Consortium Research Program Livestock & Fish

Animal Genetics Flagship; Cluster: Delivery and Use System

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Field Solutions for Sheep Artificial Insemination

Technical synthesis report

Debre Birhan and Menz, October 09th – October 29th 2015

Bonga and Doyogena, April 12th – April 28th 2016

Doyogena, July 24th – August 10th 2016

Breeding for future generations…

October 2016

IFAD INTERNATIONAL FUND FOR AGRICULTURAL DEVELOPMENT

CGIAR Science for a food secure future

ICARDA Science for Better Livelihoods in Dry Areas

SARI 2001

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Highlights

1. A total of 631 ewes in 4 locations representing over 250 households inseminated
2. New synchronization package comprising ultrasound pregnancy diagnosis immediately followed by a single injection of prostaglandin analogue developed
3. Conception rate to artificial insemination with fresh semen varied between 33 and 43%
4. The first low-infrastructure laboratory of sheep artificial insemination in Ethiopia established
5. Thirty NARS (4 female trainees) trained on reproductive technologies

Framework and Objective

Artificial insemination remains the main universal method for dissemination of improved genetics in livestock species. Artificial insemination is a staged technology with various levels of infrastructure, semen technology, technicity and field organization. Insemination using fresh semen collected in the field and relying on basic infrastructure is regarded as a promising technology for a wider delivery of improved genetics (selected rams in the Community Based Breeding Program; CBBP) under low input systems. This facilitates reaching more farmers within the communities and also reaching out to other communities. This activity aligns with the genetics flagship of CRP livestock and fish which aim to ensure that improved and appropriate sheep and goat breeds are widely available, used sustainably by women and men, and are equitably providing nutritious, affordable food and income for the poor Ethiopian small ruminant owners.

Materials and Methods

Three field trials were conducted between October 2015 and August 2016 in 4 different sites: Debre Birhan, Menz, Bonga and Doyogena.

In the first trial, a total of 131 Menz ewes in 3 different locations were selected in September 2015 for synchronization and insemination. The three locations were the Sheep Research Center in Debre Birhan (n=67), Mehal-Meda (n=42) and Molale (n=22) villages in Menz. All the selected ewes were dry, non-pregnant and had successfully lambed the previous season. No primiparous females were included and the selected ewes were subjected to the prevailing managements (grazing, feeding, housing, health care) both in the research center and the respective households. All the ewes were synchronized using progestogen impregnated sponges (45 mg fluorogestone; Syncro-part®; CEVA laboratories, Libourne France) that were inserted in the vagina for 14 days. At sponge withdrawal, each ewe received an i.m. injection of 300 I.U. of equine chorionic gonadotropin (Syncro-part PMSG®; CEVA laboratories, Libourne France). The ewes were inseminated between 52 and 55 hours after sponges' withdrawal. A total of 11 Menz rams were also selected based on their breeding values in early September in the 3 locations to be used for semen collection. All the rams originated from the ongoing CBBP. Throughout the month of September, the rams were trained on semen collection using artificial vagina.

In the second trial, a total of 300 Bonga and Doyogena ewes were selected in 4 different communities. Prior to synchronization, all the ewes were checked for pregnancy by ultrasound pregnancy diagnosis and 32 sheep were found pregnant in Doyogena. None of the sheep in Bonga was found pregnant and this demonstrates the adhesion of the farmers to the rules of using the community selected rams for mating and castrating or selling all additional, non-selected rams. All the sheep were synchronized according to the same protocol described above. A total of 8 Bonga rams and 6 Doyogena rams ranked by their breeding value were trained and used for semen collection.

In the third trial, a total of 200 Doyogena ewes were selected for insemination. The ewes were selected from 3 different communities with an average of 2-3 sheep from each household. The first 150 ewes were synchronized using the combined progestogen/eCG protocol and inseminated as described above. The remaining 50 ewes and after being diagnosed for pregnancy, immediately received each one i.m. of 5 mg of the PGF2α analogue dinoprost (1 ml Enzaprost®; CEVA laboratories, Libourne France). This option was based on our experimental work testing synchronization with one or two injections of prostaglandins (see below under preliminary results section). The ewes were inseminated between 50 and 52 hours after the injection.

Semen collection and the insemination acts included the following steps:

- Semen collection using an artificial vagina in the presence of an ewe induced in estrus;
- Measurement of the ejaculate volume and appreciation of the color and the consistency of the ejaculate. Volumes less than 0.5 ml were generally not used and watery ejaculates (low concentration) or with a distinct yellow color (suspicion of inflammation) were also discarded;
- Quick assessment of mass motility under a microscope. Ejaculates with mass motility scores less than 3 were discarded;
- Measurement of the sperm concentration using a portable spectrophotometer pre-calibrated for ram semen (ovine-caprine accuread photometer; IMV®, France). Ejaculates with a concentration less than 3 10^9 sperm ml^{-1} were discarded;
- While being processed, ejaculates were placed in a thermos flask containing water at 35-37 ˚C;
- Ejaculates were then diluted to a final concentration of 400 10^6 sperm in each straw (straw volume 0.25 ml) using a commercial extender for sheep semen (Ovixcell; IMV®, France) kept at 35-37 ˚C;
- Diluted ejaculates were then checked for individual motility under a microscope. Ejaculates with a low proportion of spermatozoa moving rapidly on a straight line (less than 40%) were not used;
- Straws were filled, then sealed with inert packing powder and immediately immersed in a thermos flask filled with water at 35-37 ˚C;

- Inseminations were carried out immediately after packing and sealing. On average, time lag between semen collection and insemination did not exceed 10-12 minutes;
- In Mehal-Meda, Molale, Bonga and Doyogena, a generator was used to provide electricity for the microscope, the photometer and to warm water;

- In the different locations, instructions were given to reintroduce the rams with the inseminated ewes a week after the date of insemination.

Ewes were considered to have conceived to artificial insemination when lambing occurred 150±5 days after insemination day. Proportions of ewes displaying oestrus were compared by the χ^2 test. The effect of the type of prostaglandin treatment on intervals to onset of oestrus and duration of oestrus were assessed using the GLM procedures of the Statistical Analysis System (SAS, 2005)

Preliminary results

Response to synchronization with prostaglandin analogue

Our results from Menz revealed the potential to synchronize Ethiopian sheep breeds with 2 single injections of a prostaglandin analogue 11 days apart (Rekik et al., 2016 - http://onlinelibrary.wiley.com/doi/10.1111/rda.12761/full). Further technical tests comparing synchronization with 2 versus 1 injection were carried out and the results from Menz sheep (Table 1; Figure 1) revealed a 70% oestrus response with a mean onset at 28.6±14.5 h after one single injection and a mean oestrous duration of 43.5±14.5 h. No differences for these 2 traits occurred when the animals were synchronized with 2 injections 11 days apart. For both treatments, a large spread characterized the distribution of oestrus which could be explained by the random stage of development of the corpus luteum prior to the injection of the prostaglandin analogue.

Table 1. Oestrous response of Menz sheep synchronized with different protocols in August

Treatment*	Number of sheep	Age (year) (mean ± s.d.)	Weight (kg) (mean ± s.d.)	Ewes (%) in oestrus	Onset (h) (mean ± s.d.)	Duration (h) (mean ± s.d)
PGF I	17	3.7±0.43	28.8±3.46	12 (70.5)	28.6±14.5	43.5±14.5
PGF II	17	3.6±0.47	29.8±4.41	12 (70.5)	32.5±10.1	47.3±17.8

P-Value	0.9121	0.4699	0.5701

* 'PGF I' 1 i.m. of 5 mg of the PGF2α analogue dinoprost (Enzaprost®; CEVA laboratories) prior to ram introduction; 'PGF II' 2 i.m. of 5 mg of the PGF2α analogue dinoprost (Enzaprost®; CEVA laboratories) 11 days apart.

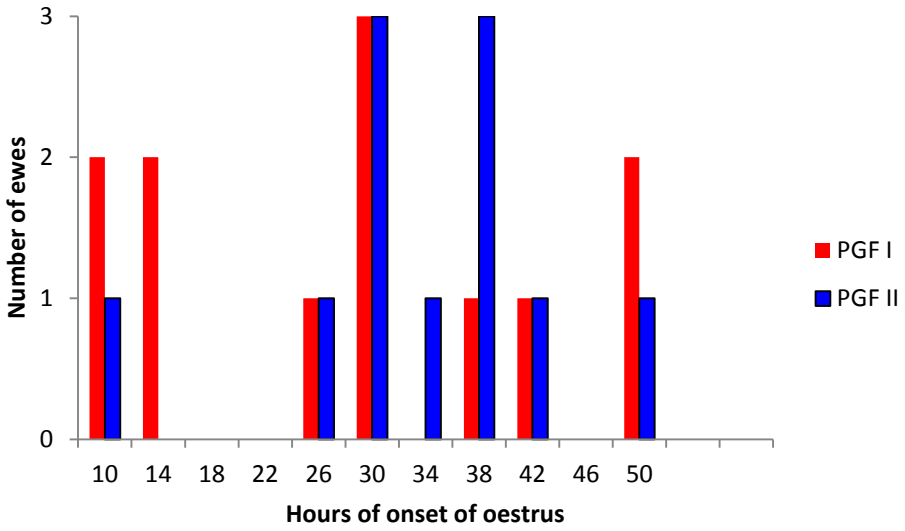

Fig 1. Oestrous distribution of Menz sheep synchronized with 1 i.m. of 5 mg of the PGF2α analogue dinoprost (Enzaprost®; CEVA laboratories) prior to ram introduction (PGF I) or 2 i.m. 11 days apart (PGF II)

Conception rate to artificial insemination

As lambing is still in place for the second trials in Bonga and Doyogena, we only report conception rates after the first trials in Menz and Debre Birhan (Table 2). The rates are considered acceptable in view of the large heterogeneity which characterizes the flocks in terms of management, feeding, body condition of the ewes at the time of insemination… In addition and despite clear instructions to select non-suckling females, some of the ewes that were synchronized were presented to insemination still suckling lambs and this is documented to negatively affect conception rates after AI. We relied on the farmers' to discard pregnant animals but apparently this was not accurate especially for early stages of pregnancy. Fortunately, the synchronized protocol used (progestogen/eCG) did not interfere with pregnancy.

Table 2. Conception rates to artificial insemination in Debre Birhan and Menz

	Debre Birhan	Mehal Meda**	Molale
Ewes inseminated	67	42	22
Ewes lambing to AI	29	10	7
Apparent conception rate to AI (%)	43.2	23.8	31.8
Ewes pregnant at the time	0	12	4

of insemination			
Actual conception rate to AI (%)*	43.2	33.3	38.8

*Actual conception rate is calculated after subtracting form the ewes inseminated the ewes pregnant at the time of insemination

Capacity development

Inauguration of the first ever laboratory for sheep artificial insemination in Ethiopia

Prior to August inseminations in Doyogena, the South Agricultural Research Centre (SARI) and ICARDA inaugurated the first field laboratory of sheep artificial insemination established in Doyogena research station and supervised by researchers of Areka Agricultural Research Centre. Inauguration took place in the presence of SARI Livestock Director, Areka research staff, ICARDA scientists, heads of Zonal and district bureau of Livestock and representatives of the farming communities. SARI implemented all the needed transformations in the laboratory, the semen collecting area and the insemination room. SARI and ICARDA collaborated to equip the laboratory with the required pieces of equipment and provided sheep insemination small equipment and disposables to make it functional. This is the first prototype of low-infrastructure laboratory remarkably situated in the middle of the target communities and is therefore well positioned to play a role in the delivery of reproductive services to support wide dissemination of improved genetics.

On-job training

On-job training was provided to at least 30 trainees (including 4 female trainees) who attended inseminations in the different locations. In Debre Birhan, the trainees were introduced to artificial vagina preparation, semen collection, semen assessment and conditioning. For the insemination act, only key persons in their respective sites were trained on the exo-cervical artificial insemination of sheep. The objective was to have in each of the sites (Debre Birhan , Bonga, Doyogena) a core team trained on rams' breeding soundness examination, ultrasound pregnancy diagnosis, ram training and semen collection, semen assessment and processing and finally the act of insemination (Table 3). The trained team in Doyogena has acquired autonomy and is now already running inseminations after synchronization with prostaglandins without any external technical backstopping.

Table 3. Members of the sheep insemination core team in the different locations

Debre Birhan and Menz	**Bonga**	**Doyogena**
Shenkute Goshme	Metsafe Mamiru	Addisu Jimma
Shambel Besutradu	Zelalem Abate	Fitsum Tessema
Aschalew Abebe	Fiseha Mengistie	Kebede Giorsos
Asfaw Bisrat	Ebadu Areb	Melese Yilma