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# Reproductive biotechnologies in Moroccan livestock: achievements, challenges, and future perspectives

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Anass Ben Moula 0000-0003-2878-2377 Mouad Chentouf 0000-0001-6802-9350 Younes Hmimsa 0000-0003-1054-8929 Hamid El Amri 0000-0001-7465-3491 Salama El Fatehi 0000-0002-6044-6499 Bouchra El Amir 0000-0003-3443-5988 Abstract This review explores the landscape of published research on reproductive biotechnologies in Moroccan livestock industry, with a particular focus on sheep and goats. While significant attention has been directed towards these species, a notable absence of research remains on other livestock species. Certain breeds of sheep and goats have also been insufficiently studied, revealing critical gaps in knowledge regarding reproductive interventions across Morocco's diverse livestock population. Although advancements in artificial insemination (AI) have improved outcomes in sheep through techniques such as semen storage and ovulation induction, essential methods like oocyte and embryo conservation, as well as embryo transfer, have been largely overlooked in Moroccan livestock research. This review underscores the necessity for a more inclusive approach. advocating for broader research that encompasses a variety of breeds within sheep and goats, while also extending beyond these species to include other overlooked categories of livestock. Addressing these identified gaps presents an opportunity to deepen our understanding of reproductive interventions and emphasizes the importance of establishing a cryobank to preserve genetic diversity and ensure livestock resilience in the face of climate change challenges. Recommendations outlined in this review propose a strategic roadmap that prioritizes expanding research to include overlooked species and breeds, integrating techniques such as oocyte and embryo conservation, and emphasizing the importance of embryo transfer studies in advancing reproductive biotechnologies within the Moroccan livestock industry.

<u>**Keywords:**</u> livestock farming; reproductive biotechnologies; Morocco; conservation efforts

Introduction

Situated in North Africa, Morocco boasts diverse agricultural landscapes, ranging from rain-fed Atlantic plains to desert areas. The varied agroecosystems have facilitated the emergence of diverse livestock species adapted to these distinct environmental conditions. The semi-arid to arid climate presents a significant challenge in ensuring food security, leading to substantial food imports.

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Livestock has historically been a crucial component of Moroccan society, playing a pivotal role in managing natural resources and providing essential animal proteins (Sraïri, 2016). Indeed, the traditional significance of livestock in Morocco is deeply ingrained in its societal structure, historically endorsed within a robust tribal framework. Livestock occupies a privileged position, fulfilling multiple essential functions in societal sustenance and cultural practices (Chaachouay and Zidane, 2023). While traditional extensive livestock production systems have demonstrated resilience during of adversitv. contemporary challenges periods necessitate innovative approaches to reinforce livestock breeding, productivity, and genetic conservation (Sraïri, 2016). Reproductive biotechnologies emerge as a promising avenue for enhancing livestock production and genetic conservation globally (Abberton, 2002).

Notably, research on reproductive biotechnologies in Morocco has primarily focused on the ovine (Tibary, 1989; Allai et al., 2016; El Amiri et al., 2017; El Khalil et al., 2018; Badi et al., 2018; Ben Moula et al., 2023a; Rahim et al., 2024), caprine (Chentouf et al., 2011; El Kadili et al., 2019a; Ouarib et al., 2021; Kchikich et al., 2023), bovine (Fihri et al., 2005; Boujenane and Boussag, 2014) and camelids (Ainani et al., 2022). Studies have predominantly highlighted techniques such as semen storage, ovulation induction, or artificial insemination (AI). However. other advanced reproductive biotechnologies, such as in vitro fertilization or embryo conservation and/or transfer, remain relatively unexplored.

The urgent need to apply reproductive biotechnology techniques in Morocco has become crucial, especially in the light of climate change impact on agricultural productivity (Williams and Hoffman, 2009; Silva et al., 2023). As Morocco navigates toward establishing its genetic bank, the imperative to master gamete and embryo production and conservation for each livestock species becomes increasingly apparent.

This review seeks to assess the current achievements, identify the gaps, and underscore the necessity of advancing research and application of reproductive biotechnologies in Moroccan livestock systems. By critically evaluating the successes, challenges, and future needs. We have tried to provide insights and recommendations to drive sustainable enhancements in livestock breeding, productivity, and genetic conservation. These initiatives are crucial to address the unique challenges posed by Morocco's diverse agroecosystems and evolving climatic conditions while safeguarding its rich genetic resources.

#### Materials and methods

A systematic approach was utilized to select pertinent research studies to ensure a thorough review of the literature concerning reproductive biotechnologies in the Moroccan livestock industry. Key databases such as PubMed, Web of Science, Scopus, and agricultural

databases like AGRICOLA were initially identified as primary sources for literature retrieval. A tailored search strategy was devised, integrating relevant keywords and Boolean operators to refine search results and capture studies specifically addressing reproductive interventions in Moroccan livestock. Clear inclusion and exclusion criteria were established to quide the screening process, ensuring that only studies meeting predefined criteria were included. Eligible materials encompassed peer-reviewed journal articles. conference proceedings, relevant reports and contributing to the understanding of reproductive interventions within the Moroccan livestock context. Studies that lacked focus on conserving animal resources and reproductive biotechnologies or were not specifically related to Moroccan livestock were systematically excluded. The synthesis of findings aimed to identify trends, gaps, and areas of consensus or disagreement within the literature landscape. By comparing and contrasting the findings, the review paper aimed to provide a comprehensive overview of research on reproductive biotechnologies in Moroccan livestock, focusing on addressing critical gaps highlighted in the abstract. An iterative approach was adopted throughout the selection process, allowing for the refinement of search strategies based on initial results and screening process feedback. This iterative process ensured that the review encompassed a broad range of relevant literature, closely aligning with the outlined objectives. Ultimately, the systematic method facilitated the comprehensive selection of research studies, enabling a thorough analysis of reproductive interventions within the Moroccan livestock industry.

# The significance of livestock farming in Morocco

Livestock farming stands indispensable as an cornerstone in Morocco, making a significant contribution to the nation's socio-economic landscape. Boasting impressive annual turnover an of approximately 35 billion dirhams (equivalent to roughly 350 million US dollars), this sector not only bolsters the country's economic prowess but also generates a substantial 95 million dollars of employment annually, offering vital livelihood opportunities across diverse regions (Sraïri, 2016)

Morocco has many traditional sheep breeds, like Beni Guil, Sardi, Boujaad, D'man, Siroua, Timahdit, Saghro, and Beni Ahsen. These breeds thrive on large grazing lands using traditional breeding methods (Gaouar et al., 2016). Similarly, caprine breeds like Barcha, Beni Arous, Daraa, Ghazalia, El Hamra, and Noir contribute to this diverse landscape. Meanwhile, cattle breeds like Brune de l'Atlas, Oulmès-Zaer, Tidili, and other imported breeds (like Holstein and Montbeliarde) are more prevalent in cultivated areas, highlighting the adaptability of Morocco's livestock to diverse environments (Boushaba et al., 2019). Camel breeds, including Marmouri, Guerzni, Khouari, and Jbeli, thrive in specific niches (Julien et al., 2021). Moreover, the poultry sector has experienced considerable growth over the past two decades and has a significant economic weight in Moroccan agriculture with 23.3 million days of labor and 9.9 billion dirhams in added value in 2019 (Smaiti et al., 2023). Notably the indigenous Beldi chicken and turkeys have demonstrated strong dynamism as highlighted in studies by Benabdeljelil and Arfaoui (2001) and Chahbi et al. (2019).

The equine sector, in turn, holds significant importance in Morocco's livestock production due to its rich cultural heritage and historical legacy. With five distinct equine breeds; Arabian Thoroughbred, English Thoroughbred, Anglo-Arabian, Barbe, and Arab-Barbe, Morocco showcases its deep connection with horses (Bokbot et al., 2021). Morocco's history is closely connected to these breeds, beginning with the Barb horse and later the Arabian horses, which played vital roles in the Moroccan cavalry. The Arab-Barbe breed, a result of this lineage combination, carries traits from both and excels in Morocco's tough environment thanks to its adaptability, resilience, and endurance (Fakir et al., 2023).

Beyond economic metrics, Morocco's livestock sector plays a pivotal role in preserving traditional farming practices, fostering social cohesion, and safeguarding cultural heritage. Additionally, by providing essential raw materials for various industries like leather and textiles, the sector forms an integral part of the country's industrial ecosystem (Dominguez et al., 2012).

Embracing modernization, technological advancements, and sustainable practices, the livestock industry in Morocco is positioned for further growth and innovation. As it evolves and adapts to market dynamics, it remains a pivotal player in the nation's economic development, ensuring food security and driving significant employment generation for the foreseeable future. Beyond its financial contributions, the significance of livestock farming in Morocco extends to its profound impact on society, culture, and the nation's pursuit of sustainable development.

# Reproductive biotechnologies used in various livestock species

#### Gamete production and conservation

The production and conservation of gametes is pivotal in livestock production and conservation efforts, by ensuring the genetic diversity and future viability of valuable animal breeds (Staric et al., 2019; Alomar, 2022). Gamete conservation allows for the storage of genetic material from superior or rare individuals, safeguarding against the loss of unique traits and helping maintain healthy populations (Manlik, 2019; Burger et al., 2023). It serves as an insurance policy, particularly for endangered or less common breeds, enabling breeders to reintroduce or strengthen desirable characteristics in breeding programs. Moreover, gamete conservation facilitates global collaboration, allowing genetic resources to be shared and exchanged, thereby enhancing the resilience and adaptability of livestock populations to changing environments and emerging challenges, ensuring sustainable livestock production, and preserving biodiversity (Zawati et al., 2015).

In Morocco, researchers have primarily concentrated on preserving semen through liquid and frozen state storage methods (Allai et al., 2016; Ben Moula et al., 2019; Kchikich et al., 2023). Notably, although extensive research has been conducted on semen preservation, investigations on oocyte storage remain unexplored and undocumented.

### Factors affecting semen production and conservation

#### Sheep and goats

In Morocco, various factors were identified to impact semen production and preservation, as outlined in Table 1. Notably, seasonal variations significantly influenced semen production in Sardi (Tibary, 1989) and Boujaad (Badi et al., 2018) sheep breeds which was higher during the summer, maintained during the autumn, and decreased during winter, with a slight increase during spring but not in INRA 180 breed (cross-bred sheep resulting from Timahdit and D'man breeds (Ben Moula et al., 2017).

Beni Arouss goats showed distinct seasonal effects on both semen production and conservation, being higher in summer and autumn and lower during winter (El Kadili et al., 2019a). In Boujaad sheep, increased age (3 years and older) in rams led to a decline in both semen quality and storage efficiency (Badi et al., 2018).

High ejaculation frequency, specifically three ejaculations every two d, negatively affected both semen production and preservation in INRA 180 sheep (Ben Moula et al., 2022). However, maintaining a rhythm of three ejaculations per week showed no significant impact on semen production and conservation. Regardless of ejaculation frequency, the initial and second ejaculations displayed superior quality in both fresh and stored states (Ben Moula et al., 2022).

#### <u>Bovine</u>

Charolais and Belgian Blue breeds (Farmed in Morocco) produced the highest semen quality, while Holstein, Piedmontese, and Oulmès-Zaer exhibited comparatively lower semen quality (Boujenane and Boussaq, 2014). The impact of age on Holstein bulls proved to be significant in influencing both semen production and its post-thawed quality (Boujenane and Boussaq, 2013). Notably, specific seasons, particularly spring and winter, were identified as optimal periods for heightened sperm production and superior quality in this breed (Boujenane and Boussaq, 2013). Additionally, semen collection once a day at one-day intervals resulted in superior monthly semen quality compared to collection intervals of 2, 3, 4, 5, and 6 d (Boujenane and Boussaq, 2013). These findings collectively provide crucial insights into the

factors affecting Holstein semen quality and production in Morocco.

Table 1. Factors affecting semen production and conservation in different livestock species in Mor	оссо
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Species	Influencing factor	Breeds	Effect	Reference	
		Sardi	Scrotal circumference and fresh and stored sperm quality were higher	Tibary 1989	
		Boujaad	from spring to autumn and lower during winter	Badi et al. 2018	
	Season	INRA 180	No effect on scrotal circumference and fresh or stored sperm quality	Ben Moula et al. 2017	
Ovine	Ejaculation frequency	INRA 180	Three ejaculations every 2d, negatively affected both semen production and its preservation. However, a rhythm of 3 ejaculations per week did not decrease semen production and conservation. The initial and second ejaculations displayed superior quality in both fresh and stored states	Ben Moula et al. 2022	
	Age	Boujaad	Increased age (3 years and older) led to a decline in both semen quality and storage efficiency	Badi et al. 2018	
Caprine	Season	Beni Arous	Scrotal circumference and fresh and stored sperm quality were higher from spring to autumn and lower during winter	El Kadili et al. 2019a	
Bovine	Season	Holstein	Fresh sperm quality was higher during winter and spring and lower during summer and autumn	Boujenane and Boussaq 2013	

#### Poultry

A study conducted by Benabdeljelil and Arfaoui (2001) is the sole comprehensive exploration of the sexual activity in Moroccan poultry, particularly focusing on the Beldi breed. Their findings define distinct patterns in sexual maturity, revealing that roosters typically reach maturity at around 154 d, while male turkeys, or toms, achieve this stage at approximately 217 d.

# Improvement of semen storage processing in small ruminants

Considerable efforts have been directed at refining semen storage techniques. In ovine species, investigations explored the influence of different extenders on sperm quality under distinct temperature conditions (15°C or 5°C) for INRA180 and Boujaad sheep breeds. The findings indicated that INRA96®, Duragen®, and skim milk extenders yielded optimal results at 15°C (Ben Moula et al., 2018a), whereas INRA96®, Duragen®, Andromed®, Ovipro®, and skim milk were identified as suitable medium for storage at 5°C (Ben Moula et al., 2018a). Beneficial effects of camel milk on enhancing the quality of preserved sperm from INRA 180 rams have been reported (Ben Moula et al., 2018b). A study highlighted that the anaerobic storage proved advantageous for chilled INRA 180 ram semen quality, particularly after the initial 24 h (Ben Moula et al., 2024).

The research on frozen semen from INRA 180 rams identified distinct sperm subpopulations (SP1 and SP2) with unique traits, aiding in selecting better-quality sperm for fertilization. Specific extenders improved thawed sperm quality, but changes in equilibration conditions negatively impacted semen quality post-thawing. These findings advance sperm freezing success in INRA 180 rams, guiding future cryopreservation improvements (Ben Moula et al., 2019). The semen cryopreservation of the Sardi ram breed was improved using *Spirulina Platensis* and *Salvia Verbenaca* extracts at −80 °C, highlighting their potential to protect cryopreserved sperm cells from oxidative stress (Ben Moula et al., 2024).

Other studies in rams and bucks focused on modifying the semen extenders for sperm preservation using natural extracts (Table 2). For instance, in Boujaad rams, liquid semen storage was improved using a variety of supplements, like argan oil (1% and 5% in Tris egg yok and skim milk extenders, respectively) (Allai et al., 2015), acetone extract from Opuntia Ficus Indica cladodes (1% in the skim milk or tris egg yok extenders) (Allai et al., 2016), cactus seed oil (1% and 2% in Tris egg yok and skim milk extenders respectively) (Allai et al., 2017), **Opuntia Ficus-**Indica cladode ethanolic extract (1% or 2% in skim milk extender) (Allai et al., 2023) and C-phycocyanin from Spirulina platensis (2.4 µg/mL in skim milk extender) (Rahim et al., 2024). In Sardi rams, Ben Moula et al. (2023) showed that Spirulina Platensis acetonic and hexanoic extracts (1.25 µg/mL), as well as Salvia Verbenaca acetonic (3.75 µg/mL) and methanolic (6.25 µg/mL) extracts, improved the efficiency of skim milk extender for improving the chilled semen quality.

In goats, research conducted on the Beni Arous breed (Ouarib et al., 2021) indicated that the Optixcell® extender imparted better cryoprotection to spermatozoa. Other works revealed notable enhancements in the quality of chilled stored semen by incorporating 0.01% of *Origanum Onites, Origanum Vulgare, Origanum Majorana*, or *Thymus Satureioides* (Kchikich et al., 2021; 2023) essential oils into the skim milk extender. Additionally, the quality of post-thawed semen notably improved specifically through the supplementation of *Origanum Majorana* (0.02%) essential oil to skim milk extender (Kchikich et al., 2023). These findings not only help optimize chilled and frozen semen quality but also underscore the potential of specific Moroccan natural additives in improving postthawed semen characteristics. Overall, these collective efforts underscore the importance of tailored approaches in semen preservation for diverse breeds, offering

#### Reproductive biotechnologies in Moroccan livestock

valuable insights that could revolutionize breeding and reproduction practices in Moroccan sheep and goat husbandry. Further research in this area holds significant promise for continued advancements in reproductive technologies and livestock breeding strategies.

Table 2. Enhanced stored semen	quality in Mor	oroccan sheep and goat breeds using natural supplements in semen exter	Iders

Species	Breed	Storage technic	Extender	Supplement	Improved parameters	Reference
Ovine		Liquid storage (5 or 15°C)	Tris-egg yok or skim milk	Argan oil (1% in Tris and 5% in skim milk)	Motility, viability, membrane integrity, abnormality, lipid peroxidation, DNA fragmentation	Allai et al. 2015
		Liquid storage (5 °C)	Skim milk	Ethanolic extract from <i>Opuntia ficus Indica</i> cladodes (1% in the skim milk)	Motility, viability, membrane integrity, abnormality, lipid peroxidation,	Allai et al. 2023
		Liquid storage (5 °C)	Tris-egg yok or skim milk	1% and 2% in Tris- egg yok and skim milk extenders, respectively	Motility, viability, morphology, peroxidation and DNA fragmentation	Allai et al. 2017
	Boujaad	Liquid storage (5 °C)	Skim milk	C-phycocyanin from <i>Spirulina platensis</i> (2.4 µg/mL in skim milk extender)	Motility, lipid peroxidation	Rahim et al. 2024
		Liquid storage (5 °C)	Tris-egg yok or skim milk	Acetone extract from <i>Opuntia ficus Indica</i> cladodes (1% in the skim milk or tris egg yok extenders)	Motility, viability, membrane integrity, abnormality, lipid peroxidation	Allai et al. 2016
		Liquid storage (4°C)	Skim milk	Spirulina platensis acetonic or hexanoic extracts (1.25 µg/mL)	Motility, viability, membrane integrity, abnormality, lipid peroxidation	Ben Moula et al. 2023
		Liquid storage (4°C)	Skim milk	Salvia verbenaca acetonic (3.75 µg/mL) and methanolic (6.25 µg/mL) extracts	Motility, viability, membrane integrity, abnormality, lipid peroxidation	Ben Moula et al. 2023
	Sardi	Freezing	Skim milk	Salvia verbenaca acetonic and methanolic (1.25 µg/mL) extracts	Motility, viability, membrane integrity, abnormality, lipid peroxidation	Ben Moula et al. 2024
		Freezing	Skim milk	<i>Spirulina platensis</i> acetonic or hexanoic extracts (1.25 and 3.75 g/mL)	Motility, viability, membrane integrity, abnormality, lipid peroxidation	Ben Moula et al. 2024
Caprine		Liquid storage (4°C)	Skim milk	0.01% of <i>Origanum majorana</i> or <i>Thymus</i> satureioides essential oils into the skim milk extender	Motility, viability, membrane integrity, abnormality, lipid peroxidation	Kchikich et al. 2021
	Beni Arous	Liquid storage (4°C)	Skim milk	0.01% of <i>Origanum</i> or <i>Onites</i> , <i>Origanum vulgare</i> essential oils into the skim milk extender	abnormality, lipid peroxidation	Kchikich et al. 2023
		Freezing	Skim milk	Origanum majorana (0.02%) essential oil into the skim milk extender	Motility, viability, membrane integrity, abnormality, lipid peroxidation	Kchikich et al. 2023

# Female sexual activity, ovulation induction, and estrus synchronization

#### Female sexual activity

#### Sheep and goats

Moroccan sheep breeds, including Beni-Guil, Sardi, Timahdit, D'man, and INRA 180 ewes, exhibit distinct patterns and variations in reproductive performance. Beni-Guil, Sardi, Timahdit, and Boujaad ewes show a seasonal anestrus phase spanning from February to May (Dewulf and Lahlou-Kassi, 1986; Chikhi and Boujenane, 2003). Besides, studies by Dewulf and Lahlou-Kassi, (1986), Chikhi and Boujenane, (2003), Boujenane (2006), Dergaoui et al. (2009), and Fadili (2011) recorded the onset of initial ovulations in these breeds post-lambing. Beni-Guil ewes exhibit their initial ovulation at around 41 d after lambing, accompanied by the appearance of estrous signs approximately at 85 d. In contrast, Sardi ewes display their first ovulation approximately at 71 d post-lambing, aligning with the onset of initial estrus observed around 132 d. The Timahdit breed showed their initial ovulation at approximately 43 d post-lambing. Interestingly, studies by Dergaoui et al. (2009) and Fadili (2011) indicated that

the reproductive performances of D'man and INRA 180 ewes were not significantly influenced by seasonal variations. Additionally, the postpartum anestrus duration was notably short, being 42 d for INRA 180 ewes and 40 d for D'man ewes (Dergaoui et al., 2009). The ovulation rate in these breeds presents significant diversity. D'man ewes displayed an average ovulation rate of 2.78 (ranging from 2.50 to 3.05), whereas Timahdite, Beni Guil, and Sardi ewes exhibited rates of 1.09, 1.02, and 1.28, respectively. Moreover, D'man ewes produced between 1 to 8 ova vs. 1 to 3 for other local breeds. In D'man ewes, a frequency distribution analysis (Boujenane, 2006) revealed percentages for various ovum numbers, as 16.0% for 1 ovum, 33.1% for 2, 24.8% for 3, 16.2% for 4, 6.42% for 5, 2.76% for 6, 0.73% for 7, and 0.14% for 8 ova.

#### Cattle and camels

In Moroccan Oulmes Zaer cattle, the average count of ovarian follicles per cow was notably high ( $22.98 \pm 8.41$ ). However, the yield of oocytes was considerably low at  $2.60 \pm 1.53$  (Fihri et al., 2005).

Camels exhibited a distinct breeding season from mid-November to mid-April, correlating with over 80-90% of observed matings (Sghiri and Driancourt, 1999). The proportion of females with active ovaries increased

#### Ben Moula et al.

significantly from 73.5% to 89% between October-December and January-May, respectively, while cystic follicles peaked in April-May. Younger females had reduced conception rates (Sghiri and Driancourt, 1999). The study found consistent follicular morphology regardless of the season, but hormonal activity varied, with early breeding season follicles displaying lower estradiol output, potentially impacting fertility (Sghiri and Driancourt, 1999). Another study showed higher expression of Kisspeptin (Kp) but lower expression of arginine-phenylalanine (RF)-amide-related peptide-3 (RFRP-3) in the camel's brain indicating the involvement of these neuropeptides in the seasonal control of the breeding activity (Ainani et al., 2020).

#### Poultry

Beldi chickens show distinct patterns of maturity and egg production. Beldi hens reach maturity at 168 d and turkey hens at around 231 d. Throughout the year, chickens lay about 78 eggs, and turkey hens produce around 69 eggs (Benabdeljelil and Arfaoui, 2001).

#### **Ovulation induction and estrus synchronization**

It is generally believed that ovulation induction and estrus synchronization could play pivotal roles in livestock management, optimizing breeding programs, and enhancing reproductive efficiency under certain conditions (Deac et al., 2024). By inducing ovulation, farmers can control the timing of mating, leading to increased conception rates and synchronized births. This process allows better genetic selection, improving the quality of the herd or flock. Additionally, heat synchronization aids in streamlining reproductive cycles among animals, facilitating more efficient utilization of resources and reducing labor-intensive tasks associated with monitoring individual heat cycles. Ultimately, these techniques not only enhance productivity but also contribute significantly to the overall profitability and sustainability of livestock operations. Thus, different experiments were performed to optimize the induction of ovulation and estrus synchronization in Moroccan livestock (El Amiri et al., 2017; 2018; El Allali et al., 2018; Ainani et al., 2022; El Kadili et al., 2022).

#### Sheep and goats

Investigations on INRA 180 ewes demonstrated improved fertility rates post-artificial insemination by inducing estrus with equine chorionic gonadotropin (eCG), revealing the efficacy of different synchronization methods (EI Amiri et al., 2017; 2018). Specifically, a comparison between two estrus synchronization methods, Chronogest® and Eazi-breed TM CIDR® (controlled internal drug release device), after AI with semen stored for more than 4 hours revealed conception rates of 59% and 67%, respectively, for ewes receiving 250 IU and 300 IU of eCG.

Studies on Beni Arouss goats during different reproductive stages highlighted the varying effectiveness of hormonal treatments versus sexually active bucks in inducing and synchronizing ovulation (EI Kadili et al., 2019b). The research emphasized the impact of these methods during anestrus and the breeding season, where hormonal treatments involving gonadotrope adenomas (FGA), eCG, and cloprostenol were more effective during anestrus. During the breeding season, the protocol involving sexually active bucks demonstrated a 100% response rate in inducing estrus and synchronizing ovulation, indicating their suitability during this period (El Kadili et al., 2022).

#### <u>Camels</u>

Studies on Marmouri and Guerzni dromedary camels highlighted the pivotal role of seminal plasma nerve growth factor beta (β-NGF) injections in triggering ovulation (Ainani et al., 2022). The injections activated specific brain neurons associated with reproduction, inducing a quick increase in circulating luteinizing hormone (LH) within hours and confirmed oocyte release, thus offering a potential method for controlling ovulation in these breeds. Research on camels using continual-release melatonin implants demonstrated advancements in reproductive activity, suggesting potential manipulations of the breeding season. The implants resulted in increased circulating melatonin levels persisting for over 12 weeks, advancing the start of reproductive activity by 3.5 months, and influencing estradiol-17ß concentrations and prolactin levels (El Allali et al., 2018).

#### Artificial insemination

There is few research on AI in Moroccan livestock, with the majority of published papers concentrating on on enhancing semen storage and refining ovulation induction to improve the effectiveness of AI in sheep breeding. The first experiments on AI were conducted by Tibary (1989), in which various synchronization treatments and hormone doses were tested to improve the fertility rate after AI of extended fresh or frozen semen. However, fertility rates remained low, ranging from 10% to 40% in D'man and 12% to 39.1% in Timahdit breeds.

Since 2018, research on AI techniques in sheep has attracted more attention, particularly with the work conducted by EI Khalil et al. (2018). In their study, the authors highlighted that the cervix of D'man ewes is less intricate and more conducive to transcervical AI. Moreover, addressing the complexities associated with the cervix in Boujaâd sheep, selection of 4-year-old ewes has been proposed as a strategy to facilitate the penetration of the AI catheter. EI Amiri et al. (2018) indicated that after optimizing semen storage, ovulation induction and heat synchronization protocols, fertility increased to 70% by using Eazi-breed TM CIDR® (controlled internal drug release device) vaginally inserted for 14 d for estrus synchronization combined with a fixed dose (300 IU) of equine chorionic gonado tropin (eCG; PMSG) for INRA 180 ewes after AI with semen stored in skim milk extender for more than 4 This achievement allowed the Moroccan hours. researcher to conduct more research to improve the AI outcome. For instance, in Sardi sheep, advancements in reproductive techniques have led to remarkable results in AI, notably achieving a 68% success rate. This milestone was attained through a strategic approach involving the use of 20 mg vaginal FGA sponges, administered for 14 d for estrus synchronization. This method was complemented by a fixed dose of eCG (300 IU) and the utilization of stored semen in skim milk fortified with 3.75 µg/mL of acetonic extract derived from Salvia verbenaca (Ben Moula et al., 2023b). Further enhancement in the success of AI was observed, increasing the success rate to an 73%. This improvement was achieved by maintaining the same

#### Reproductive biotechnologies in Moroccan livestock

ovulation induction technique while optimizing the semen storage conditions. Specifically, storing the semen in a Duragen® extender at  $15^{\circ}$ C for 5 hours before AI contributed significantly to this success rate (EI Amiri et al., 2023). Recently, Rahim et al. (2024) conducted a study revealing that incorporating C-phycocyanin from *Spirulina platensis* (2.4 µg/mL) into a skim milk extender for preserving Boujaâd ram semen at  $5^{\circ}$ C for 4 hours resulted in a 76% fertility rate. This study also implemented the same ovulation induction technique as the previous protocol developed by EI Amiri et al. (2018).

Collectively, these advancements mark a significant paradigm shift, offering promising avenues for enhancing AI outcomes in Moroccan sheep (Figure 1). The cumulative insights from research endeavors hold tremendous potential for revolutionizing sheep breeding practices, potentially augmenting agricultural productivity and positively impacting the livelihoods of farmers in Morocco.

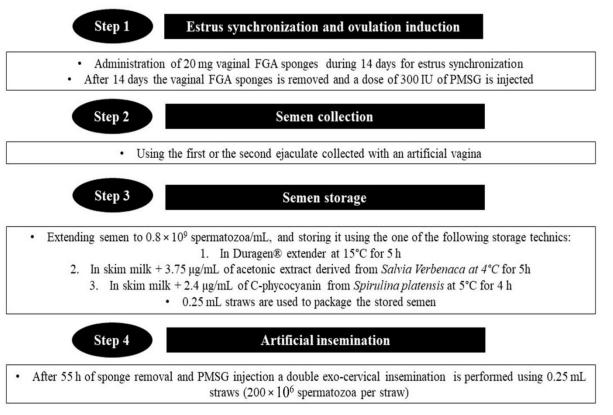


Figure 1. The adopted protocol for artificial insemination in sheep: A Moroccan perspective

#### Pregnancy detection

In a study focused on Moroccan livestock, researchers explored advanced methods for detecting pregnancy in dairy cattle and Boujaâd sheep. In dairy cattle, the study compared the accuracy of two tests (Rapid Visual Pregnancy Test (VPRT) and Bovine Pregnancy Test DG29® (ELISA-PAG)) by measuring Pregnancy-Associated Glycoproteins (PAG) in blood samples collected 28 to 35 d post-artificial insemination (Moussafir et al., 2023). These samples were tested using both methods and compared against transrectal ultrasonography after  $40 \pm 3$  d post-Al, revealing VPRT's potential as a more accurate alternative for early

#### Ben Moula et al.

pregnancy diagnosis (Moussafir et al., 2018). Triglycerides displayed significant differences between pregnant and non-pregnant Boujaâd ewes, suggesting their potential for early pregnancy detection in sheep, especially when considering mating or AI dates (Moussafir et al., 2023). These findings bear significance for enhancing reproductive management strategies, potentially optimizing breeding practices, and bolstering livestock productivity in Moroccan agriculture.

# Identified gaps, limitations, and future recommendations

Moroccan livestock research has made significant progress in reproductive biotechnologies such as semen conservation, ovulation induction, AI, and pregnancy detection. These advances have expanded the knowledge base, yet key gaps remain, underscoring the need for broader, in-depth research to enhance both practical application and understanding of these technologies in Moroccan livestock management.

### Advances and remaining gaps in reproductive biotechnologies

Ongoing research on semen production in sheep, goats, and select cattle breeds has offered valuable insights into sperm quality and storage. However, limitations persist, including restricted species diversity and insufficient exploration of factors like climate change, farming systems, nutrition, and geographical variations (Ahmad Para et al., 2020). Additionally, current studies often lack breed-specific analyses. leading to an incomplete understanding of reproductive biotechnology applications across diverse livestock breeds (Roias Canadas et al., 2020). While advancements in semen storage techniques, ovulation induction, and AI show in long-term promise, gaps efficacy. protocol standardization, and practical application remain. Future research should prioritize inclusive approaches and develop protocols tailored to different breeds to achieve more reliable outcomes in real-world conditions (Tesfay et al., 2020; Mendoza et al., 2021).

#### Limited coverage of livestock species and breeds

Research on reproductive biotechnologies has mainly focused on sheep, goats, and certain cattle breeds, with limited exploration of other species like equine, camel, and poultry. These underrepresented species require further investigation to understand their unique reproductive dynamics within the Moroccan environment. Climate, breed characteristics, and genetic variability affect responses to biotechnologies, necessitating studies that encompass a broader range of livestock species and Moroccan-specific breeds to inform efficient and inclusive management strategies (Raheem, 2017; Ben Moula and El Amiri, 2022).

#### Technology transfer and adoption challenges

The adoption of reproductive biotechnologies in Moroccan livestock is heavily influenced by socioeconomic and cultural factors. Traditional practices, local beliefs, and community socioeconomic conditions shape breeding and reproductive management decisions (Aich, 2018; Ježková and Dřevo, 2002). However, these influences are often overlooked in research. A comprehensive approach that integrates scientific advancements with local socio-cultural dynamics is essential for fostering sustainable adoption of biotechnologies. By aligning with community values and practices, researchers can enhance the acceptance and long-term viability of these technologies within Moroccan livestock systems.

#### Conclusion

In conclusion, while Moroccan livestock reproductive biotechnology has advanced significantly in areas such as semen storage and ovulation induction in sheep, substantial gaps remain, particularly in other species and breeds. Expanding research to include techniques like oocyte and embryo conservation and establishing a cryobank might play a vital role in preserving genetic diversity and enhancing resilience to climate change. A broader, inclusive approach would not only support Morocco's livestock conservation efforts but also ensure the sustained productivity and adaptability of the livestock resources in the face of environmental challenges.

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