

Estrus Synchronization in Sheep and Cattle: Overview and Some Promising Interventions in Ethiopia

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Abstract

Estrus synchronization is the process of targeting female mammals to come to heat within a short time frame usually within 36 to 96 hours. There are different protocols for estrus synchronization in cattle and sheep by which every estrus synchronization protocol has its own advantages and disadvantages. Selecting fittest protocol with animals used to reduce its disadvantage to get optimum estrus and conception rate. The protocol can be based on prostaglandin, progesterone, and gonadotrophin releasing hormone alone or in combination. In Ethiopia prostaglandin and progesterone based synchronization are practiced but prostaglandin based is more popular by which many research results used it and recommended single injection of prostaglandin followed by heat detection is more fittest protocol. Embryo loss is the major problem of estrus synchronization in Ethiopia which needs intervention like Proper nutrition, Animals before start of synchronization should be vaccinated and treatment should be given if the animal was diseased, Select animals with good body condition.

The objective of the paper is

- **General objective:** To assess different estrus synchronization methods generally accepted for sheep and cattle.
- **Specific objectives:** To see how each method of synchronization works. To give clue about best estrus synchronization method for Ethiopia.

Keywords: Estrus; Protocol; Hormone

Introduction

Estrus synchronization is manipulation of the estrous cycle or induction of estrus to bring a large percentage of a group of females into estrus at a short, predetermined time [13]. Research to understand estrus and estrous cycles was initiated in the United States by Fred F. McKenzie using sheep [9]. Estrus synchronization is very important in sheep since they do not exhibit exaggerate external estrus sign. Selection of appropriate estrus synchronization protocol should be made on the basis of management method and interest of the farmer. Estrus synchronization program can be done by using different hormones such as $PGF_{2\alpha}$, progesterone, estrogen and gonadotrophin realizing hormone. Now a day's different hormone like substances are emerging to reduce treatment days, to

reduce time consumption during heat detection and reduce labor wastage. Prostaglandin is effective on active corpus luteum so need detection of ovarian status carefully. Progesterone may reduce fertility up to 14 percent, but short time progesterone exposure less than 14 days is beneficial [6].

GnRH also become highly effective method of synchronization since it can synchronize non cycling animals by stimulating follicular activity. New methods of synchronizing estrus in which the GnRH-PG protocol is preceded by progesterone treatment offer effective synchronization of estrus with high fertility Estrus (heat) detection has been cited as the most important factor affecting the reproductive success of artificial insemination programs. However, proper control of the time of estrus is difficult, since peak estrus

activity often occurs at night, and determination of the actual onset of standing estrus may be difficult without 24h observation. Several synchronization treatments incorporate an injection of a low dose of pregnant mare serum gonadotropin (PMSG) at the end of the progestagen treatment in order to obtain a more precise and reliable synchronization of estrus since it increases the occurrence of ovulation and improves the post-treatment fertility in small ruminants [16]. Current research has focused on the development of methods that effectively synchronize estrus in postpartum beef cows and replacement beef heifers by decreasing the period of time over which estrus detection is required, thus facilitating the use of fixed timed AI [14].

In Ethiopia, estrus synchronization is an emerging technology by which most research reported were based on prostaglandin based synchronization which is an old method of synchronization as stated on [10]. In Ethiopia, studies on hormone assisted estrus synchronization in cattle started in the late 1980s by a team of researchers in the Animal Health and Reproduction Section of the International Livestock Centre for Africa (ILCA), now International Livestock Research Institute (ILRI).

The research program was initiated with a long-term objective of integrating emerging reproductive technologies, such as embryo transfer and associated techniques, to improve genetics and breeding of indigenous cattle for desirable traits and also to explore opportunities of using these technologies for genetic conservation of indigenous cattle in Africa. This focus of the research program was broadly defined as genetic improvement of cattle for milk and meat production, genetic improvement of trypanotolerant cattle breeds, genetic improvement of cattle for feed utilization efficiency.

In addition to using the ILCA research centers in Debre Berhan and Debre Zeit, scientists undertook collaborative work with the Ministry of Agriculture, particularly at the Abernosa Cattle Improvement and Multiplication Centre and the Gobe Cattle Improvement Centre [1]. Research done by LIVES project revealed that, single dose and heat detection could be a more feasible protocol than the double dose protocol for Ethiopia which takes long time, much labor and need skilled man power for estrus detection [17].

Review on different methods of synchronization and their application preconditions

There are different methods of estrus synchronization techniques, especially for cattle many technologies are emerging to in-

crease production, reduce labor and time. Effective estrus synchronization programs offer the following advantages: cows or heifers are in estrus at a predicted time which facilitates AI, embryo transfer, or other assisted reproductive techniques; the time required for detection of estrus is reduced thus decreasing labor expense associated with estrus detection; cattle will conceive earlier during the breeding period; AI becomes more practical; and calves will be older and heavier at weaning [14]. Another advantage of best synchronization method is it creates a uniform calf crop, enables more cows to breed superior sire. To get good result and more production selection of best way of synchronization method is very important but every synchronization method has an advantage and disadvantage. For example prostaglandin based synchronization is not effective for non cycling animals so for non cycling animals we should select another method which increase follicular activity such as GnRH based method [7].

Before selection of any estrus synchronization method the following precondition should be fulfilled. The animal should have good body condition; the availability of good handling facilities; high quality herd management system; the supply of enough nutrition for the herd because it affect response to the treatment; vaccinating animals to prevent reproductive disease and appointment of veterinarian or any technicians for all over activities counseling [18].

After all the above preconditions are fulfilled the animal should go for synchronization in any of synchronization methods. Cost and labor requirement for activity should also be predetermined according to 12 (Table 1).

Method	Program duration in days	Skilled labor in days	Drug administration days	Drug cost
One shot PGF _{2a}	11	11	1	Cost of PGF _{2a} only
Two shot PGF _{2a}	17	4	2	*PGF _{2a} cost
CIDR+PGF _{2a}	11	4	3	Implant cost+PGF _{2a}

Table 1: Cost of different protocols in terms of days and amount of drugs used adapted from [12].

After all cost analysis determined brand selection done according to your interest and cost also different for different brands.

The following hormones are commonly used.

Hormone	Trade/brand/name
Prostaglandin	estroPLAN, Estrumate, In-Synch, Lutalyse, Lutalyse High Con, ProstaMate, Synchsure
Progesterone based GnRH based	CIDR, MGA, Cystorelin, Factrel, Fertagyl, GONAbreed, OvaCyst

Table 2: Mostly used synchronization drugs adapted from [12].

To go for synchronization we should also know hormonal status and corpus luteum function to get better response in synchronization. The following figure discuss about general hormonal status of cattle on a month.

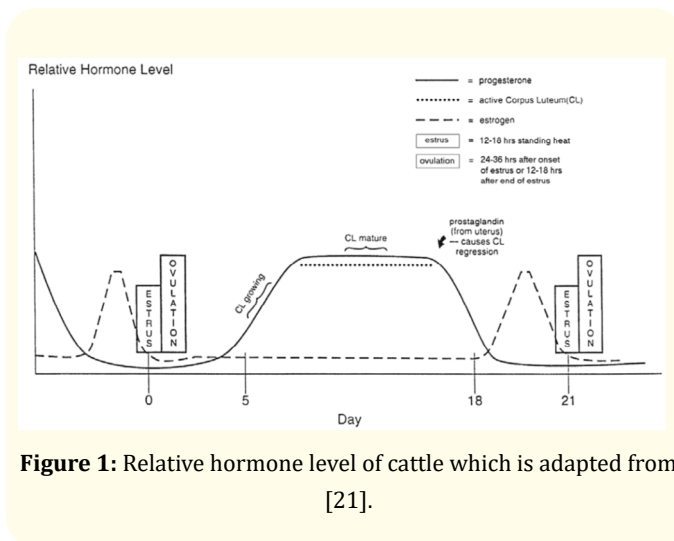


Figure 1: Relative hormone level of cattle which is adapted from [21].

Selection of the fittest method of synchronization based on combing hormonal status of the cow and synchronization hormone activity is very essential.

Review on prostaglandin based synchronization

Prostaglandin based synchronization method is an old method of synchronization with limited activity of only synchronizing cycling animals. According to [12] prostaglandin was approved for beef cattle reproduction since 1979. Prostaglandin is naturally produced in animal body which control reproductive activity by which intramuscular administration of foreign prostaglandin makes the animal come to heat within 2 to 6 days [10].

Anestrous females do not respond to prostaglandin injections. Estrous-cyclic females can respond to injections between days 7 and 16 of their cycles if they have a functional corpus luteum (CL). The CL is a gland that develops in the ovary and secretes the hormone progesterone into the cows blood. Estrous-cyclic females at days 0 to 6 and 17 to 21 of their cycles are without functional CLs and do not respond to injections. However, an estrous-cyclic female without a functional CL will respond to injections if they are given in a specific sequence [18]. Prostaglandin based synchronization is mostly practiced in Ethiopia because it needs low cost but it needs high labor and take long time. In Ethiopia labor and time too much enough but cost is the first limiting factor and skilled technicians are low in number so according to current status of the country prostaglandin based is the fittest [1].

When prostaglandin is injected to animals with silent estrus and unidentified pregnant it cause embryo loss [15] so before synchronization the animal should be checked for pregnancy by sensitive diagnostic equipment such as ultrasound, progesterone profiling using different enzyme, ELISA, detecting kit for pregnancy related glycoprotein.

One shot injection of prostaglandin

This is a low-cost, low-risk program. It requires more labor, but that also allows animals to be observed for cyclicity. Hence, the possibility of wasting semen on non cycling animals is minimized. In this program start by heat detection and artificial insemination from day 1-5 without injection of any PGF_{2α} hormone then by day 6 the technician should calculate the percentage of animals bred, if it is more than 25 percent he can proceed to PGF_{2α} injection for the next 5 days. In normal herd 90-95 percent of animals should come to heat [18].

If less than 25 percent of the herd is bred by day 6; do not proceed with the PGF_{2α} injections. This indicates that your animals are not cycling, and you should check herd nutrition and reproductive status with your local veterinarian, extension agent, or herd consultant [12].

During sheep synchronization when a single treatment of prostaglandin is given to a flock of cycling ewes, 60 to 70 percent of the flock will exhibit a synchronized estrous beginning 30 to 48 hours later. If complete synchronization of the flock is required, 2

treatments 9 days apart are needed. Combining a seven day vaginal pessary or CIDR implant of progestin with prostaglandin is also effective [3].

The overall above procedure in cattle can be demonstrated as follows.

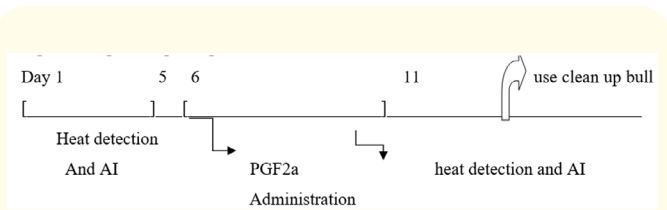


Figure 2: Single shot prostaglandin method adapted from [12].

Two shot injection of prostaglandin

This system is used to bring more cows to come to estrus during artificial insemination. PGF_{2α} is administered on the first day then the second is administered on day 13. Old research tell us the second injection should be on day 11 but recent research advise inject on day 13 for regression of more mature corpus luteum and bred more herd. Detection of estrus before or between injections is not required. All cycling cows should respond to the second injection regardless of what stage of the estrous cycle they were in when the first injection was administered. The programme may be modified with the breeding of all females exhibiting estrus subsequent to the first PGF_{2α} injection. Then the second injection is given only to the females that were not bred. This option lowers expense and handling, but results in two synchronized groups instead of one and a longer breeding period [6].

Note

Timed insemination program with this method is not recommended. A non- cycling herd attributed to nutritional or reproduction problems increase the cost of artificial insemination [12]. This method can be presented as.

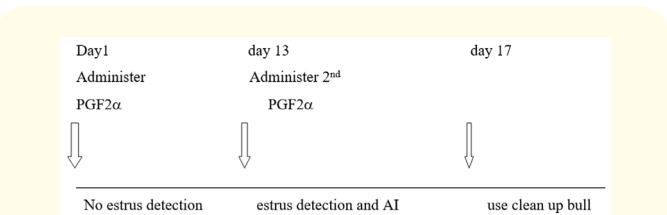


Figure 3: Two shot injection prostaglandin method.

As stated by [3] for sheep 2 shot prostaglandin can be done by the following way.

- Day 1- Inject Lutalyse at 15 mg = 3cc or Estrumate at 150 mg = 6cc (intramuscular injection)
- Day 10- Inject Lutalyse at 15 mg. = 3cc or Estrumate at 150 mg = 6cc
- Day 11- Introduce teaser ram
- Day 12-14 Inseminate ewes 10-12 hrs after ewe is marked by teaser ram (ideal time = 42 hrs after last injection). Heat lasts 18 - 36 hrs, but fertility decreases with each hr.
- Day 27-30 Watch for teaser mark and inseminate 10-12 hrs later.

Progesterone based synchronization

Progesterone is a naturally occurring hormone that functions to maintain pregnancy. This hormone also blocks estrus and ovulation during the diestrus phase of the estrous cycle. When progesterone is introduced artificially, it makes the body to feel like it is pregnant and the animal will not ovulate or come into estrous (heat). When the progesterone source is totally removed, the body realizes it is no longer pregnant and will ovulate within a very predictable period. Progestins refer to the synthetic compounds with the properties of progesterone. These substances mimic the function of the CL [18].

Synchrony of estrus occurs within 2 to 5 days following progesterone removal. Progesterone based synchronization can be done by 2 ways MGA in feed and CIDR which is vaginal implant but ear implant also practiced which is less effective and need minor surgery to implant [6].

MGA based method

Melengesterol acetate is feed additive that single animal should get 0.5mg per day for 14 days. It prevents estrus expression and block ovulation. It can be done in different ways.

- Giving MGA as a feed for 14 days then stop feeding. After removal of the feed the animal start to show estrus sign but conception rate is low during first estrus. To increase fertility we should bred on the second estrus after feed removal.
- MGA+PG way, similar to the above technique we feed the animal for 14 days then give prostaglandin injection after 19 days.

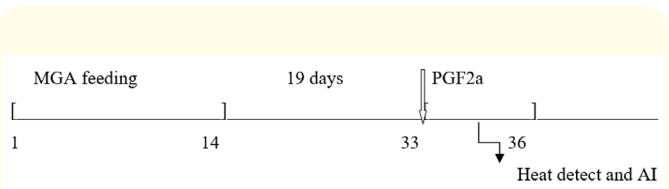


Figure 4: MGA+PG methods of synchronization which is adapted from synchronization protocol 2017.

- The above figure is functional for cattle, in sheep it is different since they are seasonal breeder which is stated as follows.
- Keep ewes away from rams for 2 months.
- Day 1-6 am Begin feeding MGA 15 mg/head every 12 hrs for 8 - 12 days.
- Day 8/12- 6 am last feeding of MGA
- Day 9/13- noon 30 hrs after last feeding inject ewes with PMSG or PG-600 and introduce teaser rams if using A.I.
- Day 10/14 -Inseminate ewes 24-36 hrs after injection either artificially or with fertile rams 1 ram to 5 ewes [3,11].

Since MGA clears the GI tract of individual ewes at different rates, it is difficult to predict the exact time of ovulation when you stop feeding MGA. This makes successful A.I. difficult. Estrus synchronization in sheep by feeding MGA should probably be reserved for mating naturally with rams until more research can be done [3].

Two injections of prostaglandin are given; one at the time of MGA removal from the feed and another 15 days following removal. This further reduces time spent in estrus detection and breeding and provides more concentrated synchrony [6].

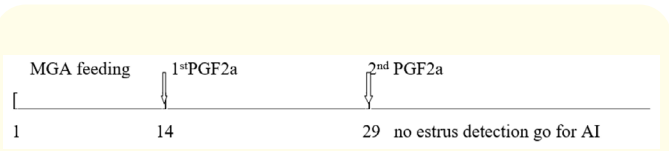


Figure 5: MGA+PG+PG method of synchronization.

CIDR based method

CIDR insert for cattle is made by molding a thin layer of silicon and progesterone mixture around a nylon spine under high tem-

perature. CIDR contains 1.38 g progesterone and is designed to maintain elevated blood concentrations of progesterone at least 2 ng/ml for up to 10 days being relatively thin; the CIDR is easily inserted into the vagina and has good retention capacity [21].

A flexible nylon tail is attached to the device to allow for easy removal. The CIDR Cattle Insert provides an exogenous source of the hormone progesterone during the 7-day administration period. Removal of the CIDR Cattle Insert on treatment day 7 results in a rapid fall in plasma progesterone levels, which results in synchronization of estrus in those animals responding to treatment [6]. To be more effective CIDR is used in combination with other hormones. The following figure shows different protocols for both timed AI and heat detect synchronization technique which is adapted from 2017 synchronization protocol.

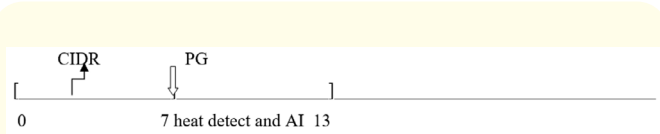


Figure 6: 7 day CIDR+PG protocol.

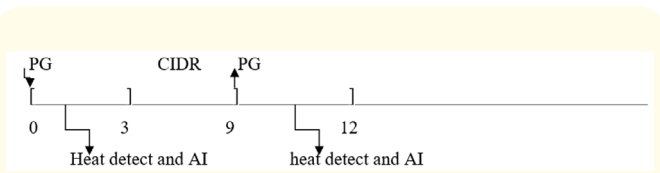


Figure 7: PG 6 day CIDR protocol.

Both the above protocol require estrus detection for timed AI we use the following protocols.

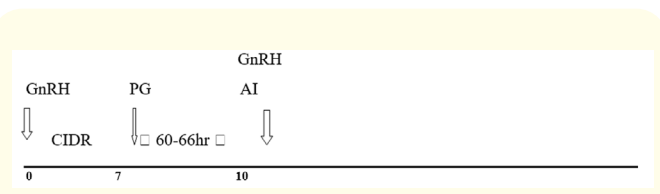


Figure 8: 7-day CO-Synch + CIDR □ Cows Perform TAI at 60 to 66 hours after PG with GnRH at TAI.

Perform TAI at 72 ± 2 hours after CIDR removal with GnRH at TAI.

Two injections of PG 8 ± 2 hours apart are required for this protocol.

injection [18]. GnRH is used in combination with other hormones to be more effective for heat detect and AI or timed AI. The following figure show different protocols which is adapted from [18].

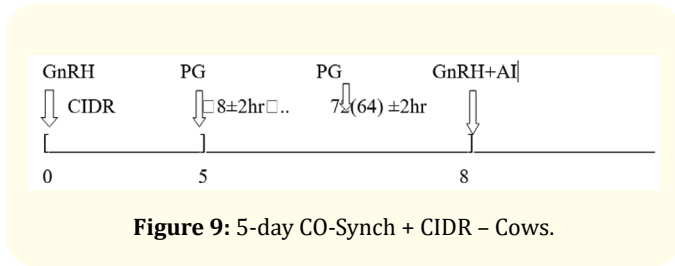


Figure 9: 5-day CO-Synch + CIDR – Cows.

CIDR is mostly used for sheep synchronization according to [3,11] synchronization can be done in the following way.

- Day 1- Insert sponge or CIDR and stay for 12 days. Lubricate sponges and CIDRs and insert at an upward angle.
- Day 6- Remove sponge or CIDR and inject PMSG
- Day 7 -Inject Prostaglandin 24 hrs after sponge removal and PMSG injection.
- Day 8- Introduce teaser ram to better synchronize ewes and to mark ewes as they come into heat.
- Day 9- Inseminate ewes at approximately 48 hours after prostaglandin injection or 10-18 hrs after ewe is marked by teaser ram.
- Day 19- Reintroduce teaser rams and then inseminates 10-18 hrs after ewe is marked or introduce fertile ram, (1 ram to 5 ewes). Ewes will recycle from 15-18 days after last breeding.

PGF2a should be handled carefully because it has cardiovascular effect in early pregnancy on humans. And also it has significant impact on individual who have respiratory problem [12].

GnRH based synchronization

Research indicates that when GnRH is given with prostaglandin to estrous cyclic and non cyclic females, the patterns of follicular development are altered, inducing ovulation.

This treatment may induce estrus in 10 to 30 percent of anestrus females. GnRH treatment is not recommended for pre pubertal heifers because these young heifers have not yet established fertile estrous cycles and have no consistent response to this hormone

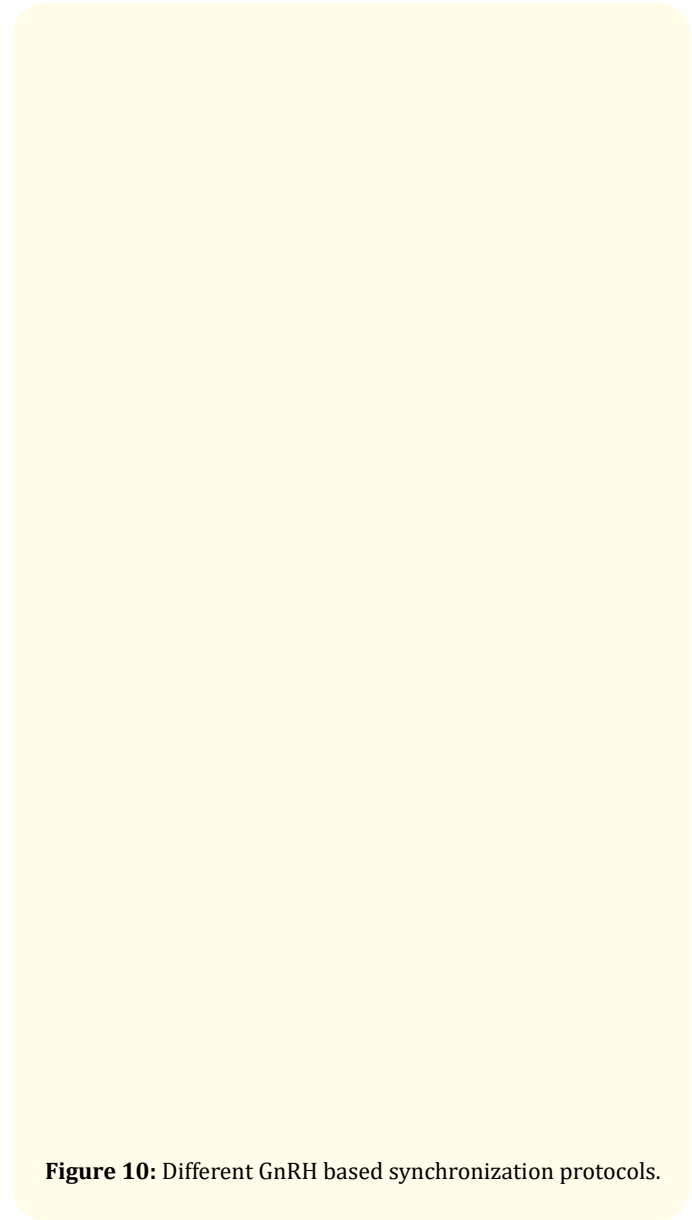


Figure 10: Different GnRH based synchronization protocols.

A modification of the GnRH-PG system designed to be used with timed insemination is a system where by the second GnRH injection is not given and insemination follows estrous detection. Because 5 to 15% of females treated with GnRH will exhibit estrus prior to the time of the PG injection 7 days later, estrus detection

should begin about 4 days after the GnRH injection and should continue for 4 days past the PG injection. This system is easy to implement with only 3 trips through the squeeze chute and it captures the advantage of initiation cycling in some anestrus postpartum cows and prepuberal heifers as the result of GnRH administration. Adding an injection of GnRH to synchronization systems that utilize feed-grade Progestogen (MGA) and prostaglandin F_{2α} (PG) appears to slightly improve the percentage of females that exhibit estrus and causes the animals that exhibit estrus to do so in a shorter time span. With this modification, GnRH is injected 7 days prior to the PG injection [8].

Review about synchronization status in Ethiopia

As stated on introduction part in Ethiopia, studies on hormone assisted estrus synchronization in cattle started in the late 1980s by a team of researchers in the Animal Health and Reproduction Section of the International Livestock Centre for Africa (ILCA), now International Livestock Research Institute (ILRI). The initial work focused on understanding the estrus physiology control of estrus cycle of indigenous zebu and Holstein Friesian x Zebu crossbred cattle. Studies focused on characterization of the estrus cycle, determination of milk/plasma progesterone profiles during the estrus cycle of heifers and cows, determination of puberty in heifers, understanding of ovarian functions during the post-partum period, embryonic mortality, luteolytic effects of different hormones (prostaglandins, progesterone releasing intra vaginal device (PRID) and progestagen ear implants), dose-response studies, effects of different preparations and does of prostaglandins on pregnancy rates [1].

Few types of synchronization interventions in some parts of the country

In Ethiopia the most widely used methods of synchronization is one shot PGF2a injection which is stated as fittest protocol for Ethiopia by different papers such as [2,17,20], etc.

Interventions on cattle estrus synchronization [19] use two shot PGF2a, he also compare different factors for PGF2a response as follows.

He also expressed the difference between local and cross breed response to single and double injection. For single dose local have 60.9% conception and 78.3 estrus rate but for double injection 63% conception and 93.3 estrus rate. Double injection in cross

	Estrus rate (%)	Conception rate (%)
PGF2a		
Single	84.2	39.6
Double	93.3	68.5
Breed		
Local	83.3	38.4
Cross	91.1	36.2
Parity		
Heifer	77.1	34.1
Cow	92.4	38.9

Table 3: Estrus and conception rate difference due to difference in protocol, breed and parity.

breeds have 74.1% conception and 93.3 estrus rate but for single injection 58.49% conception and 90% estrus rate.

According to [2] he used single and double dose injection and founded the following result. The overall estrus rate across districts was found as 83.85%. Relatively cows with parity 2(87.5%) and 3(88%) had higher estrus rate than heifers, parity 1 and parity 4 and above. Slight difference on estrus response at first injection and second injection was obtained in crossed cow/heifers. Estrus response rate of cross cow/heifers (87.04%) was found lower than that of local cow/heifers (81.58%).

Conception rate of cows or heifers had highly significant variation between AI technicians and bull sources that were inseminated to the responded cow/heifers. There was variation in terms of skill of technicians with the highest degree to influence on conception rate. On this matter T₃ T₄ and T₁ had highest skill and experience having 71.4%, 63.9% and 56.25 of conception rate followed by T₂ (35%) and then by T₅ i.e. 33.33%. As stated on his paper the quality of semen is a detrimental factor for success and failure of conception rate. In terms of degree of success of conception of semen from different bulls, Bull 75-289 was the highest (75%) followed by bull 10-211 (55.74%) and 11-289 (44.44%) and then 10-229 (40%). The least figure was recorded for bull 12-346 and null bull 11-328. The difference might be related either due to the level of skill and experience difference of AI technicians on method of thawing, site of semen deposition and heat error or quality of the semen etc.

Generally- In Ethiopia the following factors cause variation between conception and estrus rate of animal.

- **Stress:** In Ethiopia most of the times animals are in stress that makes them less productive and reduced fertility. Especially heat stress in crowded farms reduce animal reproduction since it has effect in steroidogenesis and oocyte viability [15].
- **Technician status:** To be effective highly skilled technicians should be present for synchronization as well as for artificial insemination [19].
- **Body condition score:** This is the most determinant factor for the success of synchronization. In Ethiopia most researches are done using body condition score from 1-4 but [21] stated that BCS<4 are poor candidates for synchronization and timed artificial insemination is not recommended on this animals.
- Cycling status
- Disease-different diseases are present that affect reproductive status of animals in which highly prevalent in Ethiopia such as mastitis.
- **Dietary ingredients:** Cottonseed contains gossypol that can be toxic to mammalian cells [15].
- **Sire:** Sire has a major effect on conception rates in cattle [2], little is known about the effects of sire on pregnancy losses. When evaluated, sire has been shown to have an effect on fetal loss in cattle.
- **Semen status:** Semen should be viable and enough until AI is done. In Ethiopia semen is produced in limited areas and distributed to other places that make the produced semen to deteriorate during transportation and long storage which reduce fertilizing capacity of the semen.

Intervention on sheep estrus synchronization

In Ethiopia estrus synchronization on sheep has many advantages. Some of them are listed on [22]

- Rapid distribution of improved genotypes
- To have multiple lambs at ones by super ovulation.
- Creating age uniformity and programmed breeding for selling during high market value especially for holidays in Ethiopian context.

One research done in Debre Birhan which is stated on [22] is promising intervention which should be encouraged and should distribute all over the country. The research used different meth-

ods of synchronization and founded the following result.

MAP and FGA Sponges used synchronization on black head Oga-den sheep which result in estrus rate of 91.7%, 63.1% pregnancy rate, 64.3% lambing rate by which all results fall on the normal range. Similar research was done on dorper ewes with result of 97% estrus rate, 72.3% pregnancy rate and 91.1% lambing rate.

Estrus response and fertility of Menz and crossbred ewes to single prostaglandin injection protocol was done and founded 65% estrus rate and 84.62% pregnancy rate. Estrus response and fertility of local sheep to prostaglandin based estrus synchronization protocol in south Wollo zone was done and result 82.5% estrus rate and 93.9% pregnancy rate with 1.4 litter size. Estrus response and fertility of Washera sheep to Prostaglandin treatment was evaluated and result 67% conception rate. Another research done on debre berhan sheep research center use Progestagen and prostaglandin in conjunction with GnRH with result of 82.6% conception rate [22].

Suitable synchronization methods for our country

Many researches stated that single dose PGF_{2a} injection with heat detection is more suitable for Ethiopian cattle. [17] stated that According to the current results, double administration of prostaglandin at an interval of 14 days with AI on heat detection or AI after each hormone administration could result in higher estrus response than the single shot protocol. However, the differences are not statistically significant. Moreover, the requirements of the double dose protocol in terms of time, logistics, costs and convenience to farmers may not justify its use, especially under small-holder conditions. Fixed-timed AI was also found to result in less conception rate. Thus the single dose protocol with heat detection could be a feasible option for Ethiopia.

Similarly Tadesse Gugssa on his master thesis recommended that single dose PGF_{2 α} synchronization protocol should be implemented with great care on appropriate animal selection and management to get high estrus response rate and highly requires setting proper heat detection mechanisms to maximize the conception rate of the animals. Fixed time insemination after synchronization with double dose injection protocol of PGF_{2 α} was showed low conception rate. So, to go for higher conception rate, it is better to implement heat detection after double dose synchronization protocol [19].

Conclusion

Every estrus synchronization protocol has advantage and disadvantage selecting fittest protocol with animals used to reduce its disadvantage to get optimum estrus and conception rate. Uses of modern technologies reduce risks of synchronization especially for embryo loss determination latest technologies should be used. In Ethiopia as stated by many studies single dose PGF2a administration followed by heat detection and artificial insemination is more appropriate but other technologies should be tested which increase production. Embryo loss is the major problem of estrus synchronization in Ethiopia which needs interventions like, Proper nutrition because different minerals, vitamins and proteins are very essential for organogenesis and fetal survival, Select animals with good body condition because low body conditioned animals conceive less, Animals before start of synchronization should be vaccinated and treatment should be given if the animal was diseased, Cycling status of the animal should be first checked semen viability should be checked before AI, Treatment for increasing embryo survival should be given at the end of artificial insemination. For example; Progesterone supplementation which increases embryo survival since it can maintain pregnancy, Treatment with bovine somatotropin improves fertilization and accelerates embryo quality, Induction of accessory corpus luteum with human chorion gonadotherophin.

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