Chapter

Ovine Artificial Insemination in the Maghreb Region: Present Status and Future Prospects

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Abstract

Artificial insemination (AI) plays a key role in the genetic improvement of farm animals. Although it is widely used for cattle in the Maghreb region, it is scarcely applied in sheep at farm level. This is not only due to low fertility and irregular results that range between 30 to less than 76% for both cervical AI with fresh semen and laparoscopic insemination with frozen semen in most of studied breeds and also because of low results related to conditioning of fresh, chilled and frozen rams' semen. An appropriately literature analysis was conducted to highlight the importance of sheep breeding in the Maghreb region particularly in Morocco, Algeria and Tunisia and to assess the efficiency of AI for Magrebin ovine breeds, the results related to different semen conditioning techniques and different AI procedures. The main factors affecting AI results are also presented. Finally, this chapter presents different strategies to improve AI efficiency at farm level in the future and the challenges to extrapolate experimental AI techniques to field conditions at a large scale.

Keywords: semen preservation, cervical insemination, laparoscopy, sheep

1. Introduction

Sheep farming is of great economic, social and environmental interest in all countries with a Mediterranean climate [1]. It remains an important activity in the southern Mediterranean countries particularly in Morocco, Algeria and Tunisia considering its adaptation to the majority of the countries' agro-ecosystems, which is due to the hardiness of the dominant breeds and to the flexibility of production systems in relation to socio-economic and land contexts [2–8]. Yet, breeding techniques at farm level are till nowadays basic and traditional based on pasture, which is subjected to the current issue of global warming causing severe rangeland degradation [5, 9]. This leads to substantial limited productive capacity of sheep characterized by a low annual productivity rate ranging between 0.66 and 1.24 lambs for most breeds in the Maghreb region [3, 4, 7] added to a relatively low carcass weight (about 15 kg) [10, 11] and low survival rate [7] causing a lack of red meat production.

In this framework, artificial insemination (AI) offers a powerful tool to develop ovine sector through speeding up selection programs and spreading the genetic progress. Thus, improving flocks productivity became a national objective in the Maghreb countries where several enhancing breeding programs have been initiated in order to remedy these problems starting with improving the control of

reproduction and creating national centers for ovine using AI. Ovine AI offers enormous opportunities to support the sector development, by accelerating the programs of selection and dissemination of genetic progress. AI allows out-of-season reproduction and therefore milk or lamb productions that are better distributed throughout the year in response to the needs of the market. In addition, AI allows breeders to have access to the best mated male for herd renewal while limiting health risks. Furthermore, AI permits the multiplication of genotypes and limiting applied consanguinity, without multiplying the number of progenitor in the herd [12].

In recent years, continued improvements of this method in bovine, caprine and poultry coupled with a growing demand for the application of AI at farm level, as the numerous benefits it offers, are being increasingly recognized. Yet, ovine AI has progressed rather slowly in terms of breed improvement in comparison with the aforementioned species [13, 14]. The earliest documentation on a large scale ovine AI has been reported in Russia by Miovanov in 1938 [13] than spread to China and central Europe. It was not until the 80′s that the first reports of sheep cervical AI that was documented in the Maghreb region by Khaldi and Farid in 1981 in Tunisia [15] and Manar in 1987 in Morocco [16]. Later in 1992, that was the first documented laparoscopic insemination of French breed ewes in Morocco [17]. Ever since, ovine IA has gained researchers′ interest and several works have been developed since in order to investigate factors influencing IA results in Maghrebin sheep breeds and to improve fertility rate of inseminated ewes.

According to this context, our work aims to highlight the actual situation of assisted reproduction in sheep with a special emphasis on rams′ semen collection and preservation and AI in the Maghreb region. We will first address the importance of ovine farming and its limits in this region. Then, we will review the current status of AI in sheep particularly at farm level. Finally, we will focus on future consideration to enhance assisted reproduction and to discuss how to evaluate the applicability of ovine AI at farm level in the Maghreb region. Electronic databases (Elsevier, PubMed, and Web of Science) along with some official reports and thesis documents were consulted for an appropriate literature review. A total of 55 suitable references were considered for this chapter from 1981 till now.

2. Overview of sheep farming in the Maghreb region

Sheep farming in the Maghreb region is well developed mainly due to its flexibility and hardiness of the dominant breeds as well as spatial complementarities to agricultural production [2–8, 18]. It closely depends on the climatic conditions of the year as it is based on traditional farming systems related to pasture availability.

2.1 Place of sheep breeding in the Maghreb countries

For the whole Maghreb region, the total number of sheep increased from 23 million during the 1960s to nearly 30 million in 1970s and reached 34.9 million in 1980s to stabilize around 37 million during the 1990s. The most important increase in ovine flocks has been registered from 2005 to 2019. The total number of sheep in the Maghreb region reached more than 57 million by 2019 [19]. This rapid increase in the herd, the strongest in Algeria followed by Morocco (**Figure 1**), seems to have been favored by the short-term public efforts during the droughts and the distributions of barley and fodder to resist the disastrous damages of drought and climate change on pasture [20]. In Tunisia, the evolution of the herd was fluctuating before the sixties indicating a close dependence of sheep farming on climatic conditions.

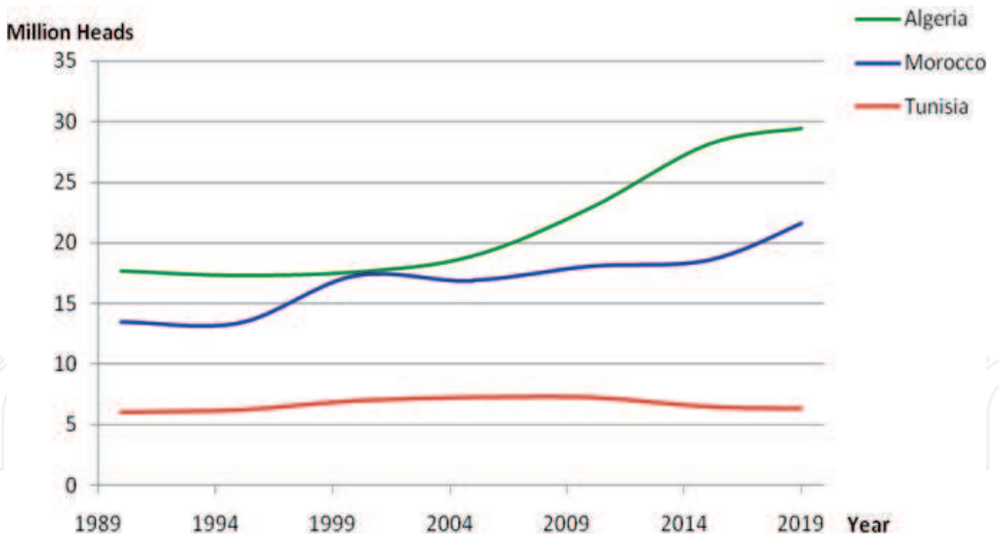

Figure 1.
Evolution of sheep stocks in the Maghreb countries (million heads).

Then through public safeguard campaigns and the subsidy for concentrated feeds, the sheep flocks have stabilized since the late eighties around 6 million head [4, 5]. The main objective of the public authorities was to increase sheep stocks and consequently to increase the production of meat for which these countries are in deficit.

Sheep farming is mainly intended for meat production in the Maghreb region, and most breeds were not selected for dairy traits, except for Sicilo-Sarde in Tunisia [21, 22]. It contributes significantly to the value of agricultural production in all Maghreb countries and insures a major part of self-sufficiency in meat consumption. For instance, ovine meat contributed with up to 48% in the total production of red meats in Tunisia estimated at 120,000 tons [4]. In Morocco, ovine stock ensures a meat production of around 130,000 tons of carcass equivalent yearly [23]. Likewise, according to official statistics of the ministry of Agriculture and Rural Development in 2017, Algeria has 26 million head of sheep and produces 325,000 tons of sheep meat, which ranked the country 5th in the world in terms of sheep meat production [8].

2.2 Characteristics of the main ovine breeds in the Maghreb region

The Maghreb region is characterized by different climatic zones from the Mediterranean coast to the oases of the Sahara. These diversified ecological conditions and climate types offered an extraordinary diversity of indigenous sheep breeds well adapted to their respective environments [24]. Except for Sicilo-Sarde breed in northern Tunisia, all sheep breeds in the Maghreb region were selected for meat and wool remains the next desirable product. Due to their proximity and commune history of the region's people, genetic flow between all Maghrebin breeds has shaped a rich livestock heritage [24, 25]. The Maghrebin sheep stock consists of several indigenous breeds with particular transboundary breeds (i.e., Algerian Hamra also called Beni-Guil in Morocco, D'Man and Ouled-Djellal). In this section, we present the main Maghrebin breeds that have been reported in the literature to receive genetic breeding programs and particularly artificially inseminated (**Table 1**).

The Algerian sheep population consists of nine breeds (Ouled-Djellal, D'man, Hamra, Rembi, Taâdmit, Sidaoun, Tazegzawt, Berbere and Barbarine), strongly adapted to harsh environmental conditions [26]. Some other non-official breeds were also reported originally introduced from Morocco and sub-Saharan Africa like Moroccan Srandi ou Sardi and Ifilene [27]. Due to the increasing farmers'

Main Breeds	Origin and current population	Description
	**Ouled Djellal** breed is the most important Algerian breed with over 11 million heads; it represents 63% of the Algerian sheep population.	The main purpose for meat production with a maximum adult weight of 80 kg for male and 60 kg for female.
	**Hamra** (also called Béni Guil in Morocco) is an indigenous breed from Algeria. This breed decreased from 3.2 million head in the 90s to less than 500000 heads in the last few years following the massive increase of Ouled Djellal breed.	This breed is reared for meat production. The adult weight is about 70 kg for male and 40 kg for female, with well adaptation characteristics for local pasture resources.
	**Berber** breed is the second main sheep in Algeria. It represents about 25% of Algerian herd with 4.5 million heads. This is probably the oldest breed in Algeria.	It is a small sheep with the values of 65 cm and 45 kg for male and 60 cm and 35 kg for female, respectively, for heights and weights.
	**Rembi** breed is an indigenous Algerian breed that represents about 11% of the Algerian herd.	The Rembi breed is considered to be the heaviest Algerian sheep with adult weights of around 90 kg for male and 60 kg for female.
	**Sardi** is an indigenous Moroccan breed. It occupies the poor rangelands of the western highlands of Morocco. It is estimated at to count 750 000 female units.	This is an excellent meat breed with an adult weight ranging from 70 to 100 kg for male and 45 à 60 kg for female.
	**Timahdite** is one of the main local Moroccan breeds with 1500000 female units and 11% of total national flock. It is located in the Middle Atlas region and well appreciated for its good conformation, carcass yield and quality of both meat and skin.	This breed is reared for meat production with an adult weight ranging between 60 to 80 kg for male and 45 to 55 kg for female.
	**Boujaâd** is one of the main five indigenous rustic breeds of Morocco. It counts for 260 000 heads, representing about 1.4% of the Moroccan ovine flock.	The Boujaâd breed is considered medium to large in size with the adult weight reaching 75 to 80 kg for the male and 45 to 60 kg for the female.

Main Breeds	Origin and current population	Description
	**D'man** breed is a Moroccan sheep well adapted to oasis ecosystem in Morocco, Algeria and Tunisia. In Morocco, it is estimated to 200 000 heads, 34 200 heads in Algeria and about 20 000 in Tunisia.	This is the most important prolific breed in north Africa. With a precocious puberty, continuous sexual activity and high prolificacy, D'man breed is very appreciated by oasis farmers; however, these animals are not resistant to harsh climate and poor pasture.
	The main meat sheep breed in Tunisia is the "**Barbarine.**" With two dominant strains, the Black and the Red Heads, it represents 64% of the total sheep herd of the country.	The **Barbarine** sheep is an indigenous breed known for its adaptation to the harsh climate conditions. This breed is easily recognizable by its large fat tail. The adult weight reaches 70 kg for male and 40 to 50 kg for female.
	**Queue Fine de l'Ouest** is originated from Algerian Ouled Djellal breed and occupies the mountains of the western Tunisia. It represents about 32% of total sheep flock in Tunisia.	This breed presents the similar characteristics compared to its Algerian origin breed. It is used for meat production purposes. The adult male could reach 80 kg and the female about 55 kg.
	**Noire de Thibar** is a Tunisian breed originated from many crosses between the Queue Fine de l'Ouest and the Black Merino of Arles. This breed represents about 2% of Tunisian sheep stock.	This breed was mainly selected for meat and wool. The adult weight is about 70 to 80 kg for male and 65 kg for female.
	**Sicilo-Sarde** is the only dairy breed in the Maghreb region. It is mainly located in northern and north western of Tunisia. With 83 000 female units, it represents about 0.5% of the national flock.	It is a light breed compared to meat breeds. The adult weight is about 35 to 70 kg for male and 25 to 45 kg for female with a dairy production of 0.7 l/ day and lactation period of 120 days.

Table 1.
Main sheep breeds in the Maghreb region.

preference to a single breed, Ouled-Djellal currently accounts for more than 63% of the Algerian sheep population. According to Mason [28], Moroccan sheep population is composed of some twenty different breeds well adapted to their variant ecosystems and tolerant to harsh climates. Currently, the most important breeds are Sardi, Timahdite, Béni Guil (also called Hamra in Algeria), D'man and Boujaâd. These breeds were phenotypically characterized and their breed standards were established since the beginning of 1980s [29]. The Tunisian sheep breeding sector is largely dominated by the indigenous fat tailed Barbarine with two different strains black headed and red headed Barbarine breed (64%), while the remaining thin tail breeds are "Queue Fine de l'Ouest" (30%), Noire de Thibar (2%) and Sicilo-Sarde

(0.5%). The main exogenous sheep breed found in Tunisia is the Moroccan prolific D'man breed, which represents about 0.25% of the total sheep population in Tunisia [30].

Despite the great genetic diversity, sheep productivity remains insufficient in the Maghreb countries. As a whole, it would be only 12 kg of lamb at weaning per ewe per year with 0.66 to 1.24 lamb/ewe/year in Morocco [3] and about 12.8 kg of lamb per ewe per year with 1.13 lamb/ewe/year in Algeria [7]. Similarly, the productivity of most Tunisian breeds was estimated about 0.8 weaned lambs per ewe per year and about 14 kg of lamb at weaning per ewe per year [4, 31] because of the low performance of the ewes and lambs. Low fertility, prolificacy, and high neonatal mortality are reported for most local breeds under extensive management system coupled with insufficient mastery of breeding techniques in terms of genetics, feeding and reproduction. Along with improving management's techniques and feeding conditions, researchers have recommended adoption of reproductive biotechnologies to improve the performance of these indigenous breeds and disseminate genetic progress [32]. Thus, several studies have been conducted to meet this need. In the following part of this chapter, we will review the most relevant works carried out related to reproductive biotechnology and AI in Maghreb ovine breeds with a particular emphasis on most important results at farm level.

3. Current state of ovine AI in the Maghreb region

The first documented studies on AI of ovine species in the Maghreb countries were reported during the 1980's following the establishment of artificial insemination centers by public authorities. Since its creation in 1975, the sheep breeding program in Tunisia has been managed by the Office of Livestock and Pasture (OEP). This program aimed to allow the dissemination of improved genes acquired by the herds controlled in sheep farms in different regions [33]. Sheep semen collection, control and conditioning are provided by the services of the Genetic Improvement Direction (DAG) of the OEP-trained pure local breed rams of the center (**Figure 2**). In Morocco, two artificial insemination centers (Fouarat and Aïn Jemaa) exist providing ovine AI services. Since the 90s, the Ministry of Agriculture has set up a laboratory for the semen storage of small ruminants at Aïn Jemaa Center. The goals assigned to this center were to produce and preserve ram semen deriving from five local breeds and several imported breeds (Ile de France, Merino and Lacaune) and the assessment of fertility of frozen semen [16]. It was only later (2006–2011) that the creation of three regional Centers of Ovine Artificial Insemination (COAI) in Algeria has led to

Figure 2.
Trained rams for semen collection a: Fat-tailed Barbarine b: Noire de Thibar (DAG, Sidi Thabet, Tunisia).

the introduction of this technique in sheep and its diffusion lately at national level [34]. Even though the creation of these centers had enhanced research on AI of local ovine breeds, the use of AI at farm level is till nowadays very limited and applied on few thousands ewes per year with little success.

3.1 Semen collection and preservation

One of the most limiting factors of the large scale use of AI in ovine selection programs is the difficulty of ram's semen preservation and cryoscopy. Thus, the use of fresh semen in trans-cervical insemination is the most common practice. For this reason, numerous studies have been recently performed with the goal of optimizing sperm cryopreservation protocols in this specie [35, 36]. However, there is paucity in studies about sperm collection procedure in Maghrebin local breeds. Semen collection from large numbers of untrained rams makes AI with fresh sperm at farm level difficult to perform. Thus, AI diffusion on a large scale relies on developing simple procedures to collect semen from untrained rams. Semen can be collected from live animals by artificial vagina (AV) or electrical stimulation (EE) [35]. Semen collection with an AV simulates natural conditions, but usually requires a preliminary training period of rams [35], whereas obtaining semen from a large number of rams, EE could be a useful and faster procedure [13]. However, most field trials conducted in Maghreb region were only performed with AV semen collection techniques (**Figure 3**) after 2-week period of rams training to ejaculate in AV [36, 37].

After collection, sperm must be diluted and cooled slowly but progressively from collection temperature (+32°C) to storage temperature (+15°C or + 5°C) in order to slow down the basal metabolism of spermatozoa from ejaculation until AI. Different extenders were used during liquid and frozen storage to improve sperm motility, viability and functional integrity of ram sperm membranes and ultimately success rate of consequent AI. In a previous work [32], different extenders were tested during liquid (15°C) and cryoscopy conservation of ram sperm. The use of

Figure 3.
Semen collection with an artificial vagina in the Tunisian " Noire de Thibar " ewe.

skimmed milk with sulfamid and antibiotics during liquid storage gave satisfactory results as sperm motility score ranged between 3.1 and 4.0 and sperm viability ranged between 52 and 71% during the mating season. These authors also tested the effect of two extenders (skimmed milk with egg yolk *vs.* Tris with egg yolk, citric acid and fructose) during frozen conservation. They used glycerol as cryoprotectant and antibiotics for both extenders. The freezing procedure was evaluated. Automated cryoscopy led to satisfactory post-thawed sperm quality with both extenders (over 3.1 motility score and 44% viable spermatozoa) recommended for intra-uterine insemination. However, manual cryoscopy caused severe damages of the spermatozoa and gave very low sperm motility and viability. Recent study [38] performed using four extenders: two based on egg yolk (egg yolk Tris and Tryladil), one based on milk (skimmed milk or Colas extender (use in equilibration and freezing)) and one to soy lecithin (Andromed) showed that the skimmed milk presented most advantageous in the preservation of the semen at 5° C. While diluents containing egg yolk have best preserved semen quality of ram INRA180 breed during the freezing procedure, it was found that ram effect was a significant factor in sperm storage in this study and it was found that sperm from ram number 2 showed the better resistance to storage, either in liquid or in frozen state [38].

To reduce the oxidative stress during storage process, several extenders and protective components have been tested with a particular emphasis on locally produced antioxidant agents in some plant extracts. Recently, in [39–41] it has been shown that the addition of argan oil and cactus seed oil with small amounts to Tris egg yolk/skim milk extenders increased the total sperm motility, progressive motility, viability and membrane integrity, and decreased the spontaneous and induced lipid peroxidation and DNA fragmentation in ram semen at 15 and 5°C temperatures. Similar effects were reported when 1% of *Opuntia ficus-indica* extract was added to extenders such as Tris or milk during liquid storage up to 72 h of storage [41].

3.2 Estrus synchronization and insemination timing

In sheep, most AIs are practiced with fresh semen on induced estrus within a few hours after the semen collection (optimum: 5 hours-maximum: 10 hours). AI efficiency is closely related to estrus induction and synchronization procedure.

Several synchronization techniques were tested for local breeds in the Maghreb region over the years. The most commune estrus synchronization procedure is based on the use of vaginal devices (sponges) impregnated with 30–40 mg of fluorogestone acetate progesterone implants for 14 days and equine Chorionic Gonadotropin (eCG) intra-muscular injection on removal day (**Figures 4** and **5**) [32, 34, 38, 42, 43].

Two synchronization treatments were tested for different Moroccan sheep breeds [44] using progesterone implant coupled with eCG or prostaglandin analog 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%

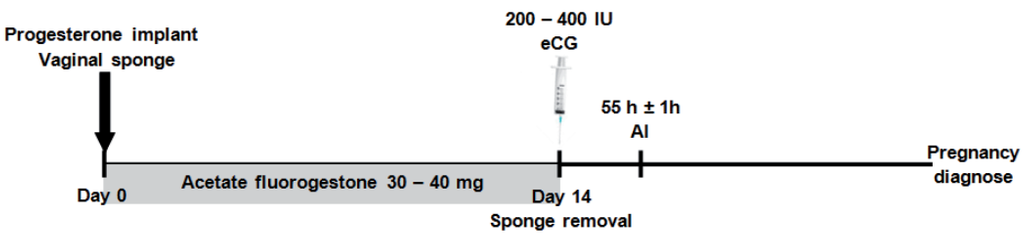

Figure 4.
Simplified estrus synchronization protocol for ewes.

Figure 5.
Intravaginal sponge insertion and PMSG injection in the Tunisian Barbarine ewe.

respectively for ewes treated with PGF2α and progesterone for Timahdite breed. A second trial focused on eCG dose (250 IU *vs.* 500 IU) in D'man and Sardi ewes, and 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.

Recently, the effect of eCG doses on fertility parameters' of Moroccan Boujaâd ewes were tested [45] and the use of 300 IU of eCG after 14 days of progesterone vaginal sponge insertion was recommended. With 400 IU, ewes had significantly higher prolificacy associated with higher neonatal death. On the other hand, eCG treatment has been associated with lower fertility rates on ewe's consecutive cycles. The use of ram effect coupled with progesterone treatment instead of eCG injection in Barbarine ewes was also tested [43]. Results showed that substituting eCG treatment by the ram effect as synchronization treatment prior to artificial insemination could lead to satisfactory lambing rates.

Most reviewed works recommended performing cervical AI after 55 ± 1 hour after progesterone sponge removal using fresh or cooled semen [32, 34, 38, 42, 45] giving satisfactory results going up to 100% of synchronized ewes and pregnancy rate varying between 52 and 88%. Earlier studies tested performing AI after 60 h of progesterone implant removal in D'Man and Timahdite breeds. At this timing, the fertility rate was low for both tested breeds with 21.7% and 13% respectively for D'Man and Timahdite ewes. Moreover, it has been shown that cervical insemination of Barbarine ewes after 55, 56 and 57 h after hormonal treatment and progesterone sponge removal gave satisfactory results with respectively 59, 48.6 and 52% fertility rates [42]. However, when AI was delayed to 58 and 60 h after hormonal treatment, fertility rate dropped to 25 and 26.5%, respectively. Thus, it was recommended that large flocks should be divided into groups of 25 ewes in order to prevent time shifts during the procedure and prolonged the interval between hormonal treatment and AI application.

3.3 Advances in ovine AI in the Maghreb region

In the Maghreb region, ovine AI is till nowadays limited to experimental centers, and AI at farm level is not yet extended. In Tunisia, few thousand ewes are yearly inseminated as part of DGA activities to limit the effect of consanguinity in small pure breed herds, particularly Sicilo-Sarde and Noire de Thibar [46]. Thus, most of the presented data in this review remain experimental. Using fresh or cooled ram

Figure 6.
Cervical AI in the Tunisian "Noire de Thibar" ewe.

semen, intra-cervical (**Figure 6**) deposition of semen techniques results in acceptable fertility rates. However, using frozen-thawed semen, at present only intrauterine insemination gives acceptable pregnancy rates that remain difficult to achieve and to disseminate at large scale because of its cost and complexity.

3.3.1 Cervical insemination

This section summarizes the main findings in cervical insemination in the Maghreb ovine breeds. The conception rate to AI is calculated as the proportion of pregnant ewes detected by ultrasound scan at early stage of pregnancy or lambing ewes from inseminated ones. Results varied widely depending on the conditions and experiment protocol. In many studies, the ewe's breed has been found to have a strong effect on the pregnancy rate after AI. In an industrial breed crossing trial, the Sardi, Timahdite and Boujaâd Moroccan local ewes were crossed with rams of Ile de France, Merinos and Lacaune [47]. Cervical insemination was performed at 56 h after the sponge removal with fresh cooled (15°C) semen of 1.6×10^9 spz/ml concentration. Results showed the superiority of Sardi breed crossed with Merinos compared to the others with fertility rate 90.48% and prolificacy of 136%, while fertility rate ranged between 42.31 and 76% for Timahdit and Boujaâd breeds crossed with Ile de France, Merinos and Lacaune rams. The male may greatly influence fertility results after cervical AI. Variation in fertility of ram ejaculates exists independently of the sperm quality and after cervical inseminations with fresh semen [47].

A significant ram's age effect on semen quality in Algerian Ouled Djellal breed was reported [48]. The concentration and the mass motility were significantly higher in adult males. As expected, adult rams had significantly higher fertility and prolificacy (74.88 and 161.87%, respectively) compared to young rams (50.08 and 120.91%, respectively). However, the age of the female did not affect their fertility. They also reported that season had a highly significant influence on the ejaculate volume but not on the concentration and mass motility of spermatozoa. In a Tunisian study [38], fertility rate of the Barbarine ewes ranged between 32 and

41% with a significant effect of animal management was reported. Similarly, fertility rates ranged between 38 and 73% and prolificacy from 1.03 to 1.24 in the same breed with a great flock effect indicating the importance of body condition and nutritional background of animals to improve success rate of AI have been reported [43].

A study on AI at farm level carried out during spring mating following induced estrus of the four main indigenous Tunisian ovine breeds: Sicilo-Sarde (SS), Noire de Thibar (NT), Queue Fine de l'Ouest (QFO) and black head (BTN) and red head (BTR) Barbarine [46] reviled that the fertility of ewes inseminated out-of-season varied from 32 to 74% with an average of 47.56 ± 9.94%. The use of chilled semen (5°C) significantly ($p < 0.01$) reduced the success rate of AI with 43.76 ± 7.56% versus 55.95% ± 9.56% using the fresh semen. The superiority of the SS breed over the Tunisian meat breeds was perceived. An important effect of breeding management was detected, showing the importance of preparing ewes before using AI [46]. 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 field study carried out on herds from the same region (Middle Atlas), the fertility rates were 60, 44 and 41.5% respectively at Irklaouen, Timahdite and Ain Leuh communes [16]. The great variation of AI results at experimental level and at farm level indicates the potential of this biotechnology on large scale and the possibility of disseminating this technique. However, much still to be done in order to get reproducible results and limits the effect of factors of variation.

3.3.2 Laparoscopic insemination

Laparoscopic intrauterine insemination (LAI) is up-to-date the only technique that guarantees adequate fertility with frozen-thawed semen. Although it is still performed on experimental level, it is particularly interesting choice to use sperm with high genetic value [32, 49] and/or when semen is imported [17, 32, 49] or post-thawing semen quality is poor [50]. At field level, several factors limit the diffusion of LAI starting with its complexity and the need for highly trained technicians and advanced equipment to perform it. This procedure is also very expensive and have other problems related to animal welfare [51]. LAI offers higher and more constant fertility rates than cervical AI. In the Maghreb region, a few LAI trials have been performed in order to improve genetic value of some local breed, particularly small group breeds with high consanguinity risk.

It has been reported the use of intrauterine inseminations between 2005 and 2007 with Sarde-frozen semen imported from Italy in order to overcome the scarcity of sires and to alleviate consanguinity hazards on the Sicilo-Sarde dairy breed in Tunisia [49]. A renewal of phenotypic variability was observed mainly at the level of the herds, which benefited the most from this crossing. These animals, of different genetic types, took advantage of the superiority of the Sardinian breed, which improved several criteria of production or conformation. Results of gene injection protocol showed that fertility, prolificacy and mortality rates ranged from 53 to 68%, 157 to 184% and from 5 to 11%, respectively. A similar experiment was conducted in Morocco to improve the genetic value of imported French breeds used for commercial industrial crossbreeding since the nineteenth century. The genetic value of these breeds has been compromised by high consanguinity, since the import of live animals is restricted, frozen semen was the ultimate alternative inseminated with laparoscopic technique. Results ranged between 38.5 and 75.5% for fertility and 126.7 and 168.2% of prolificacy depending on breed with the highest results registered in the Solognot breed [17]. The gestation rate of Noire de Thibar ewes inseminated with frozen-thawed semen of Brune Noire Suisse rams with LAI ranged between 52 and 81% [32].

Even though, LAI is still very limited in the Maghreb countries, it is considered a good way to enhance genetic value of rams particularly in insemination centers and could be a powerful tool to increase genetic selection pressure.

4. Future prospect in ovine AI

In the Maghreb region, ovine breeding is developing continuously particularly in Algeria and Morocco since small ruminants are better suited to their production systems and to their harsh climate than cattle. Thus, it is likely that there will be an upsurge in the use of AI in these species in the future, with an emphasis on improving production traits by the injection of superior genes. Introducing innovative solutions is increasingly adopted by livestock holders including acceptance of reproduction biotechnologies [52]. This would allow a large-scale diffusion of AI in the future. However, one of the greatest challenges against any genetic improvement program at national level *via* AI is the improvement of animal husbandry and management to ensure the success of such program. AI would offer little help in areas where basic husbandry skills are inadequate.

The creation of breeding centers for pregnant young ewes and rams is essential to meet the needs of sheep breeders and contribute to the genetic improvement of the herd particularly in the dairy Sicilo-Sarde breed [53], Noire de Thibar and most breeds with relatively reduced numbers. In Morocco, for the first time, a private AI center has been founded with an ambitious program but it is still not functioning as expected [16]. Currently, this center is focusing on many goat projects and capacity building reinforcement of sector stakeholder, while little is done or has to be done in sheep. Future projects will involve insemination of 2000 ewes in Boujaâd breed and should later be extended to Sardi breed.

4.1 The cost of artificial insemination

One of the most important challenges against AI diffusion at large scale in the Maghreb region is its cost. Along with improving fertility rate following AI, researcher should focus on reducing intervention cost. For instance, AI charge in bovine specie in Tunisia is estimated around 3 € for the first IA, 2.45 € for the second and 1.84 € for the third and over till successful insemination for the same cow using locally produced pure breed semen. At this cost, coupled with the value of the animal, bovine AI is very valued and applied in both dairy and meat cattle. Yet the cost is significantly higher when the breeder chooses the use of imported semen and/or sexed semen to inseminate his cows, the cost is usually accepted given the importance of the genetic value of the expected offspring. On the contrary, the ovine AI real cost remains higher with lower commercial value of the product. Coupled with the estrus synchronization treatment, AI of ewes would be very expensive. This leads to the farmers' refusal to practice this approach. The use of more natural technique such as ram effect could reduce direct charges of the technique, although natural techniques lead always to very variable results. Even though the ovine IA services provided by governmental centers of AI are until nowadays free of charges in Maghreb countries, they are still localized and offer very limited services since the use of frozen semen is yet to develop. Information about the real cost of ovine AI is not available in the Maghreb region and the study of the economic impact of this biotechnology is strongly recommended.

4.2 Sperm cryopreservation

Most studies presented in this review had a particular interest to the storage conditions at 15°C and 5°C to prolong the storage time to 8–24 h. The extended refrigeration period would reduce the dependence of the AI centers. However, the needed doses were high (around 10^9 spz/ml) [47, 48]; thus, the number of doses/ejaculate remains very low. The effect of cryconservation [37] and different cryprotectors with different extenders in liquid preservation have been studied succinctly [40–43, 45], and most studies have been performed *in vitro* conditions. Most results showed good sperm preservation in liquid up to 24 h yet further research is required to design a valid strategy for the preservation of liquid semen from rams in the medium term 48 to 72 h. The effect of cryopreservation and freezing on ovine sperm was evaluated. Three steps were tested including storage in the liquid state (5°C), equilibration with four different extenders (Tris-egg yolk, Colas (skimmed milk based extender), Tryladil and AndroMed®) and then freezing [37]. The results showed that the time of storage at 5° C, equilibration and freezing have negatively affected the sperm quality. The skimmed milk had the best results at 5°C for 48 h compared to other extenders, while Tris-egg yolk extender was the best to preserve semen quality of rams during the freezing procedure with 72% total spermatozoa motility. These experiments revealed promising results *in vitro* but further studies will be needed to evaluate the effect of cryopreservation on sperm's fertility.

4.3 Challenge of sperm deposit site: transcervical intrauterine insemination, the technique to develop

The development of a non-surgical AI procedure that could be performed efficiently and repeatedly with constant results will have tremendous implication on selection programs and genetic progress of sheep. Deposit site of semen during AI in the ewe's genital tract has direct repercussion on fertility rate. Cervical AI leads to relatively low fertility rates even when using fresh semen. The particular, highly complex structural arrangement of ewe's cervix prevents easy transcervical passage and intrauterine deposition using conventional AI catheters [54]. Since the 1970, efforts have been made in order to access the uterine lumen by the transcervical route, which would allow the use of frozen-thawed sperm [55]. The success of transcervical intrauterine insemination depends on several factors, including cervical dilatation, the design of the catheters and the used procedure. Several works had investigated all these aspects, but none of these have been tested on local Maghreb breeds. A lot have to be done in order to promote AI of the ovine specie around the world and particularly in the Maghreb region.

5. Conclusion

In summary, despite the importance and the continuous progress of the ovine sector in the Maghreb region, particularly in Algeria and Morocco, AI is not yet an operative tool for the ovine breeding development and selection. Fertility rate following AI is very fluctuant and remains low 30 to 76% in most cited literature depending on the breed, the technique and the use of fresh, chilled or frozen semen. The development of AI of sheep became crucial to enhance genetic progress of this specie and to preserve some indigenous breeds with particular rustic characteristics and well adapted to their harsh environment. Many of the published studies are conducted under experimental conditions with a low number of

animals; thus, it is difficult to extrapolate their conclusions to field conditions at a large scale. The use of frozen-thawed semen along with a non-surgical technique, if mastered, could enhance and promote the use of AI at farm level and accelerate the genetic progress of ovine. The combined protocols (modified catheters plus dilator substances) could be the beginning of the solution for transcervical insemination, but the complexity of the technique, the time spent in cervical penetration and the side effects that it produces are key factors for the success and dissemination of the TCAI and should be optimized to achieve an efficient procedure.

Conflict of interest

The author declares no conflict of interest whatsoever.

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