensity of estrus during the synchronized and induced estrus, hence could not be reviewed.

2.1.2.4 Duration of estrus:

Ali *et al.* (2009) treated Farafra ewes with two IM injections of 25 µg of GnRH on day 0 and 6 along with 15 mg of $PGF_2\alpha$ on day 7 and observed that the duration of estrus was 19.0±2.1 hrs.

Ashmawy (2012) studied the effect of Ovsynch protocol on the reproductive performance of Rahmani ewes. The ewes were equally divided into three treatment groups. All ewes in the treated groups were intramuscularly injected on day 0 with 1 ml of GnRH analogue, (Receptal) followed by intramuscular injection of 0.7 ml PGF2α analogue (Estromate) by either 5 days (G1), 6 days (G2) or 7 days (G3). A second dose of GnRH analogue (1ml) was given on day7 (G1), day8 (G2) or day9 (G3).The duration of estrus in G1, G2 and G3 group was 30.0±0.0, 34.0±3.2 and 32.0±1.6 hrs, respectively.

Kulaksiz *et al.* (2013) observed that the duration of estrus was 24.0±1.2 hrs in Fat tailed ewes with Ovsynch protocol.

Hashem *et al.* (2015) studied the effects of two estrus synchronization protocols (Ovsynch Vs double $PGF_2\alpha$ injection) on estrus activity, characterization of the ovulatory wave and fertility in ewes during the breeding season. The Ovsynch treated ewes received a GnRH analogue (4 µg of Buserelin; i.m.) on day 0 followed by $PGF_2\alpha$ analogue (10 mg of Dinoprost tromethamine; IM) on day7 and a second injection of a GnRH analogue on day 9. The double $PGF_2\alpha$ treated ewes received

$PGF_2\alpha$ analogue on day 0 and day 11. The duration of estrus in GPG-group and double $PGF_2\alpha$ group was 24.0±0.0 and 32.4±1.8 hrs, respectively.

Rekik *et al.* (2016) reported that the duration of estrus was 18.6±1.72 hrs in adult Menz ewes treated with two IM injections of 50 µg of the Gonadorelin on day 0 and 9 along with an IM injection of 1 ml of Enzaprost on day 7.

Abu *et al.* (2016) observed that the duration of estrus was 40.00±16.80 hrs in Rahmani ewes treated with two IM injections of GnRH, 1 ml on the first day (day 0) and 1.5 ml on day 11, as well as, a single injection of 125 µg of $PGF_2\alpha$ given on day 9 prior to the second injection of GnRH.

Daghash *et al.* (2017) observed that the duration of estrus was 33.75±2.65 hrs in five Egyptian ewe lambs treated with Ovsynch protocol.

2.1.2.5 Fertility rate:

Deligiannis *et al.* (2005) treated Mature dry ewes (n=28) of Karagouniko breed with GnRH analogue (8 µg/ewe; Buserelin) administered on day 7, $PGF_2\alpha$ (4 mg/ewe; Luprostiol) on day 2. Thirty-six hours later (day 1), ewes received a second dose of GnRH injection. Intrauterine insemination was performed 12–14 h after the second injection of GnRH. Fourteen ewes (50.00%) conceived at insemination and maintained pregnancy; from the remaining 14 ewes 10 became pregnant at natural service, while four, although they were mated at least two to three times but failed to conceive. The overall conception rate was 85.71 per cent.

Ali *et al.* (2009) reported that the conception rate was 50.00% in Farafra ewes treated with two IM injections of 25 µg of GnRH on day 0 and 7 along with15 mg of $PGF_2\alpha$ on day 6.

Oliveira *et al.* (2009) evaluated the efficacy of the Ovsynch protocol in Santa Ines ewes. Twenty six Santa Ines ewes were assigned to three treatments. In treatment

1, control group, estrus was synchronized with sponges containing 60 mg MAP for 14 days and 300 IU of eCG was administered on day 14. In treatment 2 (Ovsynch protocol), IM injections of 25µg of GnRH on day 0, 37.5 µg of $PGF_2\alpha$ on day 7 and 25µg of GnRH on day 9 were administered. In treatment 3, modified Ovsynch protocol was used by administering $PGF_2\alpha$ and second GnRH injections two days earlier. All ewes were mated twice at 12 hrs interval. The conception rate in treatment group 1, 2 and 3 was 37.50, 62.50 and 25.00%, respectively. They concluded that Ovsynch protocol increased the reproductive performance of ewes.

Martinez *et al.* (2011) observed that the conception rate was 18.60% in Dorper ewes treated with two IM injections of 100 µg of GnRH on day 0 and 6.5 along with an IM injection of 7.5 mg of Luprostinol administered on day 5.

Martinez *et al.* (2013) evaluated the effect of GnRH and Cloprostenol application on pregnancy and prolificacy rates on Pelibuey ewes. Forty five ewes were randomly allocated to one of three treatments: T1 (n=15), day 0: sponges with 65 mg Medroxy progesterone acetate (MPA) + 200 IU of eCG and sponge removal (day 12) + breeding by natural mating (days 12-15); T2 (n=15), day 0: 50 µg of GnRH + 7.5 mg D-Cloprostenol (day5) + 50µg of GnRH (day7) + insemination at fixed time (AIFT) 12 to 14 hrs after last injection of GnRH; T3 (n=15), 100 µg of GnRH (day 0) + 7.5 mg of Cloprostenol (day 5) + 100 µg of GnRH (day 7) + AIFT 12 to 14 hrs after last injection of GnRH. There were differences ($p<0.05$) for pregnancy rates of 60.00, 33.33 and 46.66% respectively, among the treatments T1, T2 and T3. It is concluded that the use of GnRH and D-Cloprostenol were improved the pregnancy rates.

Hashem *et al.* (2015) studied the effects of two estrus synchronization protocols (Ovsynch Vs double $PGF_2\alpha$ injection) in ewes during the breeding season. The Ovsynch treated ewes received a GnRH analogue (4 µg of Buserelin; IM) on day

0 followed by $PGF_2\alpha$ analogue (10 mg of Dinoprost tromethamine; IM) on day7 and a second injection of GnRH analogue on day 9. The double $PGF_2\alpha$ treated ewes received $PGF_2\alpha$ analogue on day 0 and day 11. Estrus response Vs pregnancy rate among estrus responded ewes in GPG-group and double $PGF_2\alpha$ group was 42.85 Vs 100.00% and 78.57 Vs 81.82%, respectively.

Daghash *et al.* (2017) reported that the conception rate was 80.00% in Egyptian ewe lambs that were treated with Ovsynch protocol.

Waheeb *et al.* (2017) treated 10 Barki ewes with an injection of 4μg of Buserelin on day 0 and 7 along with 250 μg of Cloprostenol on day 5 and recorded the estrus response and conception rate as 50.00 and 100.00%, respectively.

2.1.3 Use of Progesterone sponges plus Ovsynch:

2.1.3.1 Estrus response rate:

Greyling *et al.* (1997) reported 100.00% ERR after estrus synchronization using both FGA and MAP intra-vaginal sponges followed by 600 IU of PMSG intramuscularly.

Simonetti *et al.* (2000) treated Merino ewes with intra-vaginal sponges impregnated with different doses of MAP (Group I, 40; II, 50 and III, 60 mg). After 14 days, sponges were removed and it was concluded that there was no significant difference among the groups pertaining to ERR (79.27, 77.42 and 80.87%, respectively).

Dias *et al.* (2001) reported that ERR in Woolless ewes was 76.70, 96.70 and 34.60% with 200, 400 and 600 IU of eCG injection, respectively at 12^{th} day of 30 mg of FGA sponge removal.

Boscos *et al.* (2002) observed that the ERR was 92.50% in Chios and Berrichon ewe breeds after estrus synchronization with MAP intra-vaginal sponges in situ for 12 days followed by 400 IU of PMSG at the time of sponge removal.

Alminer *et al.* (2005) recorded the ERR as 80.00% after estrus synchronization in ewes with FGA intra-vaginal progestogen sponges in situ for 14 days followed by 600 IU of PMSG at the time of sponge removal.

Timurkan and Yildiz (2005) observed that the ERR was 100.00% after estrus synchronization with 40 mg of FGA intra-vaginal sponges in-situ for l4 days followed by IM injection of 500, 600 and 750 IU of PMSG at the time of sponge removal in Hamdani ewes during breeding season.

Ucar *et al.* (2005) stated that the ERR was 77.8±14.7% after estrus synchronization with 30 mg of FGA intra-vaginal sponges in situ for 14 days followed by 600 IU of PMSG at the time of sponge removal in Tuj ewes.

Ataman *et al.* (2006) observed that the ERR was 100.00 and 86.60% during breeding and non-breeding season, respectively after estrus synchronization with 30 mg of FGA intra-vaginal progesterone sponges in situ for 12 days followed by 400 IU of PMSG and 0.294 mg of Dinoprost tromethamine ($PGF_2\alpha$ analogue) administered intramuscularly at the time of sponge removal.

Dogan and Nur (2006) reported that the ERR was 72.20 and 88.90%, respectively during 24.0±6.0 hrs and within 120 hrs of sponge removal after estrus synchronization with 60 mg of MAP intra-vaginal sponge in situ for 12 days followed by 500 IU of PMSG administered IM 48 hrs before sponge removal.

Ali (2007) documented that the ERR was 83.30% after estrus synchronization in ewes with 40 mg of FGA intra-vaginal sponges in situ for 8 days followed by 500 IU of eCG and $PGF_2\alpha$ given intramuscularly at the time of sponge removal.

Luther *et al.* (2007) reported that the ERR in ewes was 100.00% with 30 mg of FGA intra-vaginal sponge treatment for 12 days followed by 400 IU of eCG treatment at progestin withdrawal.

Reyna *et al.* (2007) reported 100.00% ERR after estrus synchronization with 40 mg of FGA intra-vaginal sponges in situ for 12 days followed by injection of PMSG on 10^{th} day after sponge insertion.

Ustuner *et al.* (2007) recorded ERR as 18.20 and 100.00%, respectively for first 18.0±6.0 and within 140 hrs of sponge withdrawal in Awassi ewes after estrus synchronization with 30 mg of FGA intra-vaginal sponges in situ for 12 days followed by 300 IU of PMSG at the time of sponge removal and it was observed that the estrus occurred between 12-78 hrs after sponge withdrawal.

Karaca *et al.* (2009) documented that the ERR was 92.50% after estrus synchronization in ewes with 30 mg of FGA intra-vaginal sponge in situ for 12 days followed by 400 IU of eCG and $PGF_2\alpha$ (0.2 mg of Tiaprost tromethamine) at the time of sponge removal.

Koyuncu and Alticekic (2010) reported that the ERR was 95.90% during the 48-72 hrs observation period after estrus synchronization with 30 mg of FGA intra-vaginal progestogen sponges in-situ for 14 days followed by 500 IU of PMSG at the time of sponge withdrawal.

Hristova *et al.* (2011) observed that the estrus response was 100.00% and the estrus occurred between 48 and 60 hrs after estrus synchronization with 30 mg of FGA intra-vaginal sponges in-situ for 12 days followed by 500 IU of PMSG treatment at sponge removal.

Ozyurtlu *et al.* (2011) recorded that the ERR was 87.50% in Awassi ewes during non-breeding season with 30 mg of FGA intra-vaginal sponge followed by administration of 400 IU of PMSG at sponge removal.

Zonturlu *et al.* (2011) recorded the ERR as 81.00, 92.60 and 92.00%, following estrus synchronization with intra-vaginal sponge containing 30mg of FGA in situ for 12 days followed by 300, 400 and 500 IU of PMSG, respectively at the time of sponge removal.

Cavalcanti *et al.* (2012) reported that the ERR was 100.00% after estrus synchronization with 60 mg of MAP intra-vaginal sponge in situ for 6 days followed by IM injection of 300 IU of eCG and 30 µg of D-cloprostenol 24 hrs prior to sponge withdrawal.

Naderipour *et al.* (2012) reported that the ERR was 85.00% after estrus synchronization with 60 mg of MAP intra-vaginal sponges in situ for 14 days followed by 400 IU of PMSG on the day of sponge removal.

Kulaksiz *et al.* (2013) observed that the ERR was 75.0±9.9 and 77.7±10.1% in Fat tailed ewes treated with 20 mg FGA for 14 days, followed by an IM injection of 400 IU of PMSG upon the sponge withdrawal and 20 mg FGA for 8 days, followed by the PMSG injection on day 8^{th} upon the sponge withdrawal, respectively.

Martinez *et al.* (2013) reported 100.00% ERR after estrus synchronization with 65 mg of MAP intra-vaginal sponges in situ for 12 days followed by 200 IU of eCG at the time of sponge removal in Mexican Pelibuey ewes.

Bacha *et al.* (2014) documented that the ERR was 69.66% after estrus synchronization with FGA 40 mg of intra-vaginal sponges in situ for 7 days followed by 300 IU of PMSG at the time of sponge removal.

Najafi *et al.* (2014) reported that the ERR ranged from 93.00 to 100.00% after estrus synchronization in ewes with CIDR intra-vaginal device in situ for 14 days followed by 550 IU of PMSG at the time of sponge removal.

Sareminejad *et al.* (2014) reported that the ERR was 91.00±0.74% after estrus synchronization with 60 mg of MAP intra-vaginal sponges in situ for 14 days followed by 600 IU of PMSG at the time of sponge removal during non-breeding season in Arabian ewes.

Venkataramanan *et al.* (2015) studied the response to induction of estrus with intra-vaginal sponges followed by PMSG and GnRH hormones at the time of sponge removal. Intra-vaginal progesterone sponges were inserted for 12 days in all ewes. On day 11, luteolytic dose of $PGF_2\alpha$ (Lutalyse @ 2.5 ml/ewe) was administered and progesterone sponges were moved on day12. The first treatment group received PMSG @ 200 IU per animal, while the second group received GnRH @ 10 µg per animal at the time of sponge removal. All ewes in the PMSG group responded to treatment and exhibited heat where only one ewe showed estrus in GnRH group.

Kalyan *et al.* (2015) recorded the estrus response as 79.40% (ranging from 69.80 to 100.00 %) over 2 year period in various villages of Rajasthan in Malpura and Kheri ewes under field conditions after synchronization of estrus with AVIKESIL-S, cost-effective intra-vaginal sponges in situ for 12 days followed by 200 IU of eCG at the time of sponge withdrawal.

Gardon *et al.* (2015) evaluated estrus synchronization protocol based on Medroxy progesterone acetate impregnated intra-vaginal sponges with or without Equine Chorionic Gonadotropin on Merino sheep herd during the breeding season. Ewes were randomly allocated to four groups, two of ewes (E and Ee) and two of ewe lambs (L and Le).All females received 60 mg MAP (Medroxy progesterone acetate)

sponges on the first day. On the day of removal of sponge the animals of groups Ee and Le received 450 IU of eCG (Equine Chorionic Gonadotropin) and observed that the mean ERR was 92.06% and also similar in all the groups.

Murali (2017) conducted the estrus synchronization in ewes by inserting intra-vaginal sponges containing 40 mg of FGA for 12 days and injected 400 and 600 IU of PMSG on the day of withdrawal and recorded ERR as 83.33 and 100.00%, respectively.

2.1.3.2 Onset of estrus:

The interval from the end of treatment to the end of estrus were the important parameters to determine the duration of the induced estrus period and estimate the time of ovulation.

Kulaksiz *et al.* (2013) observed that the interval to onset of estrus was 51.0±0.8 hrs in Fat tailed ewes inserted with FGA sponge along with Ovsynch protocol.

Jackson *et al.* (2014) inserted CIDR for 5 days and administered 0.025 mg of Gonadorelin hydrochloride and 10 mg of Dinoprost tromethamine at CIDR insertion and CIDR removal, respectively during both transition season and anestrous season in ewes. The time intervals to onset of estrus during transition season and anestrous were 6.2 and 3.0 days, respectively.

2.1.3.3 Estrus intensity:

Banks (1964) reported the estrus signs of ewes during the 15 to 28 hrs of estrus. Female motor activity ranges through a spectrum of low to high to low intensity responses. For the first 3-5 hrs, low intensity acts included standing, head lowered and pinnae of ears somewhat flattened, swinging the head back to watch the courting ram, walking off and the standing and looking back at the ram; persistent nudging by the ram; acceptance to the male; from about the 5^{th} to 15^{th} hrs of estrus, the ewe was

notably more aggressive; soliciting behaviour takes the form of approaching the ram, nuzzling and pushing its head into the flank and scrotal regions; low intensity responses supervene from the 15th to the end of estrus, grading into the avoidance behavior characteristic of the anestrous ewe. He also explained the behavior of anestrous ewe, which was manifested either passive or active avoidance depending upon the persistence of the ram.

Parsons and Hunter (1967) developed a grading system for estrus behaviour in sheep to reduce the possibility of under-estimating duration of estrus through the reluctance of teaser rams to mount the recently mated ewes. The criterion used in all the experiments to determine the end of estrus was based on the ewe's reactions to rams exhibiting high libido. They graded the intensity of estrus at each teasing period.

Tomkins and Bryant (1974) compared the estrus behavior in Polled Dorset Horn ewes at a progestogen-synchronized estrus with 60 mg of MAP intra-vaginal sponges in situ for 13 days or at a normal estrus which were teased for a 10 min period at 4 hrs intervals by a series of different rams after 12 hrs of sponge withdrawal or during natural estrus, which include frequency of nudging, kicking, flehmen posture, mounting and service by the ram and of squatting, active soliciting, tail fanning and looking over shoulders by the ewe. They concluded that progestogen treatment produced no major differences in the manifestation of behavior at induced estrus compared to natural estrus.

Ucar *et al.* (2005) categorized the behaviours to distinguish between estrus and non-estrus ewes. They used the terms "estrus attractivity" i.e. observation shown by ram in attraction to ewe and "estrus receptivity" i.e. ewe immobility while mounted by ram to estimate estrus duration after estrus synchronization with 30 mg of FGA

intra-vaginal sponges in situ for 14 days followed by 600 IU of PMSG at the time of sponge removal.

Kalyan *et al.* (2015) used the estrus signs of ewes, restlessness, shaking of tail, slightly swollen vulva, moist and reddish vulva and vagina, seeking for ram, rubbing their body and neck against the ram and standing still during mounting in a field trial after estrus synchronization with progesterone containing vaginal sponges.

2.1.3.4 Duration of estrus:

Tomkins and Bryant (1974) reported that the mean duration of estrus in Polled Dorset Horn ewes at a normal estrus was 48.0±2.1 hrs and 51.0±3.5 hrs for ewes at a progestogen-synchronized estrus (with 60 mg of MAP intra-vaginal sponges in situ for days). Whereas, Fletcher (1971) recorded that progestogen-treated ewes had a significantly shorter estrus than normal ewes. However, Parsons and Hunter (1967) did not find significant difference between normal and treated ewes.

Greyling *et al.* (1997) reported that the duration of estrus in ewes was 25.1±17.4 and 28.3±l3.5 hrs in ewes after estrus synchronization with 60 and 30 mg of MAP intra-vaginal sponges, respectively in situ for 14days followed by 300 IU of PMSG at the time of sponge withdrawal.

Fuentes *et al.* (2001) recorded the duration of estrus in ewes after sponge withdrawal as 27.0±2.5 hrs after estrus synchronization with 45mg of MAP intra-vaginal sponges in situ for 14 days followed by 250 IU of eCG administered at the time of sponge removal.

Ucar *et al.* (2005) observed that the estrus duration based on estrus attractivity i.e. observation shown by ram in attraction to ewe was 36.57±7.06 hrs and based on estrus receptivity i.e. ewe immobility while mounted by ram was 33.14±7.7l hrs after estrus synchronization with 30 mg of FGA intra-vaginal sponges in situ for 14 days

followed by 600 IU of PMSG at the time of sponge removal. Estrus was detected at 2 hrs interval up to 102 hrs post sponge removal and concluded that progestogen sponge and PMSG combination prolonged the estrus duration when compared to duration of natural estrus.

Dogan and Nur (2006) reported that the duration of estrus in ewes after sponge removal was 28.5±2.2 hrs after estrus synchronization with 60 mg of MAP in situ for 12 days followed by 500 IU of PMSG administered 48 hrs before sponge removal.

Hashemi *et al.* (2006) documented that the interval from the end of treatment to end of estrus and duration of estrus was 51.7±7.2 and 22.11±3.4 hrs, respectively after estrus synchronization in ewes treated with 60 mg of MAP in situ for 12 days followed by 500 IU of PMSG at the time of sponge withdrawal.

Ustuner *et al.* (2007) reported that the duration and cessation of estrus in ewes was 34.91±4.3 and 67.09±6.3 hrs after estrus synchronization with 30 mg of FGA intra-vaginal sponges in situ for 12 days followed by 300 IU of PMSG at the time of sponge removal.

Zonturlu *et al.* (2011) recorded that the estrus duration was 40.82±1.21, 40.20±1.14 and 38.7±1.07 hrs in ewes synchronized with intra-vaginal sponge containing 30 mg of FGA in situ for 12 days followed by 300, 400 and 500 IU of PMSG, respectively at the time of sponge removal.

Cavalcanti *et al.* (2012) reported that the duration of estrus was 37.4±9.0 hrs after estrus synchronization with 60 mg of MAP in situ for 6 days followed by 300 IU of eCG and 30 µg of D-cloprostenol 24 hrs prior to sponge withdrawal.

Ralchev *et al.* (2012) documented that the duration of estrus was 31.2±5.98 h (ranging from 24-84h) after estrus synchronization with 30 mg of FGA intra-vaginal sponges in situ for 12 days followed by 250 IU of PMSG at the time of sponge withdrawal in Ile de France ewes.

Kulaksiz *et al.* (2013) observed that the duration of estrus was 27.2 ± 0.8 and 27.0 ± 0.8 h in Fat tailed ewes treated with 20 mg of FGA sponge for 14 days, followed by an i.m. injection of 400 IU of PMSG at the time of sponge withdrawal and 20 mg of FGA sponge inserted for 8 days, followed by the same dose of PMSG injection on day 8 upon the sponge withdrawal, respectively.

Ramirez *et al.* (2014) reported that the mean duration of estrus was 54.9 ± 8.34 h after estrus synchronization with 20 mg of FGA intra-vaginal sponges in situ for 12 days followed by i.m. injection of 500IU of eCG at the time of sponge removal in tropical hair sheep. Estrus detection was started 12 h after end of treatment and repeated every 8 h interval until all ewes reject the teaser.

Sareminejad *et al.* (2014) recorded that the estrus duration was14.77±1.11 hrs after estrus synchronization with 60 mg of MAP intra-vaginal sponges in situ for 14 days followed by 600 IU of PMSG at the time of sponge withdrawal during non-breeding season in Arabian ewes.

2.1.3.5 Fertility rate:

Husein and Kridli (2003) conducted experiment to examine the effect of progesterone prior to a GnRH-$PGF_2\alpha$ treatment on estrus and pregnancy in seasonally anestrous Awassi ewes. Twenty four ewes were randomly assigned to three groups to be pre-treated with 60 mg of Medroxy progesterone acetate sponges (group A), 600 mg progesterone sponges (group B) or blank sponges (group C) for 4 days. All ewes were injected with 100 µg of GnRH 24 hrs after sponge removal followed, 5 days later, by 20 mg of $PGF_2\alpha$ injection. Ewes were exposed to three fertile rams at the time of $PGF_2\alpha$ injection and were checked for breeding marks at 6 hrs intervals for 5 days. The conception rate in A, B and C group was 75.00, 25.00 and 0.00 %, respectively.

Kridli *et al.* (2003) reported that conception rate was 30.00 and 50.00 % with natural mating in GnRH and control groups of ewes treated with intra-vaginal sponge containing 40 mg of FGA left in place for 12 days followed by 50 µg of GnRH intramuscularly 28 hrs after sponge removal.

Kohno *et al.* (2005) observed that the conception rate was 40.70 and 55.00% by natural mating in ewes treated with intra-vaginal progesterone cream containing 500 mg of progesterone sponge into the vagina and intra-vaginal CIDR-G containing 300 mg of progesterone, respectively left in place for 12 days followed by intramuscular injection of 500 IU of eCG 24 hrs before the removal of the sponge during the non-breeding season.

Timurkan and Yildiz (2005) treated Hamdani ewes with 40 mg of FGA vaginal sponges along with 500 IU of PMSG in group 1, 600 IU of PMSG in group 2, 750 IU of PMSG in group 3 and no PMSG in group 4 (control) of ewes. The pregnancy rates with AI were 90.62, 93.75, 100.00 and 79.40 % in group 1, 2, 3 and 4 of ewes, respectively.

Zeleke *et al.* (2005) reported that overall conception rate was 78.00, 75.00, 70.20 and 60.00% with artificial insemination in ewes treated with intra-vaginal sponge containing 60 mg of MAP and 40 mg of FGA left in place for 14 days followed by intramuscular injection of 300 IU of PMSG at 24 hrs before sponge withdrawal, at sponge withdrawal, 24 hrs after sponge withdrawal and control (no PMSG treatment), respectively in Dorper breed of ewes during transition period from the natural breeding to the anestrous season. In the same experiment, they observed that conception rate was 70.60 and 74.00 % with artificial insemination in ewes treated with intra-vaginal sponge containing 60 mg of MAP and 40 mg of FGA left in place for 14 days, respectively followed by 300 IU of PMSG during transition period from the natural

breeding to the anestrous season. In the same experiment, the conception rate was 70.10% in the ewes synchronized with 300 IU of PMSG through intramuscular route and 78.80 % in the ewes synchronized with 300 IU of PMSG through subcutaneous route.

Akoz *et al.* (2006) documented that conception rate was 93.30, 92.80 and 86.70% in ewes with intra-vaginal sponge containing 30 mg of FGA for 7 days followed by 300, 500 and 700 IU of PMSG intramuscularly at sponge with drawal, respectively and also recorded that the conception rate was 92.8, 100.0 and 93.3% in ewes treated with vaginal sponges containing 40 mg of Fluorogestone acetate inserted into the vagina for 7 days followed by intramuscular injection of 300, 500 and 700 IU of PMSG intramuscularly at the sponge withdrawal, respectively in Akkaraman crossbred ewes during outside the breeding season.

Ustuner *et al.* (2007) recorded that the fertility rate was 55.60, 20.00 and 33.30% with intra-vaginal sponge containing 30 mg of FGA left in place for 6 days (short-term) followed by 300 IU of PMSG 24 hrs before, at the time of sponge removal and 24 hrs after sponge removal, respectively during breeding season upon artificial insemination.

Khan *et al.* (2007) reported that the conception rate was 75.00% in ewes treated with natural mating by intra-vaginal sponge containing progesterone (Chronogest) left *in situ* for12 days followed by 250IU of eCG intramuscularly at the time of sponge removal.

Todini *et al.* (2007) observed that the fertility rate was 83.00% by natural mating treated with intra-vaginal sponges containing 40 mg of FGA left in place for 12 days followed by intramuscular injection of 350 IU of PMSG at sponge withdrawal in seasonally anovulatory lactating Sarda ewes.

Gallab *et al.* (2008) recorded the conception rate as 83.33 and 50.00% in Rahmani ewes during breeding and non-breeding seasons, respectively treated with intra- vaginal sponge containing 40 mg of MAP left in place for 12 days followed by intramuscular injection of eCG at the time of sponge removal during non-breeding season.

Al-noaaemi *et al.* (2009) reported that the pregnancy rates was 90.0, 70.00 and 50.00 % with daily intramuscular injection of 7 mg of progesterone for 12 days followed by single injection of 50 IU eCG on last day of programme during September, March and January months, respectively in Awassi ewes.

Moeini *et al.* (2009) reported that the fertility rate was 45.80 and 37.50% by artificial insemination in ewes treated with intra-vaginal sponge containing 40 mg of FGA left in situ for 13 days followed by intramuscular injection of 400 and 600 IU of PMSG at the time of sponge removal and also 200 IU of hCG intramuscularly at the time of Al in both the groups, respectively but in control group of ewes the fertility rate was 36.00 % by artificial insemination.

Oliveira *et al.* (2009) documented that the conception rate was 37.50% in Santa Ines ewes treated with sponges containing 60 mg MAP for 14 days and administered 300 IU of eCG intramuscularly on day 14 at the time of sponge withdrawal.

Mousavy *et al.* (2009) observed that the fertility rate was 40.00% by artificial insemination in ewes by CIDR for 12 days followed by 400 IU of PMSG intramuscularly at device withdrawal and 5ml of GnRH intramuscularly at day 12^{th} of post insemination.

Gomez *et al.* (2006) recorded conception rate as 74.00% with natural mating in Manchega ewes treated with intra-vaginal sponge containing 30 mg of FGA left in

place for 12 days followed by 450 IU of eCG intramuscularly at the time of sponge withdrawal during seasonal anestrous period.

Martemucci and Dalessandro (2010) observed that the fertility rate was 36.70% in lactating cross-bred Altamurana ewes by natural mating at natural estrus during non-breeding season and they also reported that the conception rate was 60.00% in lactating adult cross-bred Altamurana ewes by natural mating treated with intra-vaginal sponge containing 40 mg of FGA for 14 days followed by intramuscular injection of 400 IU of eCG at sponge removal.

Almariol (2010) reported that the pregnancy rate was 85.00% in Libyan Barbary ewes treated with intra-vaginal sponge containing 40 mg of FGA for 12 days and followed by intramuscular injection of 500 IU of PMSG at the time of sponge removal. In another experiment, it was observed that the conception rate was 80.00 and 70.00% by natural mating in ewes treated with intra-vaginal sponges containing 40 and 20 mg of FGA, respectively kept in situ for 12 days followed by intramuscular injection of 500 IU of PMSG at the time of sponge removal.

Soria *et al.* (2011) reported that the fertility rate was 64.00 and 76.00% by natural mating in Suffolk/Hampshire anestrous ewes treated with intra-vaginal sponges impregnated with 40 mg of progesterone and 20 mg of Cronolone, respectively for 12 days followed by intramuscular injection of 400 IU of eCG 24 hrs before removal sponge during non-breeding season.

Yadiz *et al.* (2011) recorded that the pregnancy rates were 45.00 and 35.00% in ewes treated for 12 days with sponge plus 500 IU of PMSG and CIDR plus 500 IU of PMSG, respectively during early anestrous season.

Santos *et al.* (2011) noticed that the pregnancy rates were 50.00 and 79.00% in control and ewes synchronized with 60 mg of MAP vaginal sponge, 300 IU of eCG on day 7 and Cloprostenol on day 9, respectively.

Zonturlu *et al.* (2011) reported that conception rates were 82.35, 80.00 and 82.60% in Awassi ewes with natural mating treated with intra-vaginal sponge containing 30 mg of FGA left in place for 12 days followed by intramuscular injection of 300, 400 and 500 IU of PMSG, respectively at the time of sponge removal during transition period in Turkey.

Kulaksiz *et al.* (2013) studied the effects of different synchronization protocols on the reproductive parameters of fat-tailed ewes during the breeding season. Ewes were randomly divided into four treatment groups, Group 1 (long-term FGA) 20 mg FGA sponges inserted intra-vaginally for 14 days, followed by an IM injection of 400 IU of PMSG upon the sponge withdrawal, Group 2 (short-term FGA) the insertion of sponges for 8 days, followed by the PMSG injection on day 8 upon the sponge withdrawal, Group 3 (Ovsynch) an IM injection of 0.004 mg of GnRH on day 0, followed by the injection of 125 μg of $PGF_2\alpha$ on day 7 and the second GnRH injection on day 9, and Group 4 (short-term FGA plus Ovsynch) the FGA sponge inserted along with the first GnRH injection (as day zero), $PGF_2\alpha$ injection on day 7 upon the sponge withdrawal, and the second GnRH injection on day 9. Once the ewes were detected in estrus, they were then separated from the rest of flock and hand-mated with fertility-proven rams (n=4, Foreach breed) used rotationally. Estrus response Vs pregnancy rate among estrus responded ewes in Group 1, Group 2, Group 3 and Group 4 were 75.0±9.9 Vs 93.3± 6.6%; 77.7±10.1 Vs 85.7±9.7%; 82.3±9.5 Vs 42.8±13.7% and 87.5±8.5 Vs 57.1±13.7%, respectively.

Ravindranath *et al.* (2014) investigated the conception rate and frequency of single and multiple births in estrus synchronized NARI Suwarna ewes maintained under two different systems of feeding strategies. Ewes under both systems were subjected for estrus synchronization protocol for 12 days by using intra-vaginal progesterone sponges with $PGF_2\alpha$ and PMSG. They observed that the mean conception rate was 83.30 and 55.00% for scientific feeding and pastures grazing, respectively when inseminated with freshly collected and diluted semen.

Gardon *et al.* (2015) evaluated estrus synchronization protocol based on Medroxy progesterone acetate impregnated intra-vaginal sponges with or without Equine Chorionic Gonadotropin on a Merino sheep herd during the breeding season. Ewes were randomly allocated to four groups, two of ewes (E and Ee) and two of ewe lambs (L and Le). All females received 60 mg MAP (Medroxy progesterone acetate) sponges on the first day. On the day of removal of sponge the animals of groups Ee and Le groups were received 450 IU of eCG (equine chorionic gonadotropin). They reported that the pregnancy rates were 71.35 and 72.86% for ewes and ewe lambs, respectively.

2.1.4 Effect of nutrition and supplements on reproduction:

The effects of nutrition on reproduction may be associated with the metabolism of insulin, leptin, growth, glucose (energy) and protein, which in turn affected the pituitary-ovarian function. Energy was primarily available from carbohydrates (sugar, starch and fibers) and fats in the diet. In sheep and goats, high energy (glucose) was required for increased secretion of the hypothalamic peptide hormones like GnRH (Blache *et al*., 2008).

Micro minerals also played an important role in body metabolism. The minerals and vitamins like copper, zinc, selenium and Vitamin E directly influenced the estrus response, embryo implantation and fecundity rates in small ruminants. Trace

elements are needed for vitamin synthesis, hormone production, enzyme activity, energy production and other physiological processes related to growth, reproduction and health of small ruminants (Kundu *et al*., 2014).

2.2 PREGNANCY DIAGNOSIS:

Pregnancy diagnosis was performed routinely based on the non-return rate in field levels, but it may confused with persistent corpus luteum or late embryonic death and it was the most unreliable method with more error. Pregnancy was diagnosed by non-return rate and ultrasound examination by different authors was reviewed hereunder.

2.2.1 Non-return rate:

Paulenz *et al.* (2003) reported that the difference between results of overall 25 days NRR and lambing rate was 26.0%. They stated that 1.90% ewes returned to estrus later than 25 days.

Ucar *et al.* (2005) recorded that NRR and lambing rate was 71.4±18.4 and 57.1±20.2%, respectively in Tuj ewes after estrus synchronization with progesterone sponges and PMSG followed by natural service.

Ustuner *et al.* (2007) observed that 30 days NRR was 22.20% with FTAI by twice intra-cervical inseminations with freshly diluted semen at 40 and 60 hrs of sponge removal after estrus synchronization with 30 mg of FGA intra-vaginal sponges followed by PMSG injection. From 12^{th} day after sponge removal to 30^{th} day the inseminated ewes were observed for estrus signs twice daily with a teaser ram to record 30 day non return rate.

Das *et al.* (2011) reviewed early PD in small ruminants and explained non-return to estrus after breeding was one of the major sign of pregnancy and was an age-old method to diagnose pregnancy, usually practiced by the traditional sheep and goat rearing farmers. They also concluded that the ewes might not have returned to estrus

due to pathological conditions, stress, early embryonic death, gestational heat etc. Hence, this method was not a reliable method in sheep and goats; however, it could be used as a first hand diagnostic aid in conjunction with further confirmation by more accurate methods of pregnancy diagnosis.

2.2.2 Pregnancy diagnosis by ultrasound examination:

Pregnancy diagnosis in small ruminants by B-mode ultrasonography was introduced in Veterinary practice in as early as eighties and was a new tool for early pregnancy diagnosis (Buckrell *et al*., 1986; Davey, 1986 and Haibel, 1990).

Ishwar (1995) reported that with real time B-mode ultrasound scanner, pregnancy could be detected as early as 25 days of gestation and could also diagnose fetal viability, fetal numbers and pathological gestation and experienced examiners could achieve 91.00 to 100.00% accuracy.

Ganaie *et al.* (2009) documented that real-time B-mode ultrasonography was the earliest, most accurate, safest, fastest and most economical method of pregnancy diagnosis in sheep at farm level. They also described that the accuracy of trans-abdominal real time B mode ultrasonography was 68.00% accurate at 15-30 days of pregnancy and increased to 100.00% by day 61-75 and remained constant until lambing.

Das *et al.* (2011) described that pregnancy diagnosis by real-time B-mode ultrasonography has gained more attention in recent years as compared to other types as it offered an accurate, rapid, safe and practical means of diagnosing the pregnancy, fetal numbers and estimated the gestational age in livestock. False positives were rarely recorded by ultrasonographic diagnosis of pregnancy.

CHAPTER-III

3. MATERIALS AND METHODS

The present study was undertaken at Livestock Research Station, Garividi situated at an altitude of 92 meters above MSL on 83º longitude and 18º latitude. The average temperature and humidity recorded were 35ºC (19-40) and 50% (21-92), respectively. The experiment was carried out during the non-breeding season i.e., in the months of March 2020 to May 2020 to study the estrus response and fertility rate in postpartum anestrous ewes by synthetic prostaglandin (Cloprostenol sodium, Pragma®), Gynarich (Buserelin acetate) and progesterone sponges (Central Sheep and Wool Research Institute, Avikanagar). Forty eight Vizianagaram non-lactating and previously postpartum anestrous ewes apparently health were selected and divided into four groups consisted of 12 ewes each.

3.1 EXPERIMENTAL ANIMALS:

3.1.1 Management of experimental herd:

The rams and ewes were maintained separately while grazing period (7-8 hrs per day) and during their stay at shed under semi intensive system of rearing with identical conditions of housing. All the animals were fed daily with concentrate feed (pellet feed) at the rate of about 350 gm per head per day to rams and 250 gm per head per day to ewes in addition to green fodder such as Guinea grass, Napier grass, Andhra Pradesh Bajra Napier grass (APBN) etc.

Three weeks before the start of trial all the selected ewes (n=48) were flushed by giving additional feeding with 300 gm of pellet feed per head per day. Fresh drinking water facility was provided both at shed in a water trough and in the grazing area by filling the water points.

Plate 1: Experimental animals (Vizianagaram ewes and Rams)

Experimental ewes

Ram used for breeding at detected estrus

Vizianagaram ram

Apronized ram used for detection of estrus

3.1.2 Selection of ewes:

Forty eight healthy Vizianagaram ewes (Plate 1) aged 3-4 years with body weight ranging from 25 to 30 kg stationed at Livestock Research Station (LRS), Garividi, Vizianagaram district were utilized to study the characteristics of estrus response and fertility rate after estrus synchronisation with Selectsynch, Progesterone sponges plus Ovsynch and improved nutrition with mineral mixture (Minfa Gold) treatments (Plate 2) during the non-breeding season (March to May 2020).

3.1.3 Selection of rams:

Vizianagaram rams (Plate 1) having good libido were selected for natural mating of ewes at induced estrus.

3.2 EXPERIMENTAL GROUPS:

Animals of similar age group and weight were selected and randomly divided into four groups of 12 each, provided with hormonal/nutritional supplements (Plate 3)

3.2.1 Group 1 (Selectsynch, n=12):

Each ewe received Buserelin acetate @ 4µg (Gynarich) intramuscularly (day 0) followed with administration of Cloprostenol sodium @ 125 µg (Pragma, Intas) intramuscularly on day 7. On days 8, 9 and 10 estrus was detected using vasectomised teaser rams. Ewes exhibiting estrus were allowed for natural mating with proven ram.

3.2.2 Group 2 (Progesterone+Ovsynch, n=12):

In this group, progesterone sponge (0.3 gm natural progesterone, Central Sheep and Wool Research Institute, Avikanagar) was inserted intra-vaginal and GnRH analogue @ 4.0 µg was administered intramuscularly along with sponge insertion on day 0. On day 7 $PGF_2\alpha$ analogue Cloprostenol @ 125 µg was administered intramuscularly and the sponge was removed and second injection of 4.0 µg GnRH analogue was administered on day 9.

Progesterone sponges were inserted into the vagina carefully and aseptically with the applicator provided to avoid trauma and infection of the ewe's reproductive tract. The procedure was performed in a clean and dry environment.

3.2.3 Group 3 (n=12):

The ewes in this group were fed with additional 200 gm of concentrate added with Mineral mixture (Minfa Gold) consisting of vitamins and minerals @ 20 gm per day for 9 days along with the daily quantity of concentrate feed.

3.2.4 Group 4 (n=12):

These ewes were reared under conventional feeding and grazing without any hormonal or additional nutritional treatments and these ewes were considered as control.

3.3 ESTRUS DETECTION:

3.3.1 Preparation of teaser rams:

Rams used for teasing were prepared by securing the apron around the preputial region so as to prevent penile intromission and also to keep the penis well within the apron. Then paint was applied to the brisket region of ram, so as to paint the rump of the ewes that have been mounted. After synchronization of ewes, the estrus was detected by allowing the apronized rams into the flock with ratio of 1:8 between ram: ewe for about half an hour from 24 to 72 hrs after the $PGF_2\alpha$ administration with an interval of 6 hrs i.e., at 6.00, 12.00 noon, 18.00 and 00.00 hrs. Based on the presence or absence of paint on the rump of ewes, estrus was identified. All the ewes having the paint on its rump were separated to assess and record the characteristics of induced estrus.

3.3.2 Estrus response rate:

Estrus response in Vizianagaram ewes was evaluated by observing the exhibition of estrus symptoms. Percentage of ewes in estrus was calculated based on

Group 1: Selectsynch

DAY 0 Buserelin acetate 4µg 	DAY 7 Cloprostenol sodium 	 Breeding at detected estrus

Group 2: Progesterone+Ovsynch

DAY 0 Buserelin acetate (4µg)	DAY 7 Cloprostenol sodium	DAY 9 Buserelin acetate (4µg)	 Breeding at detected estrus
Insertion of vaginal sponges	Removal of vaginal sponges		

Group 3: Nutritional supplement

Feeding of additional concentrate 200 gm ewe plus mineral mixture for 9 days	 Breeding at detected estrus

Group 4: Control group

Conventional feeding and grazing	 Breeding at detected estrus

Plate 2: Grouping of animals and estrus synchronization protocols

the number of ewes that have exhibited behavioural estrus sigs after synchronization out of the total number of ewes treated.

3.3.3 Onset of estrus:

It was calculated in hours from the time of administration of $PGF_2\alpha$ injection to the time of first appearance of behavioural symptoms of estrus in ewes in Selectsynch and vaginal sponge removal/administration of $PGF_2\alpha$ injection in Progesterone+Ovsynch group. While in nutritional supplement group, the onset of estrus was calculated from discontinuation of nutritional supplements given for 9 days and exposure to apronized ram and in control group from exposure to apronized ram. The onset of standing estrus was considered as mid-way between the last negative and first positive acceptance of apronized ram, while the end of estrus as mid-way between the last positive and the first rejection of apronized ram.

3.3.4 Estrus intensity:

Estrus intensity was measured by using a score card (12 point scale) outlined by Homeida *et al.* (2009) with slight modifications as mentioned below (Table 1). These parameters were added and summation of these was quantified into weak estrus (0-4), intermediate estrus (5-8) and intense estrus (9-12) of that ewe. The score card for assessment of intensity of estrus in ewes was as follows

Table 1: Estrus intensity score card (12 points scale) for assessment of intensity of estrus

S No.	Parameters observed	Score (12 points)
1	Degree of expression of restlessness	0-3
2	Standing to be mounted	0-3
3	Vocalization	0-3
4	Swelling of vulva and mucus discharges	0-3

3.3.5 Duration of estrus:

Duration of estrus was estimated in hours from the time of first appearance of estrus symptom i.e., first acceptance of ewe to be mounted by the apronized ram to last symptom of its acceptance to be mounted by the apronized ram.

3.3.6 Degree of synchrony:

The degree of estrus synchrony from the time of administration of $PGF_2\alpha$ injection in Selectsynch, sponge withdrawal/administration of $PGF_2\alpha$ injection in Progesterone+Ovsynch group. While in nutritional supplement group, the onset of estrus was calculated from discontinuation of nutritional supplements given for 9 days and exposure to apronized ram and in control group from exposure to apronized ram to the time elapsed for onset of estrus was categorized into <24 hrs, 24-48 hrs, 48-72 hrs and 72-96 hrs.

3.4 FERTILITY RATE:

3.4.1 Non-return rates:

To detect ewes returning to estrus, all ewes were checked from day 15 to 25 after last mating using a teaser ram twice daily morning and evening. Ewes not returning to estrus were considered pregnant and recorded as the percentage of ewes that did not return to estrus at 25 days post-mating.

3.4.2 Ultrasound scanning:

Pregnancy diagnosis was performed by using ultrasonography (Chison Eco1-Chison medical technologies, China) with 3.5 MHz trans abdominal probe on day 45 of post-mating. Ultrasound scanning was performed in the shaved area of right inguinal region with right leg moved caudally for proper placement of probe.

Ewes were restrained manually in standing position near the ultrasound monitor to detect the conceptus, placentomes and fetal skeletal structures. Ultrasound gel was applied to the transducer which was paced trans abdominally in the right

inguinal region. Urinary bladder, which was visualized as an anechoic structure served as point of reference and the relevant images were freezed and recorded.

3.5 STATISTICAL ANALYSIS:

The data on different parameters was analyzed by t-test and chi-square test for differences in estrus response and fertility rate among treatment groups and also by analysis of variance (one way classification) for differences within each group using SPSS 12.0 for windows. The significance of all the parameters studied was measured at $P<0.05$ level (Snedecor and Cochran, 1994).

CHAPTER-IV

4. RESULTS

The present study was undertaken by utilizing forty eight Vizianagaram ewes aged about 2-4 years stationed at LRS Garividi, Vizianagaram District, Andhra Pradesh state during non-breeding season from March to May 2020. The trial was conducted to study the estrus response and conception rate in postpartum synchronized with Selectsynch (Group 1), Progesterone+Ovsynch (Group 2), nutrition and mineral supplement (Group 3) and untreated control (Group 4). All the ewes were examined for exhibition of estrus symptoms to record estrus response rate, onset of estrus, intensity of estrus, duration of estrus and degree of synchrony. All the ewes were bred by natural mating and conception rate was studied based on non-return rate and ultrasound examination on day 45 post-mating.

4.1 ESTRUS SYNCHRONIZATION:

The number of ewes responded to treatment (estrus response rate), time required for onset of estrus, intensity of estrus, duration of estrus and degree of synchrony were analysed and presented.

4.1.1 Estrus response rate:

The estrus response rate was 66.66 (8/12), 100.00 (12/12), 50.00 (6/12) and 33.33 (4/12) per cent in Selectsynch (Group 1), Progesterone+Ovsynch (Group 2) nutrition and mineral supplement (Group 3) and control (Group 4), respectively (Table 2 and 10 and Figure 1). The estrus response rate was significantly ($P<0.05$) higher in Progesterone+Ovsynch (100.00%) and Selectsynch (66.66%) groups when compared to nutrition and mineral supplement (Group 3) and control groups (Group 4).

4.1.2 Onset of estrus:

The mean time taken for the onset of estrus (hrs) from the time of administration of $PGF_2\alpha$ injection in Selectsynch, sponge withdrawal/administration

Table 2: Estrus response rate (%) among treatment groups in Vizianagaram ewes during non-breeding season

Group	Treatment	Number of ewes exhibited estrus	Estrus response rate (%)	Chi-square
Group 1 (n=12)	Selectsynch	8	66.66	0.000654*
Group 2 (n=12)	Progesterone+Ovsynch	12	100.00	
Group 3 (n=12)	Nutrition and nutritional supplements	6	50.00	
Group 4 (n=12)	Conventional feeding and grazing	4	33.33	

*Significant difference at P<0.05

Figure 1: Estrus response rate (%) among treatment groups in Vizianagaram ewes

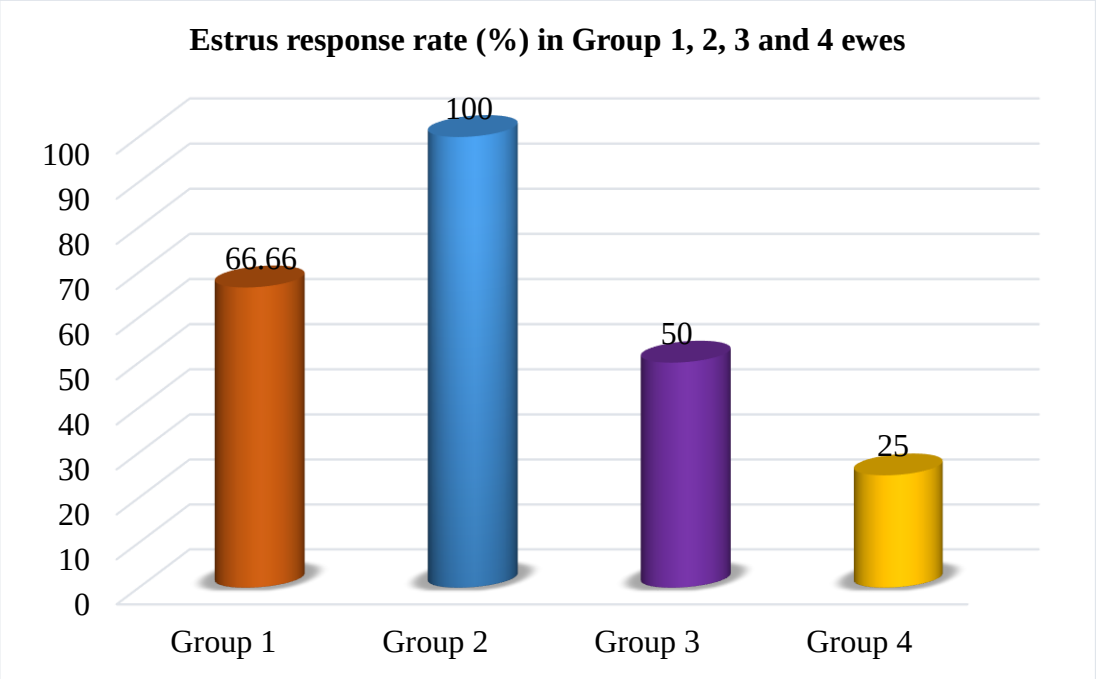
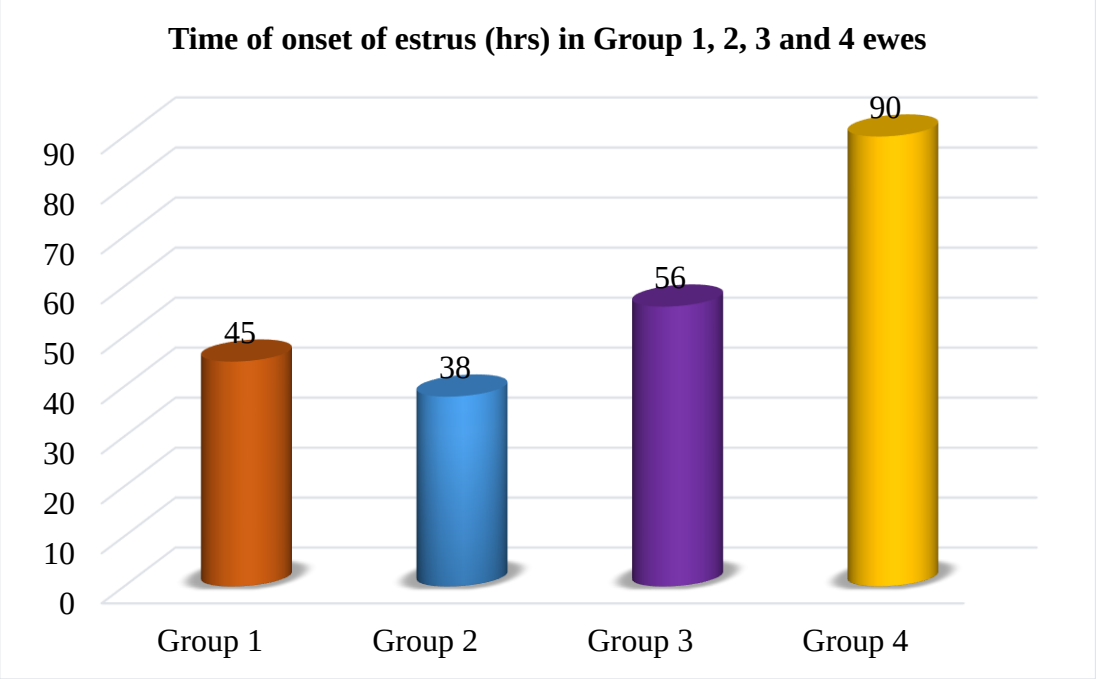


Figure 2: Time of onset of estrus (hrs) among treatment groups in Vizianagaram ewes



of $PGF_2\alpha$ injection in Progesterone+Ovsynch group. While in nutritional supplement group, the onset of estrus was calculated from discontinuation of nutritional supplements given for 9 days and exposure to apronized ram and in control group from exposure to apronized ram was 45.00±7.08 (24-72), 38.00±4.63 (24-72), 56.00±8.00 (24-72) and 90.00±18.00 (72-120) hours, respectively (Table 3 and 10 and Figure 2).

Significantly ($P<0.05$) shorter time interval to onset of estrus was observed in Progesterone+Ovsynch group (38.00±4.63 hrs) followed by Selectsynch (45.00±7.08 hrs), nutrition and mineral supplements (56.00±8.00 hrs) and control group (90.00±18.00 hrs) (Table 4).

4.1.3 Intensity of estrus:

Estrus of intensity measured in ewes on a scale of 12 (scaled of 0 to 12) was 7.50±0.65 (6-10), 8.60±0.47 (6-12), 6.10±0.24 (5-7) and 5.00±0.22 (3-5) in Selectsynch (Group 1), Progesterone+Ovsynch (Group 2), nutritional supplements (Group 3) and control (Group 4) groups, respectively (Table 5; Table 10 and Figure 3).

Among all the treatment groups of ewes, none of the ewes showed weak estrus (0-4). The estrus intensity grade was significantly ($P<0.05$) higher in Progesterone+Ovsynch (8.60±0.47) and Selectsynch (7.50±0.65) groups when compared to nutritional supplement (6.10±0.24) and control (5.00±0.22) groups. There was no significant difference between Selectsynch and Ovsynch+ Progesterone sponge groups and similarly there was no significant difference between nutritional supplement (Group 3) and control (Group 4) groups for intensity of estrus (Table 6).

4.1.4 Duration of estrus:

The mean duration of estrus was 24.00±4.82 (24-48 hrs), 26.00±2.88 (24-48 hrs), 24.00±1.64 (24-48 hrs) and 24.00±2.01 (24-36 hrs) in Selectsynch (Group 1), Progesterone+Ovsynch (Group 2) nutrition and mineral supplement (Group 3) and

Table 3: Time of onset of estrus (Mean±SE) among treatment groups in Vizianagaram ewes during non-breeding season

Group	Treatment	Number of ewes exhibited estrus	Time of onset of estrus (hrs) (Range)	Time of onset of estrus (hrs) (Mean±SE)	F-value
Group 1 (n=12)	Selectsynch	8	24-72	45.00 ± 7.08^{ab}	3.342^{*}
Group 2 (n=12)	Progesterone+Ovsynch	12	24-72	38.00 ± 4.63^{b}	
Group 3 (n=12)	Nutrition and nutritional supplements	6	24-72	56.00 ± 8.00^{a}	
Group 4 (n=12)	Conventional feeding and grazing	4	48-120	90.00 ± 18.00^{ab}	

Means bearing different superscripts (a, b) in the column differ significantly at $P<0.05$

Table 4: ANOVA for time of onset of estrus among treatment groups

Source of variation	Sum of squares	d-f	Mean sum of squares	F-value
Between Groups	8539.00	3	2846.00	6.465^{*}
Within Groups	11450.00	26	440.30	
Total	19990.00	29		

Figure 3: Intensity of estrus (hrs) among treatment groups in Vizianagaram ewes

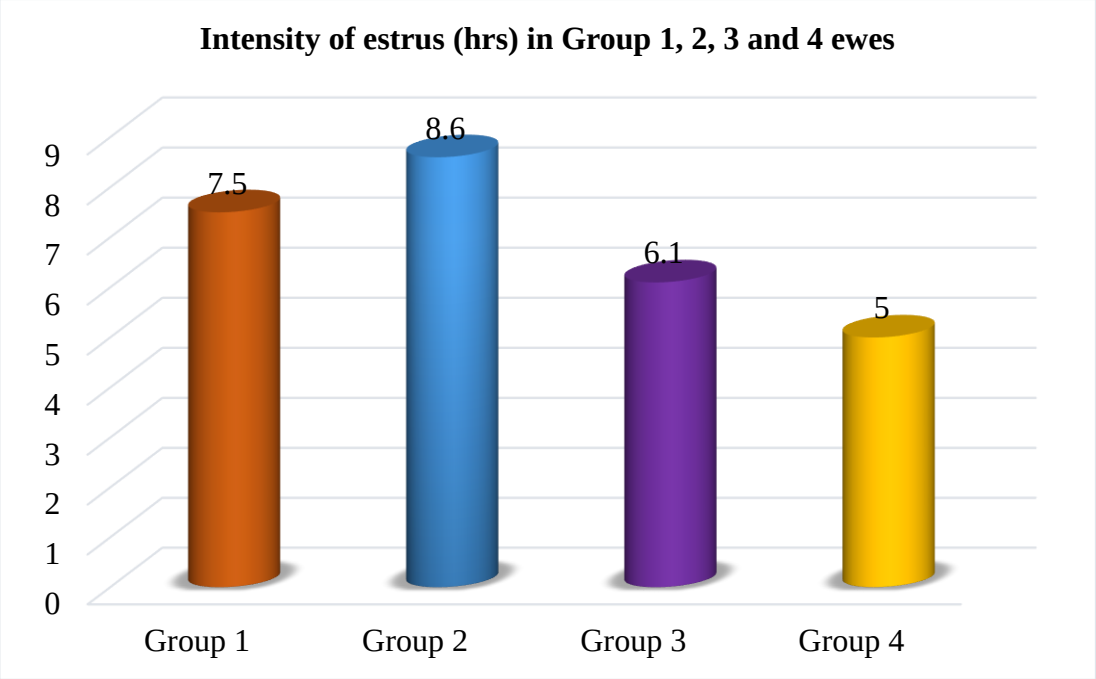


Figure 4: Duration of estrus (hrs) among treatment groups in Vizianagaram ewes

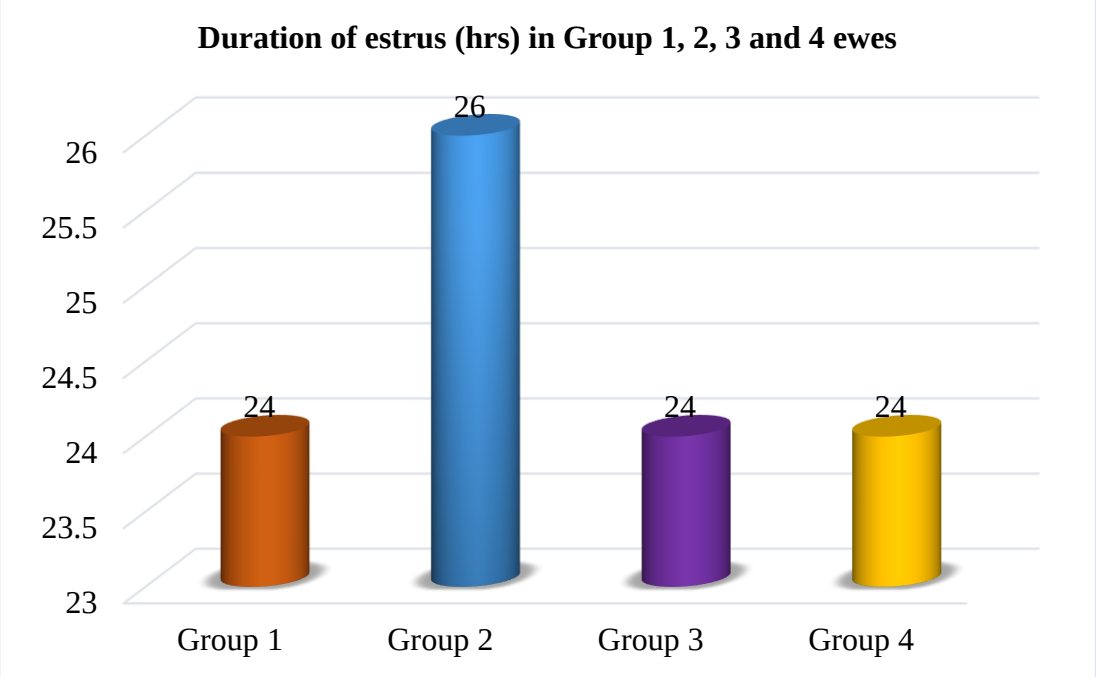


Table 5: Estrus intensity (Mean±SE) among treatment groups in Vizianagaram ewes during non-breeding season

Group	Treatment	Number of ewes exhibited estrus	Score (0-12)	Estrus intensity (Mean±SE)	F-value
Group 1 (n=12)	Selectsynch	8	6-10	7.50±0.65[a]	5.875*
Group 2 (n=12)	Progesterone+Ovsynch	12	6-12	8.60±0.47[a]	
Group 3 (n=12)	Nutrition and nutritional supplements	6	5-7	6.10±0.24[b]	
Group 4 (n=12)	Conventional feeding and grazing	4	3-5	5.00±0.22[b]	

Means bearing different superscripts (a, b) in the column differ significantly at P<0.05

Table 6: ANOVA for estrus intensity among treatment groups

Source of variation	Sum of squares	d-f	Mean sum of squares	F-value
Between Groups	33.175	3	11.058	5.875*
Within Groups	52.700	28	1.882	
Total	85.875	31		

control (Group 4) groups, respectively (Table 7; Table 10 and Figure 4). There was no significant difference among the treatment groups pertaining to duration of estrus (Table 8).

4.1.5 Degree of synchrony:

The degree of estrus synchrony from the time of administration of $PGF_2\alpha$ injection in Selectsynch, sponge withdrawal/administration of $PGF_2\alpha$ injection in Progesterone+Ovsynch group. While in nutritional supplement group, the onset of estrus was calculated from discontinuation of nutritional supplements given for 9 days and exposure to apronized ram and in control group from exposure to apronized ram to the time elapsed for onset of estrus was categorized into <24 hrs, 24-48 hrs, 48-72 hrs, 72-96 hrs. The incidence of estrus induction in <24 hrs, 24-48 hrs, 48-72 hrs, 72-96 hrs were 0.00 (0/12), 16.66 (2/12), 50.00 (6/12) and 0.00% (0/12) in Selectsynch group, 0.00 (0/12), 25.00 (3/12), 58.33 (7/12) and 16.66% (2/12) in Progesterone+Ovsynch group, 0.00 (0/12), 8.33 (1/12), 33.33 (4/12) and 8.33% (1/12) in nutrition and mineral supplements group and 0.00 (0/12), 0.00 (0/12), 8.33 (1/12) and 25.00% (3/12) in control group, respectively (Table 9).

4.2 CONCEPTION RATE:

The conception rates were 50.00 (6/12), 66.66 (8/12), 33.33 (4/12) and 25.00 (3/12) per cent in Selectsynch (Group 1), Progesterone+Ovsynch (Group 2), nutritional supplements (Group 3) and control (Group 4) groups, respectively (Table 11 and Figure 5). Significantly ($P<0.05$) higher conception rate was recorded in Progesterone+Ovsynch group (83.33%) when compared to Selectsynch (50.00%), nutrition and mineral supplements (66.66%) and control (25.00%) groups.

The percentage of ewes conceived among estrus responded ewes in Selectsynch, Progesterone+Ovsynch, nutritional supplement and control groups was 75.00 (6/8) and 66.66 (8/12), 66.66 (4/6) and 75.00 (3/4) per cent, respectively (Table

Table 7: Duration of estrus (Mean±SE) among treatment groups in Vizianagaram ewes during non-breeding season

Group	Treatment	Number of ewes exhibited estrus	Duration of estrus (hrs) (Range)	Duration of estrus (hrs) (Mean±SE)	F-value
Group 1 (n=12)	Selectsynch	8	24-48	24.00±4.82	0.951
Group 2 (n=12)	Progesterone+Ovsynch	12	24-48	26.00±2.88	
Group 3 (n=12)	Nutrition and nutritional supplements	6	24-48	24.00±1.64	
Group 4 (n=12)	Conventional feeding and grazing	4	24-36	24.00±2.01	

Table 8: ANOVA for duration of estrus among treatment groups

Source of variation	Sum of squares	d-f	Mean sum of squares	F-value
Between Groups	106.875	3	35.625	0.951
Within Groups	1049.000	28	37.464	
Total	1155.875	31		

Table 9: Degree of estrus synchrony at different times for onset of estrus (hrs) among treatment groups in Vizianagaram ewes during non-breeding season

Group	Treatment	Number of ewes exhibited estrus	Time to onset of estrus after sponge withdrawal/PGF_2 alpha injection			
			<24 hrs	24-48 hrs	48-72 hrs	72-96 hrs
Group 1 (n=12)	Selectsynch	8	0.00	16.66 (2/12)	50.00 (6/12)	0.00
Group 2 (n=12)	Progesterone+Ovsynch	12	0.00	25.00 (3/12)	58.33 (7/12)	16.66 (2/12)
Group 3 (n=12)	Nutrition and nutritional supplements	6	0.00	8.33 (1/12)	33.33 (4/12)	8.33 (1/12)
Group 4 (n=12)	Conventional feeding and grazing	4	0.00	0.00	8.33 (1/12)	25.00 (3/12)

Table 10: Estrus response, time for onset of estrus, duration of estrus and intensity of estrus among treatment groups

Parameter	Group 1 (Selectsynch)	Group 2 (Progesterone+Ovsynch)	Group 3 (Nutrition and nutritional supplements)	Group 4 (Conventional feeding and grazing)
Estrus response rate (%)	66.66	100.00	50.00	25.00
Time of onset of estrus (hrs) (Mean±SE)	45.00 ± 7.08^{ab}	38.00 ± 4.63^{b}	56.00 ± 8.00^{a}	90.00 ± 18.00^{ab}
Estrus intensity (Mean±SE)	7.50 ± 0.65^{a}	8.60 ± 0.47^{a}	6.10 ± 0.24^{b}	5.00 ± 0.22^{b}
Duration of estrus (hrs) (Mean±SE)	24.00±4.82	26.00±2.88	24.00±1.64	24.00±2.01

11 and Figure 6). There was no significant difference in the conception rate among the ewes that exhibited estrus response.

4.3 PREGNANCY DIAGNOSIS:

Early pregnancy diagnosis was estimated on the basis of non-return rates and ultrasound examination on day 45 post-mating.

4.3.1 Non-return rate (NRR):

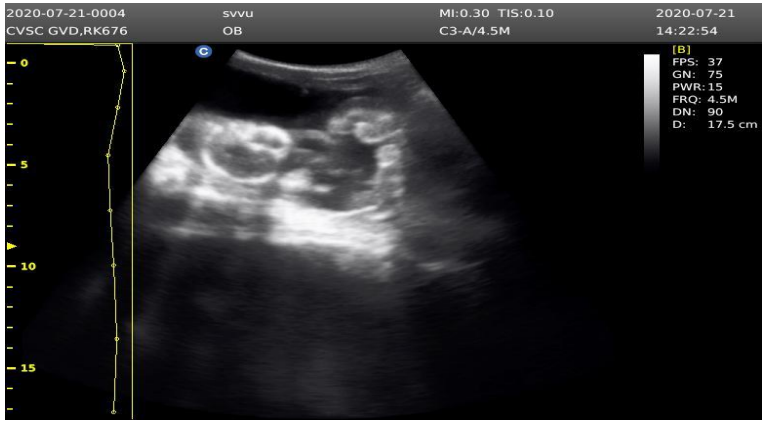
The conception rates on non-return basis were 50.00 (6/12), 66.66 (8/12), 33.33 (4/12) and 25.00 (3/12) in Group 1, 2, 3 and 4, respectively. The overall non-return rate (NRR) was 43.75 (21/48) among all the treatment groups (Table 11).

4.3.2 Pregnancy diagnosis by trans abdominal Ultrasound examination:

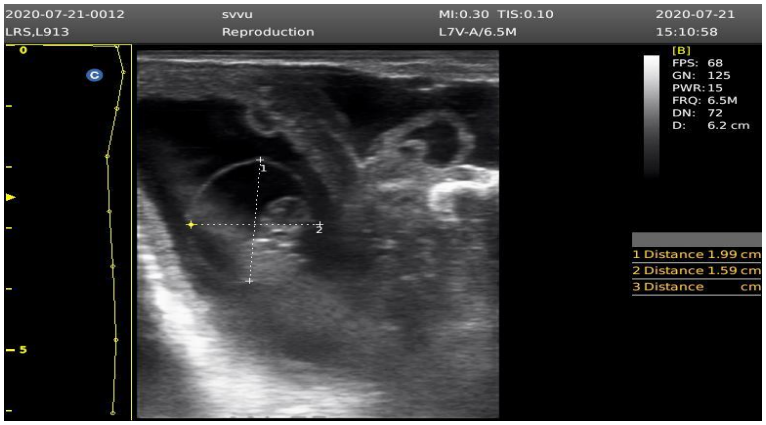
Early pregnancy diagnosis was carried out with ultrasound examination using 3.5 MHz trans abdominal probe on day 45 (Plate 4) post-mating. Pregnancy was confirmed by visualizing uterine fluid, presence of placentomes and fetal skeleton. The overall conception rate by using ultrasonography was 39.58% (19/48) Vs 43.75% (21/48) non-return basis among all treatment groups (Table 11). When the efficacy of NRR pregnancy diagnosis method compared with ultrasound method of pregnancy diagnosis revealed that the false positive percent was 4.17 with NRR method of pregnancy diagnosis.

In the present study, the highest estrus response rate was observed in Progesterone+Ovsynch (100.00%) followed by Selectsynch (66.66%) group of ewes with early onset of estrus in Progesterone+Ovsynch (38.00±4.63 hrs) group of ewes. The duration of estrus in ewes was similar among all the treatment groups. On the other hand, higher estrus intensity grade was noticed in Progesterone+Ovsynch (8.60±0.47) followed by Selectsynch (7.50±0.65) group of ewes. The lower estrus response rate (33.33%), longer time interval for onset of estrus (90.00±18.00 hrs) and lower estrus intensity grade were noticed in control (5.00±0.22) group of ewes.

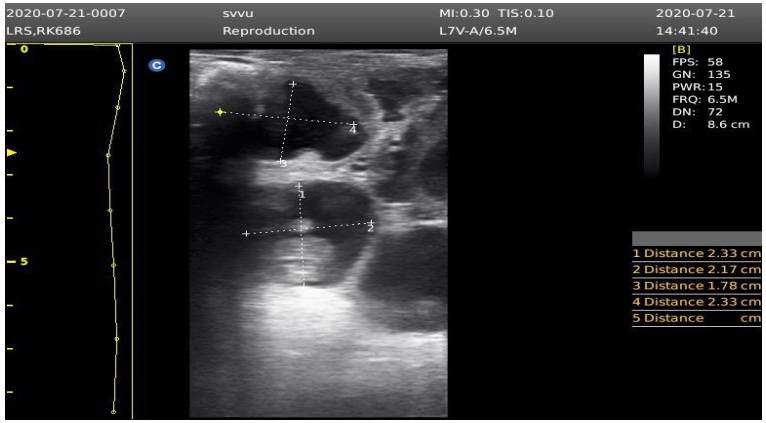
Plate 3: Ultrasonography images of 45 days of pregnancy in Vizianagaram ewes



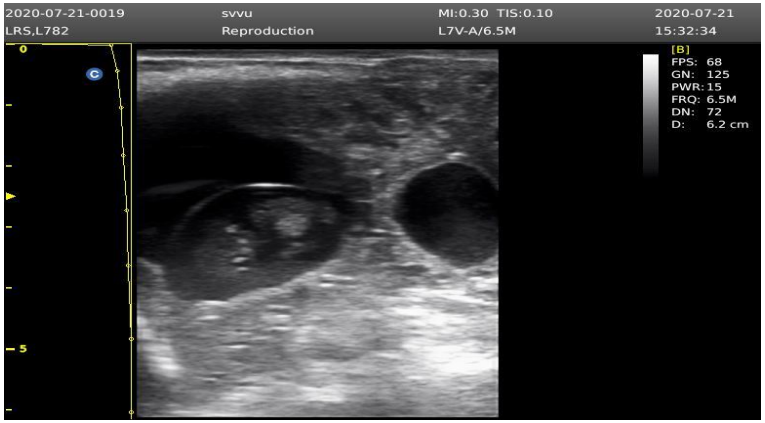
Anechoic uterine fluid with appearance of fetal parts



Anechoic amniotic vesicle with appearance of embryo proper



Compartmentalization and anechoic amniotic vesicles with appearance of embryo proper



Anechoic uterine fluid and amniotic vesicle with appearance of embryo proper

Table 11: Conception rate (%) among treatment groups in Vizianagaram ewes during non-breeding season

Parameter	Group 1 (Selectsynch)	Group 2 (Progesterone+Ovsynch)	Group 3 (Nutrition and nutritional supplements)	Group 4 (Conventional feeding and grazing)	Overall conception rate (%)	Chi-square
Conception rate (%) on non-return basis	50.00 (6/12)	66.66 (8/12)	33.33 (4/12)	25.00 (3/12)	43.75 (21/48)	0.003*
Conception rate on ultrasound examination on day 45 post mating	50.00 (6/12)	66.66 (8/12)	25.00 (3/12)	16.66 (2/12)	39.58 (19/48)	
Per cent ewes conceived among estrus responded ewes	75.00 (6/8)	66.66 (8/12)	66.66 (4/6)	75.00 (3/4)	76.66 (23/30)	0.434

Figure 5: Conception rate (%) among treatment groups in Vizianagaram ewes

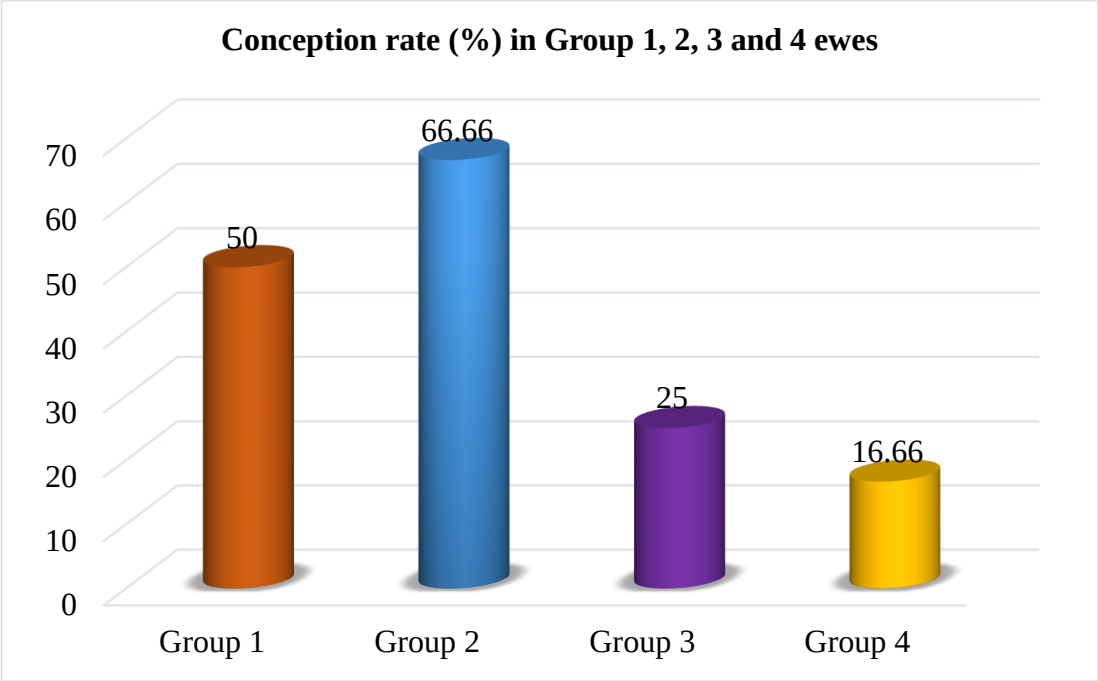
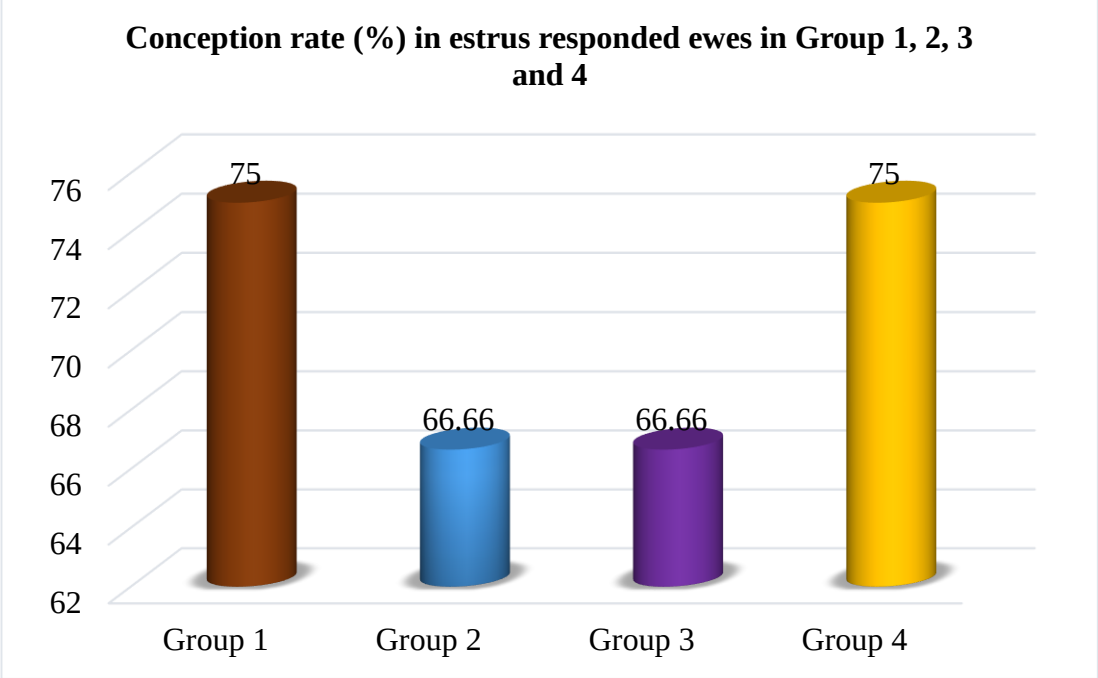


Figure 6: Conception rate (%) in estrus responded ewes among treatment groups in Vizianagaram ewes



CHAPTER-V

5. DISCUSSION

The present study was undertaken in forty eight Vizianagaram ewes aged about 2-4 years stationed at Livestock Research Station, Garividi, Vizianagaram District, Andhra Pradesh during the non-breeding season from March 2020 to May 2020. The trial was conducted to study the estrus response and conception rate in Selectsynch (Group 1), Progesterone+Ovsynch (Group 2), nutrition and nutritional supplement (Group 3) and conventional feeding and grazing (Group 4, control) groups. All the ewes were examined for exhibition of estrus signs to record the estrus response rate, onset of estrus, estrus intensity, duration of estrus and degree of synchrony. All the ewes, which expressed estrus were bred by natural mating with a fertile ram and the conception rate was studied based on non-return rate and ultrasound examination on day 45 post-mating.

The efficacy of each group and among the groups were analysed in terms of estrus response rate, onset of estrus, intensity of estrus, duration of estrus, degree of synchrony and conception rate. The efficacy of each protocol was discussed critically here under.

The estrus response rate was 66.66 (8/12), 100.00 (12/12), 50.00 (6/12) and 33.33 (4/12) per cent in Group 1, 2, 3 and 4, respectively. The estrus response rate was significantly ($P<0.05$) higher in Progesterone+Ovsynch (100.00%) and Selectsynch (83.33%) groups when compared to nutrition and nutritional supplement and control groups.

In the present study, the estrus response rate in Progesterone+Ovsynch group was 66.66%. Ali *et al.* (2009) who also reported similar estrus response rate as 68.80% in Farafra ewes. However, higher estrus response rate than the present study was recorded in the studies of Abu *et al.* (2016) in Rahmani ewes (93.33%), Rekik *et*

al. (2016) in Menz breed ewes (73.3%), Daghash *et al.* (2017) in Egyptian ewes (100.00%) and Kulaksiz *et al.* (2013) in Fat tailed ewes (82.30%). On the contrary, lowered estrus response rates were observed by Martinez *et al.* (2011) in Dorper ewes (25.50%), Ashmawy (2012) in Rahmani ewes (30.00%), Hashem *et al.* (2015), Lone *et al.* (2016) and Waheeb *et al.* (2017) in infertile Corriedale sheep (28.57 to 50.00%). The variations in estrus response rate might be attributed to differences in breed, dose used and type of hormone analogue (Ashmawy, 2012), lactation status, nutrition, agro-climatic conditions of the region and season of study (Omontese *et al.*, 2010).

In the present study, the estrus response rate in Progesterone+Ovsynch was 100.00%. Higher estrus response in the present study consonance with the reports of Husein and Kridli (2003) and Martinez *et al.* (2011) with addition of progesterone prior to GnRH and $PGF_2\alpha$ administration. Kulaksiz *et al.* (2013) and Jackson *et al.* (2014) also conducted similar studies by addition of progesterone between GnRH and $PGF_2\alpha$ administration. In contrary to the present study, Bacha *et al.* (2014) recorded lower estrus response rate (supplementation of progesterone between GnRH and $PGF_2\alpha$ administration). Variations in the estrus response rate might be due to differences in the time and duration of administration of progesterone in combination with GnRH and $PGF_2\alpha$ as well as the type and dose of the hormones utilized.

In the present study, Progesterone+Ovsynch had higher estrus response rate than the Selectsynch, nutrition and nutritional supplement and conventional feeding and grazing groups. Administration of progesterone source between the GnRH and $PGF_2\alpha$ treatments might had effectively delayed estrus and ovulation allowing better synchrony (Tomkins and Bryant, 1974 and Todini *et al*., 2007), which might be the reason for better estrus response rate in Progesterone+Ovsynch group. Sponges impregnated with progesterone provided estrus synchronization by exerting negative

feedback on luteinizing hormone (LH) secretion that inhibited the endocrine events leading to the maturation of pre-ovulatory follicles and ovulation (Wildeus, 2000 and Jackson *et al*., 2014).

In the present study, Group 3 (nutrition and nutritional supplements) and Group 4 (control) ewes had shown estrus response rate of 50.00 and 33.33 per cent, respectively. Perusal of pertinent literature revealed no reports related on mineral supplementation for better reproductive activity in ewes during breeding and non-breeding seasons.

The present study recorded mean time taken for the onset of estrus (hrs) from the time of administration of $PGF_2\alpha$ injection in Selectsynch, sponge withdrawal /administration of $PGF_2\alpha$ injection in Progesterone+Ovsynch group. While in nutritional supplement group, the onset of estrus was calculated from discontinuation of nutritional supplements given for 9 days and exposure to apronized ram and in control group from exposure to apronized ram was 45.00±7.08 (24-72), 38.00±4.63 (24-72), 56.00±8.00 (24-72) and 90.00±18.00 (72-120) hours, respectively. Significantly ($P<0.05$) shorter time interval to onset of estrus was observed in Progesterone+Ovsynch group (38.00±4.63 hrs) followed by Selectsynch (45.00±7.08 hrs), nutritional supplement (56.00±8.00 hrs) and control (90.00±18.00 hrs) groups.

The mean interval from the time of $PGF_2\alpha$ injection to the time of first appearance of behavioural estrus in Selectsynch group was 45.00±7.08 hrs. Ali *et al*. (2009) and Oliviera *et al*. (2009) reported the time for onset of estrus as 41.0±9.6 hours after $PGF_2\alpha$ injection on day 7 in Farafra ewes and Santa Ines ewes, respectively.

Interval to onset of estrus upon the sponge removal/$PGF_2\alpha$ injection (Progesterone+Ovsynch) among the synchronized groups was markedly shorter (45.00±7.08 hrs and 38.50±4.63 hrs) when compared with the findings of Kulaksiz *et*

al. (2013) who reported the time for onset of estrus as 52.80±4.87 hrs in Nellore Jodipi ewes. Similarly, Jackson *et al*. (2014) observed longer duration for onset of estrus than that of the present study. The variations in the time for onset of estrus (hrs) in the Progesterone+Ovsynch group might be due to the differences in sample size, progesterone source and duration of sponge administration, breed and season.

In the present study recorded 6 out 12 ewes exhibited estrus at 50.00±8.00 hrs after the discontinuation of nutritional supplements. In control group, during the non-breeding season 4 out 12 ewes had exhibited estrus after 90.00±18.00 hours after exposure to the apronized ram. Minerals and vitamins were considered as the deciding factors for higher reproductive rates and they helped to optimize the body condition, body metabolism and reproductive activity in terms of estrus response, estrus intensity and improved the fertility rate (Kundu *et al*., 2014). No pertinent literature on nutritional supplementation for better reproductive activity in ewes during breeding season and non-breeding season was available for comparison.

Estrus intensity was measured in ewes on a 12 point scale (scale of 0 to 12) and was determined as 7.50±0.65 (6-10), 8.60±0.47 (6-12), 6.10±0.24 (5-7) and 5.00±0.22 in Group 1, 2, 3 and 4, respectively. Among all the treatment groups, none of the ewes showed weak estrus (0-4). The estrus intensity grade was significantly ($P<0.05$) higher in Progesterone+Ovsynch (8.60±0.47) and Selectsynch (7.50±0.65) groups when compared to nutritional supplement (6.10±0.24) and control (5.00±0.22) groups. There was no significant difference between Selectsynch and Progesterone+Ovsynch groups and similarly, there was no significant difference between nutritional supplement (Group 3) and control (Group 4) groups for intensity of estrus. The frequently observed estrus signs in intense estrus ewes included signs like nudging, kicking, flehmen posture, mounting and service by the ram and by the ewe were squatting, active soliciting, tail fanning and looking over shoulders.

Tomkins and Bryant (1974) compared the estrus behaviour in Polled Dorset ewes progestogen-synchronized with 60 mg of MAP intra-vaginal sponges as nudging, kicking, flehmen posture, mounting and service by the ram and of squatting, active soliciting, tail fanning and looking over shoulders by the ewe. They concluded that progestogen treatment produced no major differences in the manifestation of behaviour compared to natural estrus. Homeida *et al.* (2009) observed that the estrus intensity in Naeimi ewes synchronized with 10 mg of $PGF_2\alpha$ (Dinoprost tromethamine) was 6.0±0.1. Kalyan *et al.* (2015) recorded the signs of estrus in ewes as, restlessness, shaking of tail, slightly swollen vulva, moist and reddish vulva and vagina, seeking for ram, rubbing their body and neck against the ram and standing still during mounting in a field trial after estrus synchronization with progesterone containing vaginal sponges.

The mean duration of estrus was 24.00±4.82 (12-48 hrs), 26.00±2.88 (24-48 hrs), 24.00±1.64 (24-48 hrs) and 24.00±2.01 (24-36 hrs) in Group 1, 2, 3 and 4, respectively. There was no significant difference among the treatment groups pertaining to the duration of estrus.

The estrus duration recorded in the present study with Selectsynch treatment was longer (24.0±4.82) than the duration of estrus (19.0±2.1) observed by Ali *et al*. (2009) in Farafra ewes.

In the present study, duration of estrus observed with Progesterone+Ovsynch treatment was 26.50±2.8 hrs. Similar results were reported (28.00±1.88hrs) by Kulaksiz *et al*. (2013) in Nellore Jodipi and Fat tailed ewes during the breeding season, respectively.

The mean estrus duration recorded in Group 3 and 4 ewes was 24.00±1.64 (24-48 hrs) and 24.00±2.01 (24-36 hrs), respectively.

The degree of estrus synchrony from the time of administration of $PGF_2\alpha$

injection in Selectsynch, sponge withdrawal/administration of $PGF_{2}\alpha$ injection in Progesterone+Ovsynch group. While in nutritional supplement group, the onset of estrus was calculated from discontinuation of nutritional supplements given for 9 days and exposure to apronized ram and in control group from exposure to apronized ram to the time elapsed for onset of estrus was categorized into <24 hrs, 24-48 hrs, 48-72 hrs and 72-96 hrs. The incidence of estrus induction in <24 hrs, 24-48 hrs, 48-72 hrs, 72-96 hrs as 0.00 (0/12), 16.66 (2/12), 50.00 (6/12) and 0.00% (0/12) in Selectsynch group, 0.00 (0/12), 25.00 (3/12), 58.33 (7/12) and 16.66% (2/12) in Progesterone+Ovsynch group, 0.00 (0/12), 8.33 (1/12), 33.33 (4/12) and 8.33% (1/12) in nutrition and mineral supplements group and 0.00 (0/12), 0.00 (0/12), 8.33 (1/12) and 25.00% (3/12) in control group, respectively.

The conception rates were 50.00 (6/12), 66.66 (8/12), 33.33 (4/12) and 25.00 (3/12) per cent in Group 1, 2, 3 and 4 groups, respectively. Significantly ($P<0.05$) higher conception rate was recorded in Progesterone+Ovsynch (Group 2) adopted group of ewes (66.66%) when compared to Selectsynch (50.00%), nutritional supplement (33.33%) and control (25.00%) groups. The percentage of ewes conceived among estrus responded ewes in Selectsynch, Progesterone+Ovsynch, nutritional supplement and control groups was 75.00 (6/8), 66.66 (8/12), 66.66 (4/6) and 75.00% (3/4), respectively. There was no significant difference in the conception rate among the ewes that exhibited estrus response.

It was observed that references on efficacy of Selectsynch protocols are very scanty and the schedule of experiment was different in terms of dose of the hormone used, breed variation of ewes, season and location of experiment. In the present study, 6 out of 12 ewes were conceived in Selectsynch group (50.00%).

The conception rate in the Progesterone+Ovsynch group recorded in this study was 66.66% (8/12). The present study was in partial agreement with the studies

of Husein and Kridli (2003), Khan *et al*. (2007) and Kulaksiz *et al.* (2013) who recorded conception rate in ewes with 75.00, 75.00 and 57.10 per cent, respectively. Similarly, Khan *et al*. (2007) reported a conception rate of 75.00 per cent in ewes with progesterone sponges and gonadotropin treatment. Akoz *et al*. (2006) documented the higher conception rates were 93.30, 92.80 and 86.70% in ewes with intra-vaginal sponges (30 mg of PGA for 7 days) with 300, 500 and 700 IU of PMSG in Akkaraman crossbred ewes during non-breeding season as compared to present study.

In the present study (nutrition and nutritional supplements) 33.33 per cent (4/12) of Group 3 ewes were conceived, while only three out of the 12 ewes of control group had conceived. Several earlier studies had indicated that trace elements were needed for vitamin synthesis, hormone production, enzyme activity, energy production and other physiological processes related to growth, health and reproduction (Kundu *et al*., 2014).

The overall non-return rate (NRR) was 66.66% (32/48) among all the treatment groups. The results of the present study were in partial agreement with the reports of Ucar *et al.* (2005) who recorded the overall non-return rate (NRR) was 71.40 per cent. On contrary with the observation of Paulenz *et al.* (2003) and Ustuner *et al.* (2007) who reported lower NRR as 26.00 and 22.00 per cent, respectively. The variable results might be due to variations in heat detection methods or ability of heat detection by rams and season of study.

Early pregnancy diagnosis was carried out with ultrasound examination using 3.5 MHz trans-abdominal probe on day 45 post-mating. The overall conception rate by using ultrasonography was 39.58% (19/48) Vs 43.75% (21/48) on non-return basis in all treatment groups. When the efficacy of pregnancy diagnosis by NRR method was compared with ultrasound method, it revealed that the false positive

percent was 4.17 with NRR method of pregnancy diagnosis.

In the present study, real time ultrasound examination through trans-abdominal scanning the placentomes appeared like echogenic densities along the uterine wall and was taken as a positive sign for pregnancy at 45 days post-mating (Buckrel, 1986).

Basic principle of controlled breeding includes bringing cyclic and non-cyclic animals into uniform stage of reproduction and thus resulting in synchronized estrus. Administration of GnRH followed by $PGF_2\alpha$ therapy after 7 days assisted to induce estrus, which could be synchronized with GnRH after two days of $PGF_2\alpha$ administration. The protocol without second GnRH was referred to as Selectsynch whereas the same with second GnRH was Ovsynch, which was very successful and popular as reported by Turk *et al*. (2008) and Yadiz *et al*. (2011). Prostaglandins were available in natural and synthetic forms with different chemical nature and luteolysis occurred with Cloprostenol sodium @125 µg.

The Selectsynch protocol was adopted on 12 non-cyclic Vizianagaram ewes during the non-breeding season and they responded to the hormonal protocol. Selectsynch protocol resulted in 66.66 per cent ERR, with onset of estrus at 45.00±7.08 hours after the $PGF_2\alpha$ administration and the duration of estrus was 24.00±4.82 hours. 6 out of 8 ewes, which responded for treatment exhibited estrus between 48 to 72 hours (degree of estrus synchrony). The initial injection of GnRH in Selectsynch protocol provoked a preovulatory like LH surge to ovulate the dominant follicle available on the ovary or initiated a new follicular wave and induced ovulation and the CL already present was unaffected. The developed follicle is supported by the GnRH for maturity and further ovulation, while the developed CL was unaffected and was more developed by day 7, which was lysed by the $PGF_2\alpha$ administered on day 7 and resulted in estrus.

Ewes in estrus after Selectsynch protocol were promptly identified and natural mating for 2 to 6 days with fertile rams. In the present study, 66.66% (8 out of 12) postpartum anoestrus ewes exhibited estrus with administration of 4 µg of Buserelin acetate on day 0 and 125 µg of Cloprostenol sodium on day 7. Estrus response rate was 68.80% as reported by Ali *et al*. (2009) in Farafra ewes with 25µg of GnRH and 15 µg of $PGF_2\alpha$ on day 6. On the contrary to the present observations, Martinez *et al*. (2013) recorded much lower estrus response rate. Variations in the ERR might be due to differences in the administration of different types of gonadotropins their sources, dosage, season and breed of ewes.

Selectsynch protocol was successful in inducing estrus but the protocol necessitates heat detection, which was easily achieved by using ram in the present study. Ewes in estrus after Selectsynch protocol were frequently identified and natural mated with fertile rams for the subsequent 2 to 6 days. Hence, Selectsynch protocol could be a appropriate tool to induce estrus with acceptable conception rates during non-breeding season in Vizianagaram ewes.

However, due to the lack of synchrony of ovulation, the progesterone based synchronization regimes were required for the detection of estrus. During the past decade, considerable efforts were made to develop protocols for estrus and ovulation synchronization in small ruminants (Kridle *et al*., 2003). Ovsynch and Cosynch protocols synchronized the preovulatory gonadotropin surge and resulted in relatively synchronous ovulation.

In the present study, trail was conducted during the non-breeding season by using Progesterone+Ovsynch treatment, which resulted in 100.00% estrus response with early onset of estrus (38.00±4.63hrs) and estrus duration of 26.00±2.88 hrs, which was intense. The conception rate recorded was significantly higher ($P<0.05$) when compared to the other treatment groups (66.66 Vs 50.00, 33.33 and 25.00%).

The higher estrus intensity observed in the Progesterone+Ovsynch could be due to better formation of dominant follicle than in other groups.

In the present study, Group 3 ewes, which received mineral mixture supplementation had shown ERR of 50.00 per cent with onset of estrus as 2-3 days (56.00±8.0 hrs) with moderate estrus signs. Ewes treated with mineral supplement during non-breeding season responded poorly (50.00%) than the Selectsynch and Progesterone+Ovsynch group (50.00% Vs 66.66% and 100.00%), but slightly better than the control group (50.00% Vs 33.33%).

The better results in terms of estrus response, degree of synchrony and improved fertility were observed in the present study with Progesterone+Ovsynch and Selectsynch treatments could be due to attainment of synchronized follicular wave by administration of gonadotropin releasing hormone (GnRH) analogue and administration of $PGF_2\alpha$ analogue removed the suppressive effect on the existing dominant follicle by withdrawal of progesterone.

Acceptable estrus response and conception rate were achieved with Selectsynch treatment in ewes during non-breeding season. A higher ERR (100%) and conception rate (66.66%) were recorded with Progesterone+Ovsynch treatment when compared to other treatments in the present study in Vizianagaram ewes during the non-breeding season. The results of the present study indicated that Progesterone+Ovsynch (GPG) and Selectsynch (GnRH+$PGF_2\alpha$) could be a used as effective tools to augment the fertility in anestrous ewes during the non-breeding season.

CHAPTER-VI

6. SUMMARY

The present study entitled “Studies on certain hormonal and nutritional strategies to improve synchronization of estrus and fertility in ewes under farm conditions during the non-breeding season” are summarised as follows.

This study was undertaken to determine the efficacy of three different synchronization protocols in Vizianagaram ewes. A total of 48 non-pregnant, healthy and aged about 2 to 4 years ewes were selected**.** The selected ewes were randomly divided into four groups of twelve each designated as Selectsynch (Group 1), Progesterone+Ovsynch (Group 2), feed supplements (Group 3) and untreated control (Group 4). In Selectsynch group (Group 1), each ewe was administered with GnRH analogue @ 4.0 µg on day 0 and $PGF_2\alpha$ analogue Cloprostenol @ 125 µg on day 7 intramuscularly. In Progesterone+Ovsynch group (Group 2) progesterone sponge was inserted intra-vaginal and GnRH analogue @ 4.0 µg was administered on day 0. On day 7 $PGF_2\alpha$ analogue cloprostenol @ 125 µg was administered intramuscularly and the sponge was removed and second injection of 4.0 µg GnRH analogue was administered on day 9. In feed supplements group (Group 3), each ewe was fed with additional 200 gm of concentrate added with Mineral mixture (Minfa Gold) consisting of vitamins and minerals @ 20 gm per day for 9 days along with the daily quantity of concentrate feed. The ewes in control group were provided with conventional feeding and grazing without any additional nutrition or hormonal protocol.

Ewes were observed for the symptoms of estrus by using teaser ram daily 4 times with an interval of 6 hours for the duration of 30 minutes for five days after the treatments. Pregnancy diagnosis was conducted on the basis of non-return rates and ultrasound examination on day 45 of post-mating.

The estrus response rate was 66.66 (8/12), 100.00 (12/12), 50.00 (6/12) and 33.33 (4/12) percent in Selectsynch (Group 1), Progesterone+Ovsynch (Group 2), nutritional supplement (Group 3) and Control (Group 4), respectively.

The mean time taken (hrs) for the onset of estrus from the time of administration of $PGF_2\alpha$ injection in Selectsynch, sponge withdrawal/ administration of $PGF_2\alpha$ injection in Progesterone+Ovsynch group. While in nutritional supplement group, the onset of estrus was calculated from discontinuation of nutritional supplements given for 9 days and exposure to apronized ram and in control group from exposure to apronized ram was 45.00±7.08 (24-72), 38.00±4.63 (24-72), 56.00±8.00 (24-72) and 90.00±18.00 (72-120) hours, respectively. Significantly ($P<0.05$) shorter time interval to onset of estrus was observed in Ovsynch+progesterone sponge group (38.00±4.63 hrs) followed by Selectsynch (45.00±7.08 hrs), nutritional and mineral supplement (56.00±8.00 hrs) and control (90.00±18.00 hrs) groups.

Estrus intensity measured in ewes on a scale of 12 (0 to 12) was 7.50±0.65 (6-10), 8.60±0.47 (6-12), 6.10±0.24 (5-7) and 5.00±0.22 (3-5) points in Selectsynch (Group 1), Progesterone+Ovsynch (Group 2), nutritional supplement (Group 3) and Control (Group 4) groups, respectively. The estrus intensity grade was significantly ($P<0.05$) higher in Progesterone+Ovsynch (8.60±0.47) and Selectsynch (7.50±0.65) groups when compared to nutritional supplement (6.10±0.24) and Control (5.00±0.22) groups.

The mean duration of estrus (hrs) was 24.00±4.82 (24 -48), 26.00±2.88 (24-48), 24.00±1.64 (24-48) and 24.00±2.01 (24-36) in Selectsynch (Group 1), Progesterone+Ovsynch (Group 2), nutritional supplement (Group 3) and Control (Group 4) groups, respectively. There was no significant difference ($P>0.01$) among the groups pertaining to duration of estrus.

The incidence of estrus induction in <24 hrs, 24-48 hrs, 48-72 hrs, 72-96 hrs as 0.00 (0/12), 16.66 (2/12), 50.00 (6/12) and 0.00% (0/12) in Selectsynch group, 0.00 (0/12), 25.00 (3/12), 58.33 (7/12) and 16.66% (2/12) in Progesterone+Ovsynch group, 0.00 (0/12), 8.33 (1/12), 33.33 (4/12) and 8.33% (1/12) in nutrition and mineral supplements group and 0.00 (0/12), 0.00 (0/12), 8.33 (1/12) and 25.00% (3/12) in control group, respectively.

The conception rates were 50.00 (6/12), 66.66 (8/12), 33.33 (4/12) and 25.00 (3/12) per cent in Selectsynch (Group 1), Progesterone+Ovsynch (Group 2), feed supplement (Group 3) and Control (Group 4), respectively. Significantly ($P<0.05$) higher conception rate was recorded in Progesterone+Ovsynch group (66.66%) when compared to Selectsynch (50.00%), nutritional supplement (33.33%) and control (25.00%) groups. The overall conception rate by using ultrasonography was 39.58% (19/48) Vs 43.75% (21/48) based on non-return rates among all treatment groups under the study.

It was concluded from the present study, that acceptable estrus response and conception rate were achieved with Selectsynch treatment. However, higher ERR (100%) and conception rate (66.66%) were recorded with Progesterone+Ovsynch treatment when compared to other treatments of the present study in Vizianagaram ewes during the non-breeding season. The results of the present study indicated that Progesterone+Ovsynch (GPG) and Selectsynch (GnRH+$PGF_2\alpha$) could be a used as effective tools to augment the fertility in anestrous ewes during the non-breeding season.

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