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Artificial insemination in Moroccan sheep: present and perspectives

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Abstract. Despite the large number and the importance of sheep in Morocco, artificial insemination (AI) in this species is not yet an operative tool for the ovine breeding development and selection. In the actual context of climatic change, many countries consider AI as a major tool for the genetic improvement and the selection of specific traits of interest for sustainable development. In fact, the first trials in Morocco started in 1987 and have continued at a very slow pace until 2000. Recently, an integrated study has been carried out in a collaborative approach merging INRA-Settat-Morocco and INRA-Nouzilly-France. The present work aims at highlighting the findings resulting from this collaboration. Two cycles of three years each have been set up. The Studies concerned the characterization of males and females reproductive features (hormonal profiles, estrus synchronization protocols, seasonal variations of semen production and quality, semen conservation, effects of different diluents, exo-cervical AI trials). However the results obtained in the present study could not alone promote the technique. The currently biggest and most urgent challenge for the country is to gather all national and international experts, take into account the experience of our Mediterranean neighbors in order to advance the AI activity in sheep and not to forget involving the producers at every step.

Keywords. Artificial insemination – Sheep – Morocco – Present – Perspectives.

L'insémination artificielle au Maroc : présent et perspectives

Résumé. *Malgré l'importance des effectifs ovins au Maroc, l'insémination artificielle (IA) dans cette espèce n'est pas encore un outil efficace pour le développement et la sélection des races ovines. Dans le contexte actuel de changement climatique, de nombreux pays considèrent l'IA comme un outil majeur pour l'amélioration génétique et la sélection des caractères d'intérêt assurant un développement durable. Les premiers essais au Maroc ont commencé en 1987 et ont continué à un rythme très irrégulier jusqu'à 2000. Récemment, une étude intégrée a été réalisée dans le cadre d'un projet collaboratif groupant l'INRA-Settat-Maroc et l'INRA-Nouzilly-France. Le présent travail a pour objectif de présenter les résultats émanant de cette collaboration. Deux cycles de trois ans chacun ont été mis en place. Les études mises en place ont porté sur la caractérisation des traits reproducteurs des males et femelles Boujaâd (profils hormonaux, protocoles de synchronisation des chaleurs, variations saisonnières de la production et la qualité du sperme, conservation du sperme, effet de différents dilueurs sur la qualité de la semence, essais d'IA exo-cervicales). Cependant, les résultats obtenus dans la présente étude ne peuvent pas à eux seuls promouvoir la technique. Le défi actuel et le plus urgent pour le pays est de rassembler toutes les expertises nationales et internationales, de prendre en compte l'expérience antérieure de nos voisins Méditerranéens pour faire progresser l'activité d'IA chez les ovins, sans oublier d''impliquer les éleveurs à chaque étape.*

Mots-clés. *Insémination artificielle – Ovin – Maroc – Présent – Perspectives.*

I – Introduction

Morocco has set up a plan for the ovine production as early as in 1980. This has led to a big success in terms of the sheep genetic improvement through the organization of farmers and the establishment of performance recording. At the same time, the National Commission for selection has done a good job for breeding homogenization and farmers' awareness about the breed-

ing purity concept. In 1996, Boujenane highlighted that the biggest challenge that Morocco is facing today is to assure the genetic connections between herds and his statement still true today. The connection could be easily achieved through the use of artificial insemination (AI) and the control of different factors that influence the outcome of AI (the semen production, the conservation and the quality, the oestrus synchronization, the insemination itself, and many other factors).

The early development of AI in sheep on a large scale began in 1938 in Russia (Foote, 2002) where the collective farms provided an ideal arrangement for establishing AI programs. Then, this technique spread to central Europe and became widely applied commercially in France and Brazil. It has to be highlighted that despite this method is originated (in 1332) from the Arab people who used to use the sperm from stallions belonging to rival groups to inseminate their own mares, its first use in the Moroccan sheep was not documented before 1987 (Manar, 1987; Tibary, 1988). The reason for the slow development of AI in sheep worldwide has been due not only to irregular fertility results but also the difficulties faced in the application of enhancements such as the use of frozen-thawed sperm (Anel *et al*., 2006). In order to analyse the specific constraints of developing the AI activity in the Moroccan sheep, the present review aims to present the past, the present and some perspectives of this technique.

II – Learning from the past

1. Research experiments

The first study (Manar, 1987) was carried out as three experiments: (i) in the breeding season, the response of two breeds (Timahdit and D'man) to two synchronization treatments (Progesterone / PMSG and prostaglandin) were compared, (ii) in the breeding season, ewes from two breeds Sardi, D'man and their crosses (D X S) were treated by progesterone / prostaglandin and PMSG and compared, and (iii) the effect of different PMSG doses (250 or 500 IU) after progesterone treatment of D'man and Timahdit breeds has been analyzed. In these three experiments, AI was performed by using fresh semen. However, the overall fertility results were low and did not exceed 30%.

The second experimental study (Tibary, 1988) concerned artificial insemination using fresh or frozen extended semen on ewes synchronized with progesterone implants (375 mg) and PMSG (400 IU) or Prostaglandin F2α analogue (2.5 mg). The semen was collected from D'man and Timadit rams and diluted 1:9 in the extender based on Pipes-Tris, glucose, egg yolk and cooled to 5°C. All ewes (D'man n = 46 and Tmahdit n = 46) were inseminated twice with 0.5 ml of extended semen (approximately 150 million sperm cells). AI was performed 60 h after progesterone implant removal or 56 and 66 hours after the second prostaglandin injection. Lambing rate of D'man ewes was 34.9% and 21.7% (respectively for ewes treated with PGF2α and progesterone) while this rate was 39.1% and 13% (respectively for ewes treated with PGF2α and progesterone for Timahdite. The same trial has been performed in D'man, Sardi and D'man X Sardi crosses. Another assay was performed to study the effect of PMSG dose (250 IU *vs*. 500 IU) in D'man and Timahdit ewes, the results showed a very low fertility after AI (from 10 to 40 in D'man and 12 to 28.5 in Sardi respectively for 250 and 500 IU).

2. Field trials

Two artificial insemination centers (Fouarat and Aïn Jemaa) exist in Morocco. Since the 90s, the Ministry of Agriculture has set up a laboratory for the semen storage of small ruminants at Ain Jemaa center. The goals assigned to this center were to produce and preserve ram semen deriving from five local breeds and also from imported breeds (Ile de France, Merino and Lacaune) and the assessment of fertility of frozen semen (Manar and Bouayoune, 2000). Many trials have been performed during the period ranging from 1990 to 2000.

An agreement between FAO and the Ministry of agriculture has been signed to export 1000 doses of frozen D'man semen to Egypt (in 1991) and 2000 doses to Iraq (in 1994). This exportation has been preceded by a fertility assessment that involved the frozen semen collected (900 x 10^6 spz/ewe) and ewes from Sardi breed. The tests showed a low fertility rate (30%). In another field trial aiming to improve the productivity of Timahdite breed, the fertility results obtained were as high as 60% of lambing rate while in a filed study carried out on herds from the same region (Middle Atlas) the fertility rates were 60%, 44% and 41.5% respectively at Irklaouen, Timadit, and Ain Leuh communes.

During the last decade, the genetic value of animals imported from France in the 19th century has decreased enormously. A field trial of artificial insemination under laparoscopy in a Moroccan-French program for the genetic improvement and the promotion of French ovine breeds (Vallet *et al.,* 1995) has been done to solve this problem. In 1992 and 1994, 2401 ewes were inseminated using the endoscopy technique. The program concerned the following breeds: Ile de France (OIF), Mérinos Précoce (MP), Meat Lacaune (LV), Noire du Velay (NV), Berrichon du Cher (BCF) Caussenard du Lot (CdL), Solognot (S). A synchronization program was used for oestrus induction and ovulation. AI was realized 55 h ± 1 h or 62 h after sponge removal. Frozen straws containing each 100 million spermatozoa were used. The results of fertility and prolificacy obtained are summarized in Table 1.

Table 1. Cumulative results per breed after AI of French ovine breeds (1992-1994)

Breed	OIF	MP	LV	NV	BCF	CdL	S	Total
Number of ewes inseminated	1031	772	224	180	87	78	29	2401
Fertility (%)	65.6	48.6	59.8	58.3	58.6	38.5	75.9	58
Prolificacy (%)	162.9	145.1	161.2	160.0	186.3	126.7	168.2	157.5

Source: Vallet *et al.,* 1995; Manar and Bouayouane, 2000.

The last large field trial that the Ministry of Agriculture and the National Association of sheep and goat breeders in Morocco (ANOC) have performed (Hammada, 1995) concerned a program of artificial insemination in industrial breed crossing. In this trial the ewes were belonging to Sardi, Timahdit and Boujaâd breeds while the ram breeds were Ile de France (OIF), Mérinos Précoce (MP) and Lacaune (LC). Artificial insemination was compared to natural mating. Synchronization was performed by applying a vaginal sponge (FGA 40 mg) for 14 days and an injection of 300 IU of PMSG (in June 1995) combined to a cervical insemination at 56 h after the sponge removal. The semen was fresh cooled (15°C) and had a concentration of 1.6 x 10^9 spz/ml. The results of fertility and prolificacy obtained are summarized in Table 2.

Table 2. Results of IA in field trials

Farm localization	Ewe breeds	Ram breeds	Fertility (%)	Prolificacy (%)
Tit Mellil, Bouskoura, Benslimane, Skhrate, Bouznika, Had Soualem	Timahdit (n = 685)	OIF, MP, LC	42.31% to 76%	107 to 146
Benslimane	Sardi (n = 21)	MP	90.48%	136
Sehou	Boujaâd (n = 59)	OIF, MP, LC	56.6%	117

Source: Hammada, 1995.

III – What is the situation today?

1. Research experiments at INRA: case study of Boujaâd breed

After the trials cited above, a huge gap (from 2000 to 2006) has been recorded in term of AI use in Moroccan sheep. Since 2006, an integrated study has been carried out in a collaborative project bringing INRA-Settat-Morocco, INRA-Nouzilly-France and other partners together. Two cycles of three years each have been set up. They involved the two institutions, the Institute of Agronomic and Veterinary research (IAV Hassan II), ANOC and the Regional Department of Agriculture Ministry (DPA of Khouribga). The study concerned the characterization of male and female features (hormonal profiles, protocols of oestrus synchronisation, seasonal variations in semen production and quality, semen conservation, effect of different diluents, exo-cervical AI trials). The results derived from the two projects, dealing with (i) the use of assisted reproduction techniques to improve productivity in Boujaâd sheep and (ii) the optimization of some factors that could affect the result of artificial insemination, are summarized as follow:

- The testicular size was maximal (scrotal circumference, testicular length and diameter, epididymal diameter were respectively 31.33 cm, 9.51 cm, 6.22 cm and 3.01 cm) in May-June. It decreased to reach its lowest value during November-December (Derqaoui *et al.,* 2010),
- The quality of semen, monitored during two years, was at its maximum in July (Individual motile = 92.78%) and at its minimum during January (Individual motile = 67.78%) (Derqaoui *et al.,* 2010),
- The assessment of different extenders, storage temperature, storage duration, anti-oxydant supplementation, efficiency of fresh *vs* frozen conservation started and is still in progress,
- The profiles of seminal plasma proteins in rams showed a significant difference between breeding and non-breeding season,
- The anatomy of the cervix is explored and still in progress in Boujaâd and D'man (as control) breed,
- The preovulatory surge of LH in ewes after sponge removal appeared later than expected (range from 20 h to 52 h after sponge removal, with a large number of ewes between 36 h and 52 h),
- Fertility was slightly higher with 300 IU of PMSG than with 400 IU (88% *vs* 83%, n = 34) in natural mating (El Amiri *et al.,* 2010),
- Artificial insemination with fresh semen (0.8×10^9 two times) gave a fertility rate ranging from 45 to 55% (n = 34).

2. AI in ANOC conservation programs

The National Association of sheep and goat in Morocco (ANOC) and the sheep breeding Technical Institute (ITELV) in Algeria are carrying out a project funded by FAO for Hamra / Beni guil sheep development and preservation as a part the Global Plan of Action for Animal Genetic Resources strategy. The tested rams that will have good performances will be selected and transferred to the AI center of Ain Jemaa. In Morocco, AI will be performed in 23 farms using a cervical insemination while the laparoscopy technique will be used in Algeria on 200 ewes belonging to the sheep breeding Technical Institute. ANOC does not have this unique and ambitious program, it is also expected to create a new AI center and to renew the Center of Aïn Jemaa.

3. AI as a part of the private sector

For the first time, a private AI center has been founded in Morocco. It has an ambitious program but it is still not functioning as expected (Manar, personal communication). Today, this center is focusing on many goat projects, while little is done or has to be done in sheep. In this latter species, future projects will involve insemination of 2000 ewes in Boujaâd breed and should later be extended to Sardi breed. Today, the major activity in this center is the capacity of building reinforcement.

IV – Conclusions

The current biggest and urgent challenge for the country is to gather all national expertise and learn from the experience of neighboring countries to advance AI in sheep while taking care of involving producers/breeders at every step. All efforts cited above will become useless if the problem is not treated under a muti-institutional and a multi-disciplinary approach.

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