# Assisted reproductive technologies in cattle in Cameroon

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# Keywords

cattle, reproduction control, cestrus synchronization, artificial insemination, embryo transfer, Cameroon

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# Summary

Background: Assisted Reproductive Technologies (ARTs) include artificial insemination (AI), embryo transfer (ET), multiple ovulation embryo transfer (MOET), estrus synchronization and superovulation, laparoscopic ovum pick-up (LOPU), in vitro maturation (IVM), in vitro fertilization (IVF) and in vitro culture (IVC) (collectively known as in vitro embryo production, or IVEP), intracytoplasmic sperm injection (ICSI), cryopreservation of sperm, cryopreservation of oocytes and embryos, sperm and embryo sexing, embryo splitting, embryo cloning, nuclear transfer (NT), gene transfer, and marker-assisted selection (MAS). Emerging technologies include microfluidics, three-dimensional printing of cell culture materials, organoid culture, livecell imaging, new advances in cryopreservation, and artificial intelligence. Aim: This study aims to present an update and overview of ARTs in Cameroon with a focus on cattle. Results: In Cameroon, several methods have been used to synchronize the estrus in cattle using progestins (PRIDND Delta & CIDR-B), prostaglandins (PGF), and GnRH. Adding progesterone to the CoSynch protocol improves the fertility of local cows. Since the first artificial insemination was performed in 1942 with fresh semen, numerous genetic improvement projects using fixed-time AI with frozen semen have improved the genetics of local breeds, with an overall pregnancy rate of 49.5% (32.8–57). The main constraints facing AI in Cameroon are the availability and cost of liquid nitrogen for transporting semen, especially in rural zones. Several studies have been done on the ovarian potential of local cattle for *in vitro* embryo production. Much of the research was done using slaughterhouse ovaries with a potential ranging from 55 to 60% of selected oocytes for in vitro embryo production (grades I and II) using the slicing technique. Conclusions: The use of ovum pick-up procedures guided by ultrasound to collect oocytes from both fertile and infertile genetically valuable cows, IVM, IVF, IVC, and other ARTs (in vivo embryo production, embryo transfer, embryo splitting, cloning, production of transgenic animals, and emerging technologies) is not yet widespread in Cameroon. There is an urgent need for stakeholders in Cameroon to develop and update policies and guidelines to help address ethical concerns regarding ARTs.

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## ■ INTRODUCTION

In Cameroon, livestock plays a crucial role in the primary sector, contributing 13% to the country's Gross Domestic Product (GDP). Livestock, which primarily includes cattle, small ruminants, pigs, poultry, goats, and other non-conventional animals, is the main source of income for 30% of the working population. Among the species mentioned above, cattle hold a prominent position, with an estimated population of around 10 million head, and cattle farming meets 61% of

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the national demand for meat. In Cameroon, there are both zebu and taurine cattle. Among the zebu, the most important in terms of numbers are the red-coated Mbororo (also known as Red Fulani, Djafoun, or Rahadji) (28%), followed by the white-coated Mbororo (White Fulani or Akou) (25%), and the Gudali of Adamawa, which includes the subtypes Ngaoundere (Peuhl or Foulbe), Banyo (Banyole), and Yola (Mayne) (19%). Finally, the Massa, Arab Choa, and other Sahelian zebus represent a fairly heterogeneous group (25%). The taurine cattle (2%) mainly include the Muturu in the Southwest, the Namchi in the North in Faro, the Kapsiki in the Far North, and the Kouri in the Far North around Lake Chad. Imported breeds (Holstein and Montbéliarde) remain very rare, with only 5% of breeders owning them (Kouamo & Pa-ana, 2017; Kouamo *et al.*, 2019). National cattle breeding contributes 54% of all locally produced meat products

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consumed by the population. Cattle breeding produces 110,000 tons of meat and 174,000 tons of milk annually, all of which is consumed locally, and contributes approximately CFA 950 billion ( $\leqslant$ 1,448 million) to per capita GDP (Tsapi *et al.*, 2022).

Despite the diversity of breeds raised, the national coverage rates for the meat and milk needs of the population remain very low. According to the Ministry of Livestock, Fisheries and Animal Industries, Cameroon produced 176,600 tons of milk in 2023 (a 2% increase from 2022). The national supply is estimated to be 185,570 tons per year, which is far below the demand of around 300,000 tons for an estimated population of 29,894,253 people. This results in an annual deficit of nearly 120,000 tons, which is partially offset by imports of dairy products. The latest report from the National Institute of Statistics (INS) on foreign trade shows that 20,596 tons of milk and milk products costing CFA 40.6 billion (eq. 62 million euros, and 17,217 tons of powdered and concentrated milk costing CFA 35 billion (eq. 53.3 million euros), were imported in 2023 to address the shortfall in national production.

The pastoral livestock production system in Cameroon faces many constraints, including limited access to inputs, degraded pasture lands, limited feed resources, and insufficient water points for herds – all of which have been worsened by climate change. Other major constraints involve health and sanitary problems, which contribute to the prevalence of endemic animal diseases, particularly reproductive diseases (WBG, 2023). Animal productivity largely depends on their reproductive performance; hence regular and successful reproduction is a key to profitable animal production.

Over the past 10 to 20 years, new biotechnologies have been developed that are on the verge of revolutionizing reproductive processes in both humans and animals. In agriculture, modern techniques in assisted reproductive technology (ART) are being used for the introduction, improvement, and preservation of livestock genetics and the enhancement of animal reproductive efficiency. Modern embryology and ART technologies have facilitated the development of methods to transfer either desired single genes or entire genomes from desirable individuals or embryos. In addition, rapid advances in laboratory techniques for manipulating embryos have made it possible to screen embryos for genetic defects or highly desirable quantitative traits using molecular markers (Hasler, 2023). In cattle, ART can be defined as techniques that manipulate reproductive-related events and/or structures to achieve pregnancy, with the final goal of producing healthy offspring in female cattle. The ART techniques include artificial insemination (AI), estrus synchronization and superovulation, in vitro fertilization, in vivo and in vitro embryo production, embryo collection, embryo transfer, embryo cryopreservation, embryo splitting, cloning, production of transgenic animals, and preimplantation genetic diagnosis (Table I) (Chandra & Sharma, 2020; Baldassarre, 2021; Mikkola et al., 2024). The progress made over the past 20 years (timed AI, genomic selection for fertility in both bulls and cows, automated estrus detection, chemical and ultrasonographic pregnancy diagnosis, and gender selected semen) will continue over the next 20 years as most of these technologies can be optimized further. Improving embryo technologies and increasing our understanding of embryonic loss may present the greatest challenges for the future. The derivation of oocytes, sperm, and embryos from pluripotent stem cells may yield a vast supply of gametes and embryos from genetically superior animals, radically changing future reproductive management (Lucy & Pohler, 2025). According to Pascottini et al. (2025), the main areas of rapid development that are expected to further advance over the next 20 years to improve fertility and management of ruminants include: i) genetic selection (including improved phenotypes for use in breeding programs), ii) nutritional management (including transition cow management), iii) control of infectious diseases, iv) rapid diagnostics of reproductive health, v) development of more efficient ovulation/estrus synchronization protocols, vi) assisted reproductive management (and automated systems to improve reproductive management), vii) increased implementation of sexed semen and embryo transfer, viii) more efficient handling of substantial volumes of data, ix) routine implementation of artificial intelligence technology for rapid decision-making at the farm level, x) awareness of climate change and sustainable cattle production, xi) new (reproductive) strategies to improve cattle welfare, and xii) improved management and technology implementation for male fertility. This study presents an update and overview of ARTs in cattle production and research, specifically in Cameroon.

# ■ MATERIAL AND METHODS

The literature search covered publications up to 2025 to include both foundational and current research, considering their relevance to the topic, methodological rigor, and focus on cattle ARTs. A broad search using the keywords "Cameroon", "Cattle", "Livestock sector", "Genetic improvement of cattle in Cameroon", "Assisted reproductive technologies", "Artificial insemination of cattle", "Embryo transfer of cattle", "Genetic selection of cattle", "Bovine oocytes", "Ovum pick-up", "In vitro and in vivo embryo production", "Embryo technologies", "Factors affecting OPU", "Hormonal stimulation", "Embryo cryopreservation", "Adoption of cattle breeding technologies", "Challenges/constraints

Table 1: Classification of assisted reproductive technologies in cattle /// Classification des techniques de reproduction assistée chez les bovins

| First generation  | Second generation                         | Third generation   | Fourth generation   | <b>Emerging technologies</b>  |
|---|---|--|---|---|
| Artificial insemination including estrus synchronization. | Embryo transfer including superovulation. | Multiple ovulation embryo transfer (MOET), Estrus synchronization and superovulation, Laparoscopic ovum pick-up (LOPU), In vitro maturation (IVM), In vitro fertilization (IVF), In vitro culture (IVC) collectively known as in vitro embryo production (IVEP), Intracytoplasmic sperm injection (ICSI), Cryopreservation of sperm, Cryopreservation of oocytes and embryos, Sperm and embryo sexing, Embryo splitting, Embryo cloning. | Nuclear transfer (NT),<br>Gene transfer and marker-<br>assisted selection (MAS),<br>Production of transgenic<br>animals,<br>Preimplantation genetic<br>diagnosis. | CRISPR-based genome editing, Microfluidics, Artificial tissues, Three-dimensional culture, Non-invasive assessmen of cellular function, Time-lapse imaging and new advances in cryopreservation, Artificial intelligence in the ARTs. |

in the use of breeding technologies", "ARTs", and "Ethical issues of ARTs in cattle" was performed. These searches were performed multiple times combined with the terms ARTs and Cameroon. We should note that limited published data on ARTs in Cameroon were available.

### ■ ARTIFICIAL INSEMINATION

# History of AI in Cameroon

Artificial insemination (AI) is the reproductive biotechnology that has improved the production potential of local breeds such as *Bos indicus* and taurus. In Cameroon, the first insemination was carried out in 1942 with fresh semen (average fertility of 30.1%) and in 1969 with imported frozen semen (average fertility of 51%) on Gudali cows at the Wakwa zootechnical station in Ngaoundere (Mandon, 1948; Lhoste & Pearson, 1976). Cattle genetic improvement programs using AI were implemented mainly in the Adamawa and Northwest regions, not only by IRAD but also by state structures such as the Ranch of the Animal Production Development Company (SODEPA) in Jakiri. Between 1969 and 1973, these programs became widespread, and encouraging results were obtained. In relation to the number of cows inseminated (n=911), the overall fertility rates (pregnancies controlled or observed) were 44.6% (1969-1970; n=148), 54.7% (1970-1971; n=400), 54% (1971-1972; n=174), and 48% (1972-1973; n=189), with an average fertility rate of 51% for the four years of the preliminary experiment. In the 2000s, SODEPA launched a cattle AI program in Jakiri in the Northwest region of Cameroon that achieved varying degrees of success. Between 2022 and 2023, milk production in Cameroon increased from 174,000 tons to 176,000 tons. Yet despite this increase, Cameroon continues to face an average production deficit of nearly 120,000 tons of milk each year. This imbalance between supply and demand is offset by dairy product imports, which cost the Cameroonian government nearly CFA 20 billion (€30.489 million) per year. To reduce this financial loss, Cameroon acquired 495 dairy cows from France between 2020 and 2023 to boost local milk production. They were pregnant Montbéliarde heifers whose milk production is estimated at 40 liters of milk per day. They are a highly prized breed in sub-Saharan Africa due to the quality of their milk and their adaptation to difficult climatic conditions. This acquisition was made within the framework of the Livestock Development Project (Prodel), financed by the World Bank (PNN, 2023). Artificial insemination was chosen as the tool to intensify animal production. This reproductive technique, already widespread in Europe, America, and Asia, has for some time been experiencing renewed interest in developing countries, including Cameroon, due to an increasingly rapid population growth and an ever-increasing demand for animal products. Kathambi et al. (2025) reported that farmers in Africa have reported advantages of using AI over natural mating including, but not limited to, accelerated genetic turnover and improvement, lower cost relative to bull mating, forgone cost of rearing bulls, and decreased risk of disease transmission. Today, AI is gradually being taken up by non-governmental organizations (NGOs), individuals, and government projects (PAPA and Prodel) for dairy production. However, crossbreeding and the selection of breeds are often conducted haphazardly, without a clear plan to control the distribution of crossbreeding outcomes. As a result, this approach can pose a potential risk to the integrity of existing genetic material. Cameroon currently has about forty inseminators through Prodel who work mainly in the northern and northwestern regions of the country.

# Management of AI in Cameroon

Given the constraints related to the manifestation and detection of estrus, AI is most often performed after hormonal induction using progesterone (PRID<sup>ND</sup> Delta, CIDR<sup>ND</sup>). This process may be combined with other treatments, such as prostaglandin (PgF2 $\alpha$ ), equine

Chorionic Gonadotropin (eCG), and Gonadotrophin-Releasing Hormone (GnRH, also known as gonadoliberin), depending on the specific protocols used (Kouamo et al., 2020; 2021; Lopez-Gatius, 2022). The overall pregnancy rate achieved is 49.5% (ranging from 32.8% to 57%). This allows the insemination of a larger number of animals. The overall pregnancy rate obtained (49.5%) is higher than those reported in Niger (29.9 to 46.6%, Bos indicus type animals: Moussa-Garba et al., 2014) and Burkina Faso (42.7%, Gudali zebus: Zongo et al., 2012). In the Adamawa region of Cameroon, a total of 943 females (181 heifers and 762 cows) were inseminated between 2019 and 2022. Three distinct genetic groups were identified. The first group included Bos indicus of the Gudali breed (503), while the second group consisted of crossbred animals from Bos indicus  $\times$  Bos taurus (241). The third group was made up of Bos taurus animals (199), including 22 Holsteins and 177 Montbéliarde. Three therapeutic groups were used for estrus synchronization: the first group utilized the CoSynch protocol, a double injection of PGF2α 11 days apart and a single injection of PGF2α (n=140; 14.8%); the second group employed the CoSynch + progesterone protocol (n=433; 46%); and the final group combined progesterone with eCG (n=370; 39.2%). With an average apparent fertility index of 2.3, the overall pregnancy, abortion, and twinning percentages were 49.5%, 8.3%, and 6.7%, respectively. Studies show that pregnancy percentages were higher in Holstein and Montbéliarde Bos taurus (60.8%) and first generation Bos indicus × Bos taurus crossbreeds (57.6%) compared to Gudali Bos indicus (41.1%; p=0.0005), respectively (Kouamo et al., 2024). Fertility was satisfactory (60.8%) in *Bos taurus* type cattle (n=199), but remained low for local Bos indicus type breeds (41.1%; n=503). Cows that were stalled, cows those under 4 years old, cows with a body condition score (BCS) > 3, and cows with a waiting period from 120 days to one year obtained a better fertility (p < 0.05). The season, the type of semen used, and parity had no influence on the occurrence of pregnancy and calving (p > 0.05). However, the AI-technician was a significant factor (p = 0.001) (Kouamo et al., 2024). Bayemi et al. (2015) conducted a study in Cameroon using a long protocol that involved two injections of PGF2α administered 11 to 14 days apart. Their findings showed an embryo loss of 28% in heifers and 20% in multiparous cows (p > 0.05), as determined through progesterone assays and rectal palpation, during a pregnancy period of 3 weeks to 3 months. The 11-day interval protocol included double fixed-time insemination at 72 and 96 hours after the second PGF2 $\alpha$  injection. The conception rate was 52%. In Chad, it was 30% (Zeuh et al., 2014). The study recommended that farmers do not synchronize animals with poor BCS. The authors recommended using the two-dose regime in areas where inseminators are not easily available.

## Factors influencing reproductive performance

## Reproductive pathologies

In our context, most of the animal are non-cycling and suffer from many genital abnormalities. A study was conducted of 501 genital tracts of female zebus collected from Ngaoundere Municipal Slaughterhouse (NMSH) to determine the prevalence of genital abnormalities. A total of 292 (58.28%) specimens had abnormalities. The highest percentage of pathological conditions were observed in the ovaries (39.6%), followed by those in the uterus (15.4%), oviduct (2.8%), and vulvo-vagina (0.6%). The pathological conditions observed in the ovaries included anestrus (25.2%), repeat breeding (8.4%), ovarian cysts (3.8%), double and multiple ovulation (1.2%), oophoritis (0.4%), ovarobursal adhesions (0.4%), and ovarian abscess (0.2%). Those in the uterus included mucometra (7.8%), metritis (5.6%), hydrometra (1.6%), and lymphosarcoma (0.4%). Oviduct abnormalities included parovarian cysts (1.4), hydrosalpinx (0.6%), lymphosarcoma (0.4%), salpingitis (0.2%), and double oviduct (0.2%) (Kouamo et al., 2016). Recently, a study conducted in Yaounde (Cameroon) revealed that the overall prevalence of genital organ pathologies in cows at the slaughterhouse was 51.4%, and the most represented were anestrus (19.6%), mucometra (10.8%), metritis (7.2%), and ovarian cysts (2.8%) (Kouamo et al., 2025). In this context, and to increase fertility, prostaglandin has been used as a co-treatment in effective progestogen-based synchronization protocols in cattle for both natural mating and AI/timed AI situations (Lucy et al., 2001; Islam, 2011; Kouamo et al., 2024). Furthermore, given the breeding methods, which in sub-Saharan Africa – and Cameroon in particular - remain mostly traditional, the majority of hormonal synchronization protocols are accompanied by the systematic insemination of treated females (Kouamo et al., 2021, 2022, and 2024). Our studies demonstrated that the association of the CoSynch protocol with progesterone was the best method for synchronizing estrus in local cows, with a pregnancy rate of 57%. The addition of progesterone to the CoSynch protocol decreases the number of premature ovulations that often are observed between the first GnRH injection and the PGF2 $\alpha$  injection, and thus helps to increase the pregnancy percentage at induced estrus (Kouamo et al., 2022). Furthermore, progesterone intake during terminal follicular growth tends to decrease the risk of embryonic mortality between days 32 and 60 after AI (Stevenson et al., 2015; Kouamo et al., 2024).

# Educational level of farmers and limited resources

Despite its many advantages, AI is not yet widely used, and is still limited to the Northwest and Adamawa regions. In sub-Saharan Africa, and particularly in Cameroon, the educational level of farmers, the potential of available resources (cultivated or natural pastures, rain distribution, water availability, ecological environment, etc.), the low success rate of AI (81% of the farmers), the cost of the technology (79% of the farmers), and the sex ratio in favor of males after AI operations (50% of the farmers) (Table II) are factors that partially explain why the use of modern reproductive techniques (estrus synchronization and AI) are not applied on a commercial scale (Kouamo *et al.*, 2019). Most farmers operate on a small scale, with limited financial resources, inadequate infrastructure, and inconsistent access to information. Kouamo *et al.* (2009) reported that the cost of AI after estrus synchronization using PRID- PGF2α-eCG for 12 days in Senegal was CFA 33,797 (51.5 EUR).

## Problem of semen conservation

The main constraints identified by AI technicians were semen conservation (100%), negligence of breeders (100%), and unavailability of semen (50%) (Kouamo *et al.*, 2019c). Liquid nitrogen is widely used as a cryoprotectant in semen storage due to its extremely low temperature of -196 °C. By rapidly cooling and keeping the semen at such low temperatures, the vitality and motility of sperm cells are maintained for a long time. Unfortunately, the availability and cost

of liquid nitrogen for transporting semen, especially in rural areas in Cameroon, remain limiting factors. Researchers must develop new methods for preserving bovine semen without using liquid nitrogen. A success story has been described in Bambui (Cameroon), where a team overcame this limitation by developing a chilled semen processing methodology using egg-yolk and coconut water in which sperm can survive for up to seven days. The initial average motility is around 75%, and decreases to 60%, but the motility is still sufficient for insemination purposes. AI is being conducted when farmers report cows in heat after estrus synchronization (IAEA, 2010). In addition, Kaneko et al. (2014) developed and proposed a new preservation method that enables sperm from wild animals to be stored for a long time in a refrigerator at 4 °C. Sperm are freeze-dried in a solution containing 10 mM Tris and 1 mM EDTA. Using this method, liquid nitrogen is not required for the storage and transportation of sperm. Furthermore, freeze-dried sperm can be easily and safely transported worldwide at room temperature. In addition, it is possible to preserve samples at room temperature for short periods without the need for liquid nitrogen or dry ice (Kaneko et al., 2014). Sperm preservation is useful for applying assisted reproduction and we should continue researching this topic in order to overcome this constraint.

# The use of sexed semen

To solve the problem of the sex ratio, AI technicians may suggest to breeders the use of sexed semen. There are currently two commercial approaches that exploit the differences in DNA content between X and Y spermatozoa. After quantifying the fluorescence, the cells are either sorted into X and Y fractions, or the spermatozoa of the undesired sex are ablated (Kouamo & Kharche, 2014a; Faust et al., 2016). Today, millions of doses of sexed semen are produced annually from thousands of sires worldwide (Hasler, 2023). In many countries, there has been a rapid increase in the sale of sexed semen over the past 6 to 7 years. The increased use of sexed semen has several benefits for the cattle industry. In dairy herds, when genetically superior females of the herd are inseminated with sexed semen for replacement heifer production, average to low-ranked females can then be inseminated with beef semen. This has marked consequences for the economy of dairy producers and for animal welfare. The welfare consequences of using sexed semen are both direct and indirect. The direct consequences include reduced dystocia and stillbirth through the use of X-sorted semen (Norman et al., 2010). Indirect consequences arise from the increased use of beef semen, driven by the sexing of semen in dairy farming. This helps reduce the production of surplus male dairy calves and improves calf welfare (Crowe et al., 2021). Semen sexing technology should be used in dairy farms in Cameroon to accelerate genetic gain and increase sustainability.

**Table II:** Constraints related to the adoption of artificial insemination (AI) and accompanying measures desired by farmers in the Adamawa, North, and Far North regions of Cameroon /// Contraintes liées à l'adoption de l'insémination artificielle et mesures d'accompagnement souhaitées par les agriculteurs des régions de l'Adamawa, du Nord et de l'Extrême-Nord du Cameroun

| Variables                          | Modalities            | Adamawa (%) | North (%) | Far North (%) | Total (%) |
|------------------------------------|-----------------------|-------------|-----------|---------------|-----------|
| Breeder constraints on adopting Al | High cost             | 25 (46.3)   | 8 (14.8)  | 10 (18.5)     | 43 (79.6) |
|                                    | Poor success rate     | 27 (50)     | 8 (14.8)  | 10 (18.5)     | 45 (83.3) |
|                                    | *Bad Phenotype        | 0 (0)       | 0 (0)     | 2 (3.7)       | 2 (3.7)   |
|                                    | Sex ratio             | 13 (24.1)   | 6 (11.1)  | 9 (16.7)      | 28 (51.9) |
| Assistance measures wished         | Subvention            | 30 (55.6)   | 10 (18.5) | 12 (22.2)     | 52 (96.3) |
|                                    | Veterinary assistance | 14 (25.9)   | 4 (7.4)   | 6 (11.1)      | 24 (44.4) |

Kouamo et al., 2019. \*Some breeders, particularly those in the Far North region of Cameroon, remained attached to phenotypic traits and in particular prefer white animals with horns /// Certains éleveurs, en particulier ceux de la région du Grand Nord du Cameroun, restent attachés aux caractères phénotypiques et préfèrent notamment les animaux blancs à cornes

# Future challenges

There are many constraints that hinder the improvement of the zootechnical performance of inseminated cows in Cameroon. The survival and good growth of the products are therefore affected. The establishment and sustainability of the development of this livestock will depend largely on the future of crossbred animals within the production environment. This environment is characterized by the availability of feed resources, the prevalence of various pathologies to which they may be more susceptible than local breed animals, the technical skills of agro-pastoralists, the availability and access to extension services, and the financial resources available to producers to bear the investment costs related to intensification. AI operations should be considered as a component of a strategy for intensifying milk production through a change in animal management methods. These methods are based on stabling, adequate coverage of the animals' feed and health needs, and proper management of cow reproduction. To achieve this, it is necessary to strengthen the capacities of producers and extension agents in the field of animal production intensification. Crossbreeding should be viewed as a genetic improvement strategy, and should be part of a global strategy for developing milk production. This requires planning with clearly defined objectives regarding the number of crossbred cows, expected production, genotype (degree of blood desired), the crossbreeding scheme to be used, and the marketing of production. A good crossbreeding program should include a choice of exotic breeds that are compatible for crossbreeding with local varieties, such as Gudali, White Fulani, and Red Fulani. It also should include a genetic crossbreeding scheme tailored to each breed and its environment, based on the management of a "selection core to be determined". This scheme should be constituted based on criteria developed in a participatory manner, and detail related costs. Additionally, the program should outline the management methods for animals included in the crossbreeding initiative, provide a minimum kit of infrastructure and equipment for participating breeders, and establish a plan to enhance the AI capabilities of the managers and staff involved. Finally, a monitoring plan should be implemented to oversee the program's execution throughout its duration.

# ■ EMBRYO PRODUCTION

# General consideration

Over the past three decades, embryo technologies have played a key role in supporting farm animal breeding programs, and thereby increasing the productivity of livestock. Cattle are the most important species for the world embryo industry, representing 95.2% of all embryos collected or produced in 2022, followed by sheep (2.0%), horses (1.8%), and goats (0.9%) (Viana, 2024). Moreover, embryo cryopreservation facilitates the movement and marketing of bovine germplasm, enabling the safe worldwide movement of livestock. The importance of embryo technologies will continue to rise. Somatic cell nuclear cloning and the production of gene-edited animals are both technologies that depend on the production of embryos in the laboratory and can contribute to the genetic improvement of livestock (Goszczynski et al., 2023). The potential of embryo technologies to enhance genetic improvement and fertility has been restricted by inefficient methods for producing and transferring embryos. The possibilities presented by transferable embryos, combined with the existing challenges to optimizing their use, suggests that research aimed at improving embryo technologies should be a national priority for countries where cattle production plays a significant role in the economy (Hansen, 2024). There are widening opportunities to rethink the technological basis for many current practices related to the production and transfer of embryos. There have been explosive advances in bioengineering fields, such as microfluidics, three-dimensional printing of cell culture materials, organoid culture, live-cell imaging, and cryopreservation. Moreover, artificial intelligence will certainly have a role in embryo technologies. Examples of emerging technologies include microfluidics (Alkan *et al.*, 2023; Ferraz & Ferronato, 2023), artificial tissues (Gargus *et al.*, 2020), three-dimensional culture (Ferraz & Ferronato, 2023), non-invasive assessment of cellular function (Sciorio *et al.*, 2022), time-lapse imaging (Magata, 2023), and new advances in cryopreservation (Table I) (Pomeroy *et al.*, 2022). Even a technique as central to the field as transcervical embryo transfer could be re-engineered to avoid possible damage to the reproductive tract (Hansen, 2024). Embryos can be produced *in vivo* or *in vitro*. The advantages and disadvantages of *in vivo* and *in vitro* embryo production in cattle are summarized in Table III.

# In vivo embryo production

In vivo embryo production in cattle can be achieved through multiple ovulation and embryo transfer (MOET). Most embryo transfers in cattle are performed after a hormonal treatment to stimulate superovulation in donor cows, thereby maximizing the number of embryos recovered during the collection process. In cattle, there are two generally accepted methods of superovulation. One method consists of administering a single injection (2,000–2,500 U, IM) of equine chorionic gonadotropin (eCG), typically on day 10 of the estrus cycle (with day 0 being defined as the day cows are observed in estrus), followed 2–3 days later by two injections of prostaglandin F2alpha (dinoprost or cloprostenol) 12–24 hours apart. The second method is to induce superovulation through serial administrations of follicle-stimulating hormone (FSH). FSH is typically administered IM over 4–5 consecutive days, twice daily in decreasing doses. A typical 4-day FSH treatment protocol is as follows: day 1, 4 ml every 12 hours; day 2, 3 ml every 12 hours; day 3, 2 ml every 12 hours; day 4, 1 ml every 12 hours (total volume = 20 ml). Treatments typically begin on day 10 after estrus. On the third or fourth day of FSH treatment, a luteolytic injection of prostaglandin F2alpha is administered and repeated 12 hours later. Estrus can be expected 36-48 hours later, and the AI can be scheduled based on the observed heat (12 and 24 hours after the onset of estrus) or by appointment (72, 84, and 96 hours after the administration of PGF2alpha) that is accompanied by the administration of an agent to trigger ovulation (e.g., human chorionic gonadotropin or gonadotropin releasing hormone). Embryo collection is done on day 7 of the cycle when uterine stage embryos (morula and blastocysts) are expected to be recovered.

In general, MOET allows the production of good quality embryos, (i.e., embryos that have a strong capacity to develop to term after being transferred to synchronized recipients), and high cryotolerance (i.e., ability to survive to the freezing and thawing procedures) (Table III). However, this method is expensive and the number of embryos produced is often highly variable and unpredictable due to the donor effect (Galli, 2001). To our knowledge, no study on this technique has been reported in Cameroon.

## In vitro embryo production

In vitro embryo production (IVEP) is another method that has the potential to enhance the multiplication of superior bovine germplasm. This technique effectively uses sexed semen technology and genomic selection indices at the embryonic stage to produce calves with high genetic potential. Basically, in vitro embryo production involves several phases. The first step involves the collection of oocytes, which can be obtained either from abattoir derived materials or from live animals through the Ovum pick-up (OPU) technique. Next, good quality oocytes are selected and incorporated into the in vitro maturation system. Following this, the sperm and matured oocytes are prepared for in vitro fertilization, and the embryos obtained are cultured up to the expanded blastocyst stage of development.

**Table III:** Advantages and disadvantages of *in vivo* and *in vitro* embryo production /// Avantages et inconvénients de la production d'embryons in vivo et in vitro

|               | In vivo embryo production  | In vitro embryo production   |
|---------------|--|--|
| Advantages    | <ul> <li>Relatively low fixed costs are required for MOET.</li> <li>Higher quality of <i>in vivo</i> produced embryos, as evidenced by higher implantation rates after transfer and cryosurvival.</li> <li>Good pregnancy rates.</li> <li>Few embryo/fetal.</li> </ul> | <ul> <li>- A significant increase of embryos from high genetic value females because oocytes can be recovered from prepubertal (2 to 8 months), pregnant (up to 3 months) and even dead or slaughtered cows.</li> <li>- Provides an excellent source of low cost embryos for basic research, embryo biotechnology studies (nuclear transfer, transgenesis, embryo sexing and stem cells) and all kinds of embryo research which need large numbers of embryos for manipulation.</li> <li>- Used as a strategy for the rescue of some endangered animal species by interspecific embryo transfer.</li> <li>- Frequency of collections: every 2-3 weeks.</li> <li>- A reduction in the semen required for embryo production.</li> </ul>  |
| Disadvantages | <ul> <li>Frequency of collections: every 35-60 days for MOET.</li> <li>Requires the administration of hormones to stimulate follicular development and the subsequent stimulation of luteolysis.</li> </ul>  | <ul> <li>- High investment: requires specific laboratory expertise and equipment.</li> <li>- The cow is the incubator for <i>in vivo</i> embryo production. It requires a large number of embryos to cover fixed costs.</li> <li>- Lower pregnancy rates.</li> <li>- More embryo/fetal losses: <i>In vitro</i> derived embryos usually have darker coloration, a lack of compactness of the cellular mass, alteration in the ratio of the inner cell mass to trophoblast cells, lower total cell number, greater mixoploidy, and alterations in gene expression and cell metabolism. These alterations may be involved in the low rate of embryo cryosurvival and phenotypic disorders observed in fetuses and offspring derived from <i>in vitro</i> produced embryos.</li> </ul> |

### Oocyte collection

■ ANIMAL PRODUCTION AND ANIMAL PRODUCTS

To set up the techniques and produce large quantities of average genetic merit embryos, significant amounts of material can be obtained at low cost by collecting ovaries from slaughterhouses. These ovaries are shipped to the laboratory (dry or in warmed saline) where the contents of the follicles are aspirated or sliced. The type of needle used and the aspiration vacuum are important factors in determining the number and quality of the oocytes collected. In cows, good results can be achieved using an 18-gauge needle connected to a 3 cm Hg vacuum. Alternatively, the ovaries may be sliced with a razor blade and washed with phosphate buffered saline (PBS) to collect the oocytes. An average of 5 to 10 oocytes can be collected from each bovine ovary (Mermillod et al., 1992). After aspiration or slicing, the collected fluid is screened under a stereomicroscope to select the oocytes. Only cumulus oocyte complexes (COCs) with complete and compact cumulus investment should be selected (Figure 1). Oocytes also can be collected from live females through the OPU technique in cattle. OPU is mainly done by transvaginal aspiration of follicle content under ultrasonographic control.

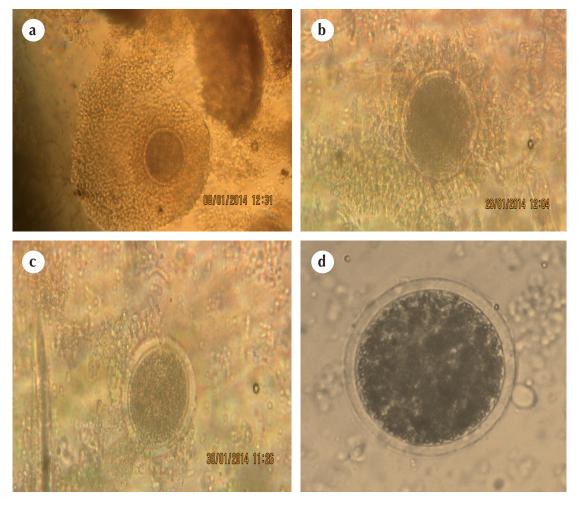
In Cameroon, several studies have been conducted on the ovarian potential of local cattle for in vitro embryo production. Much of the research was done using slaughterhouse ovaries, and the use of OPU procedures guided by ultrasound to collect oocytes from both fertile and infertile genetically valuable cows has not yet become widespread. To our knowledge, the first study on IVEP in Cameroon was done by Kouamo et al. (2014c). An abattoir study was conducted to evaluate the ovarian potential of 201 local zebu cattle from Ngaoundere, Adamawa region (Cameroon) for IVEP. The results indicated an average of 16.75±0.83 follicles per ovary (Table IV). The small, medium, and large follicles were 8.39±0.60, 8.14±0.43 and 0.21±0.02, respectively. Oocyte recovery was 10.97±0.43 per ovary (65%) using the slicing technique. Oocytes graded I, II, III, and IV were 3.53±0.19 (32.21%), 2.72±0.15 (24.82%), 2.24±0.15 (20.43%), and 2.47±0.20 (22.54%), respectively (Figure 1). Oocytes with a quality (grade I and II) acceptable for in vitro maturation constituted 57.15% of the harvest. The ovarian potential of local cows for in vitro embryo production remains average in Cameroon (Kouamo et al., 2014c) (Table IV).

#### In vitro maturation

In vitro maturation of oocyte is an ART that enables oocytes to be matured ex vivo. It is the second step of in vitro embryo production. Like in vivo, both nuclear and ooplasmic maturation is required to ensure normal fertilization and embryo development in vitro. The maturation of oocytes is a complex biological process where the primary oocytes resume meiosis, progress from the diplotene stage of prophase of the first meiotic division to metaphase of the second meiotic division with the breakdown of germinal vesicle, the formation of meiotic spindle, the extrusion of the first polar body and the expansion of surrounding cumulus cells. The maturation rate of oocytes in vitro is assessed by various methods, such as staining the oocytes (M-II stage), identifying the extruded first polar body in the perivitelline space, and evaluating the degree of expansion of cumulus cell mass (Kouamo & Kharche, 2014b). Oocytes are matured in TCM 199 supplemented with 10% serum in the presence of gonadotrophins at 38.5 °C in 5% CO<sub>2</sub> for 24 hours. Researchers are using FSH doses ranging from 0.1  $\mu$ g/ml to 10  $\mu$ g/ml, LH doses ranging from 3  $\mu$ g/ ml to 100  $\mu$ g/ml, and estradiol doses of up to 1  $\mu$ g/ml to determine the developmental potential of in vitro produced embryos. Gonadotropins are present because they support the maturation of oocyte and induce major changes in their protein profiles. Commercial eCG is less expensive than FSH and LH. Kouamo and Kharche (2014b, 2017) reported that the supplementation of 20 IU/ml eCG and 20 IU hCG in maturation media significantly increased the maturation rate. Another approach to improving oocyte maturation in vitro involves adding to the culture medium specific cell signaling ligands that play a role in maturation in vivo. These ligands include amphiregulin, insulin-like growth factor 1, estradiol, progesterone, androstenedione, neuroregulin 1, and natriuretic peptide C (Hansen, 2024). Zhang et al. (2023) reported that C-X-C motif chemokine ligand 12 acts on oocytes during maturation to increase the percent that become blastocysts following fertilization or parthenogenetic activation.

### In vitro fertilization of oocytes

Fertilization involves the activation of the oocyte by the sperm, which results in the union of male and female gametes and the formation of pronuclei and zygote. *In vitro* matured oocytes are co-incubated



**Figure 1:** Quality of oocytes. (a) Quality 1: oocyte surrounded by a compact cumulus with more than three layers and presenting a homogeneous ooplasm; (b) Quality 2: oocyte surrounded by a compact cumulus with one or two layers and presenting an ooplasm with an irregular appearance, with a darker zone visible at its periphery; (c) Quality 3: the cumulus has a layer of irregular and less compact cells and the ooplasm is less regular with darker areas; (d) Quality 4: The cumulus is fully expanded or even absent (naked oocytes) and the ooplasm is irregular with dark areas (Kouamo et al., 2014). /// Qualité des ovocytes. (a) Qualité 1: ovocyte entouré d'un cumulus compact à plus de trois couches et présentant un ooplasme homogène; (b) Qualité 2: ovocyte entouré d'un cumulus compact à une ou deux couches et présentant un ooplasme d'aspect irrégulier, avec une zone plus sombre visible à sa périphérie; (c) Qualité 3: le cumulus présente une couche de cellules irrégulières et moins compactes et l'ooplasme est moins régulier avec des zones plus sombres; (d) Qualité 4: le cumulus est complètement expansé, voire absent (ovocytes nus) et l'ooplasme est irrégulier avec des zones sombres (Kouamo et al., 2014)

**Table IV:** Ovarian potential of local cattle for in vitro embryo production (IVEP) in Cameroon /// Potentiel ovarien du bétail local pour la production d'embryons in vitro au Cameroun

| References            | Breed of animals                            | Number of animals | Average number of follicles/ovary | Average number of oocytes/ovary) | Selected oocytes for IVEP<br>Grades (G) I and II/cow<br>(%) | Oocyte index* |
|-----------------------|---|-------------------|-----------------------------------|----------------------------------|---|---------------|
| Kouamo et al. (2014)  | White Fulani, Bokolo,<br>Red Fulani, Gudali | 201               | $16.7 \pm 0.8$                    | 10.9 ± 0.4 (n = 4,411)           | 6.2 ± 0.3 (57.15%)  | 2.2           |
| Kouamo et al. (2015)  | Gudali                                      | 81                | 18.0±1.0                          | 11.9±0.7 (n=1929)                | 13.8±0.8 (58%)  | -             |
| Kouamo et al. (2017c) | Gudali, Red Fulani,<br>White Fulani, Bokolo | 353               | 13.6±0.9                          | 5.0±4.1 (n=3581)                 | 9.0±5.5 (58.28%)  | 2.3           |
| Kouamo et al. (2018)  | Gudali, Red and White<br>Fulani, Bokolo     | 496               | 18.7 ± 12.6                       | $7.8 \pm 5.8$ (n = 7687)         | $8.6 \pm 6.5 (55.1\%)$                                      | 2.5           |
| Azafack et al. (2019) | Gudali, Red and White<br>Fulani             | 95                | $13.9 \pm 3.4$                    | $9.5 \pm 0.4  (n=1822)$          | 11.3 ± 1.0 (59.13%)   | 2.3           |
| Azafack et al. (2020) | Gudali, Red and White<br>Fulani             | 195               | $17.0 \pm 6.5$                    | $7.6 \pm 0.4  (\text{n}=3045)$   | 9.2±0.3 (60.09%)  | 1.0           |

<sup>\*</sup> The overall quality of oocytes was calculated as an index using the formula [(G I x 1 + G II x 2 + G III x 3 + G IV x 4) / Total number of oocytes recovered]. Index values close to 1 reflect good quality oocytes /// La qualité globale des ovocytes a été calculée sous forme d'indice à l'aide de la formule suivante : [(G I x 1 + G II x 2 + G III x 3 + G IV x 4) / Nombre total d'ovocytes récupérés]. Les valeurs proches de 1 reflètent une bonne qualité des ovocytes.

with frozen thawed in vitro capacitated spermatozoa in a fertilization medium (Brackett and Oliphant (BO) medium or Tyrode's modified medium) for 24–48 hours at 38.5 °C in 5% CO<sub>2</sub>. In vitro capacitation of bull spermatozoa is carried out by incubating motile spermatozoa separately, using either the swim-up or Percoll gradient techniques, using frozen thawed semen in media containing sperm motility enhancers such as a combination of heparin and caffeine or calcium ionophore (Kouamo & Kharche, 2015a). While intracytoplasmic sperm injection (ICSI) has not been widely implemented in commercial bovine IVF programs, IVF still provides opportunities to use relatively low numbers of sperm to produce viable embryos. This allows for the utilization of high value semen and may provide significant opportunities when coupled with gender separated semen. Given the variability in the ability of sperm to support embryonic development, one strategy for improving outcomes of in vitro fertilization is to devise new methods for selecting sperm for fertilization. The selection of sperm based on rheotaxis, for example, increased the proportion of cleaved embryos that developed into blastocysts compared to oocytes from sperm isolated through centrifugation (Yaghoobi et al., 2024). Another strategy is to mimic sperm-oviductal interactions that occur in vivo. The co-culture of sperm, oviductal epithelial cells and oocytes in a system called "oviduct-on-a-chip" resulted in a reduced incidence of polyspermy and parthenogenesis (Ferraz et al., 2017).

# Culture of embryos in vitro

Current techniques for culturing farm animal embryos from the 1-cell stage to early blastocyst seem to fall into one of two categories. The first category involves a co-culture system using a complex tissue culture medium with various cell types such as oviductal epithelial cells, granulosa cells, and even cells of a tissue culture cell line such as BRL cells. A significant amount of effort to improve these media has concentrated on the type of co-culture cells. The second category uses a cell-free system based very often on a simple medium such as synthetic oviduct fluid (SOF) supplemented, to varying degrees, with amino acids, vitamins, serum, and other components. Although many types of culture media have been developed independently, three formulations serve as the foundation for most cattle culture media used today: SOF, Charles Rosenkrans medium with amino acids (CR1AA), and potassium simplex optimized medium (KSOM), each developed using slightly different approaches. Typically, SOF is used as a sequential medium, whereas CR1AA and KSOM are often used as a single-step medium (Kouamo & Kharche, 2015b; Krisher & Herrick, 2024). The addition of oviductal fluid (Lopera-Vasquez et al., 2017) or several specific growth factors whose gene is expressed in the oviduct or endometrium (activin A, colony stimulating factor 2, C-X-C motif chemokine ligand 12, hepatoma-derived growth factor, insulin-like growth factor 1, WNT5A and WNT7A) can increase the percentage of in vitro produced embryos that develop to the blastocyst stage (Hansen, 2024). Embryos at the blastocyst stage are at a level of development that is suitable for both transfer and cryopreservation.

# Factors influencing embryo production in Cameroon

Overall, the potential of local cattle ovaries for *in vitro* embryo production remains average (55 to 60% of oocytes grades I and II) in Cameroon. However, by acting on certain factors such as diet, the season, and the oocyte collection technique, it is possible to obtain a better yield of oocytes that can be selected for *in vitro* maturation, *in vitro* fertilization, and *in vitro* culture.

In our context, many factors affected the follicular population, yield, and quality of oocytes.

# Effect of ovarian factors (ovarian localization, corpus luteum, size and weight of ovary) on the follicular population, oocyte number, and grade

The effect of ovarian factors on the follicular population and oocyte yield of local zebu cows has been studied. The right ovaries are heavier and offer more numbers of follicles and oocytes than the left ones (p < 0.05) (Azafack et al., 2020). In all domesticated ruminants, the right ovary is usually more active than the left. Local paracrine and autocrine factors and differences in lymphatic drainage between the right and left ovaries may contribute to the observed variation in their activities (Habibizad *et al.*, 2021). The right ovaries are also more functional in ruminants due to the presence of the rumen, which reduces blood supply to the left ovary, and consequently GnRH. The yield and quality of oocytes from follicles increased with the weight (g) and size (cm) of the ovaries. The ovaries with corpus luteum had more medium (3-8 mm) follicles (Kouamo *et al.*, 2014).

# Effect of non-ovarian factors (breed, age, body condition, physiological status, and pregnancy length) on the follicular population, oocyte number, and grade

The follicular population and oocyte recovery rates were higher in cows that were under 10 years old with a BCS of 3, non-pregnant, or carrying a fetus in the first trimester of pregnancy (p < 0.05) (Kouamo *et al.*, 2014).

# Effect of nutritional parameter on follicular population and oocytes recovery rate

Local zebu cows with a BCS of 4-5 and high levels of albumin and phosphorus had the best follicular population and oocyte yield. In Cameroon, the BCS is a useful tool for selecting female oocyte donors for IVEP. The BCS had a positive influence on the follicular population, consistent with other findings (Alves et al., 2014). In fact, a negative energy balance may have direct consequences on the hypothalamic-pituitary axis in a cow by reducing the secretion of pituitary gonadotropins (FSH) responsible for follicular development. Basal folliculogenesis is essentially controlled by growth hormones such as Insulin Growth Factor-1 (IGF-1). During an energy deficit, follicular growth slows due to a decrease in concentrations of IGF-1. High plasma levels of IGF-1 resulting from improved nutrition increases the sensitivity of granulosa cells to FSH stimulation. In addition, our results indicated that serum phosphorus levels could be an important supplementary indicator to distinguish cows with good quality oocytes. The cows selected should not suffer from undernutrition because this could adversely influence oocyte yields (Kouamo et al., 2015; Azafack et al., 2019).

# Effect of harvesting technique, metabolic profile, season, and stage of the sexual cycle on follicular dynamics, quality, and oocyte yield

The slicing technique allows the best yield of oocytes acceptable for IVEP (Kouamo *et al.*, 2017). Saleh (2017) reported that slicing methods produce a higher number of oocytes with moderate quality and moderate embryo production, while aspiration methods yield a moderate number of oocytes with higher quality and good embryo production. The dry season negatively affects follicular population, oocyte yield, and metabolic parameters of local cattle. Elevated environmental temperatures negatively affect a cow's ability to display natural mating behavior, as it reduces both the duration and intensity of estrous expression. Heat stress alters endocrine patterns and reduces follicular development and oocyte quality (Polsky & von Keyserlingk, 2017). Estrus and præstrus are the optimal phases of the sexual cycle for the emergence of large follicles and good quality oocytes, respectively (Kouamo *et al.*, 2017, 2018; Azafack *et al.*, 2020).

# Evaluation of bovine (Bos indicus) ovarian potential preserved at 5°C for in vitro oocyte production

Oocytes can be easily recovered from ovaries collected at the slaughterhouse. The ovaries taken postmortem from animal carcasses in slaughterhouses are the largest source of primary oocytes obtained at low cost for the large-scale production of bovine embryos in vitro. Ovaries should be transported to the laboratory within 1 hour in Dulbecco's PBS at a temperature between 35 and 37 °C in order to preserve the developmental competence of oocytes. At low temperatures, the metabolic activities of cells are known to slow down or stop completely. In contrast, cellular autolysis can occur in ovaries during a long period of transportation at high temperatures. Many authors have reported, in several species, that ovary storage at 38 °C for several hours decreased the rate of blastocyst formation (Fukui et al., 1991; Soler et al., 2005). In our context, many oocytes are lost due to the distance between research laboratories and slaughterhouses, and the poor condition of roads and vehicles increases the transport time. Our results indicated that the ovaries of zebu cows could be preserved at 5 °C for 9 hours without any significant change in biochemistry, yield, or the quality of selectable oocytes for in vitro maturation (Kouamo et al., 2019a).

# Constraints related to the development of embryo technologies in Cameroon

Since the birth of the first IVEP calf in 1982, cattle IVEP has made significant progress. However, some residual shortcomings are still limiting the larger commercial use of this promising technique in Cameroon.

# Laboratory equipment

In vitro embryo production requires the presence of an incubator that maintains a controlled temperature and gas atmosphere, and laboratory equipment including microscopes, a laminar flow hood, analytical balances, hemostats (or clamps), graduated cylinders and embryo filters, a slide warmer, centrifuges, pipettes, and sterile consumables such as filters, straws, media and other instruments for handling oocytes and embryos. Unfortunately, reproductive biotechnology laboratories in Cameroon are not currently equipped to handle the different phases of *in vitro* embryo production (IVM, IVF and IVC), and this constitutes one of the limiting factors to the use of this technology on a large scale.

### Cost of the technology

The high cost of embryo production and transfer is the main disadvantage. The cost for hormones and drugs is approximately €172 per donor, and €85.8-257.5 for the flush procedure. Transfer fees per embryo vary between €81 for non-surgical procedures to €128 for surgical ones. The cost of providing good quality recipients should not be overlooked. This averages out to around €257-429 per recipient (Ross, 1992). When modeling the expected costs for an embryo produced and transferred, the expenses can reach nearly €1,717, when the probable fertility is only 10%. However, when the probable fertility is 60%, the cost of the embryo is close to €257. This technology seems to be viable on average or high-scale systems, showing a superovulatory response of 60 to 80% with 4-6 transferrable embryos. Yet in small-scale farming, due to the reduced number of donors and/or recipients, the costs surpass the economic feasibility of the technique (Sanchez et al., 2015). Moreover, the low rates of embryo production and survival prevent this technique from becoming widely adopted.

# Very low number of qualified personnel

Embryo transfer represents the second generation of reproductive biotechnologies following AI. Bovine embryo transfer takes place after embryo production, either through embryo flushing or *in vitro* fertilization (IVF). Most embryo transfers in cattle are performed after a

hormonal treatment to superovulate donor cows and to maximize the recovery of embryos during the collection procedure. Both surgical and non-surgical methods of embryo transfer can be effective. Under most circumstances, non-surgical transfer is greatly preferred. Nevertheless, the main challenge with non-surgical transfer is the difficulty in mastering this technique. It is necessary to be able to palpate ovaries accurately in order to select the side of ovulation. Pregnancy rates are markedly reduced if embryos are transferred to the uterine horn contralateral to the corpus luteum. Recipients also should be rejected if there is no corpus luteum present or if any pathology of the reproductive tract is noted. All of this requires specific knowledge and skills. Embryo transfer requires trained personnel for successful implementation. To date, it is still not widely used in Cameroon, despite its many advantages. The very low number of qualified personnel is a significant limiting factor. Kathambi et al. (2025) reported that many AI and embryo transfer programs in Africa have failed due to this issue. An emphasis should be placed on the continuous training of staff on the latest advances in assisted reproduction technologies.

# Potential development prospects

Cattle breeding in Cameroon is characterized by a largely traditional herd management system, limited technical skills in embryo technologies (both at the level of breeding farms and selection/dissemination institutions), and a lack of laboratory equipment for embryo production. Given this context, MOET, *in vitro* embryo production, and other embryo technologies such as cryopreservation of oocytes and embryos, embryo sexing, embryo splitting, and embryo cloning should not be considered at this stage as solutions of interest for the genetic improvement of cattle in the country. Nevertheless, this technology should be considered in a research component. The research could help reduce the gap with other countries, train highly qualified personnel, equip laboratories with cutting-edge technology, finance research in embryo technology, and produce embryos at a lower cost in order to carry out large-scale embryo transfer in Cameroon.

# ■ ETHICAL ISSUES

ARTs are attracting increasing interest and promise radiant solutions to the various concerns and aspirations of scientists and society. They are fundamental for cattle breeding and sustainable food production. Along with genomic selection, these technologies help reduce the generation interval and accelerate genetic progress. However, we must remain vigilant and exercise caution regarding certain aspects of these manipulations of genetic material because unpleasant surprises could compromise the entire future of humanity. Modern reproduction technologies may decrease agro-biodiversity, raising concerns from both an anthropocentric and an ecocentric environmental ethics standpoint. Moreover, reproductive technologies such as genetic modification may affect the ecosystem more directly. Technology and society interact, and technologies carry with them implicit moral codes (Swart, 2014). Many countries have adopted moratoriums or laws in response to concerns about the potential implications of ARTs for life and biodiversity. Unfortunately, in Cameroon, there is no law regulating the manipulation of embryos and genes of domestic and farm animals. There is an urgent need for stakeholders in Cameroon to formulate cultural and context-specific guidelines to help address some of these ethical concerns. Cameroon needs to develop and update policies and guidelines for introducing, implementing, and utilizing new breeding technologies. National and local governments should lead these efforts, supported by stakeholders such as private institutions and organizations. These policies and guidelines should outline requirements for integrating breeding technologies into existing and new programs, preventing technological overlap in specific regions, and facilitating efficient monitoring and evaluation (Kathambi et al., 2025).

### ■ CONCLUSION

Several reproductive technology tools have been developed for cattle. In Cameroon, estrus synchronization and fixed-time AI are the most widely used and contribute significantly to the development of cattle production by accelerating genetic gain and increasing the value of bovine livestock. The ovarian potential for *in vitro* embryo production is average and research must continue on IVM, IVF, and IVC to assess the number and quality of blastocysts. Embryo transfer and other embryo technologies are not yet widely used. National and private institutions must focus on equipping laboratories and training qualified personnel to stay up to date with new ARTs. In so doing, they will be able to benefit from the various advantages that these technologies offer for improving animal production in Cameroon.

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### **Conflict of interest**

The author declares that there is no conflict of interest.

## **Author contributions**

JK: Manuscript design and writing.

### **Ethics approval**

Approval from an ethics committee regarding the use of animals was not necessary for this study because data were collected from previously published sources.

# **Declaration of Generative AI in the writing process**

The author did not use any artificial intelligence-assisted technologies in the writing process.

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### Résumé

Kouamo J. Techniques de reproduction assistée au Cameroun

Contexte : Les techniques de reproduction assistée comprennent l'insémination artificielle ; le transfert d'embryons ; le transfert d'embryons à ovulation multiple ; la synchronisation des chaleurs et la superovulation ; le prélèvement d'ovocytes par laparoscopie, la maturation in vitro (MIV), la fécondation in vitro (FIV) et la culture in vitro (CIV) collectivement appelés production d'embryons in vitro (PEIV), injection intracytoplasmique de spermatozoïdes, cryoconservation de spermatozoïdes, cryoconservation d'ovocytes et d'embryons, sexage des spermatozoïdes et bissection d'embryons, division embryonnaire, clonage d'embryons, transfert nucléaire, transfert de gènes et sélection assistée par marqueurs. Les technologies émergentes comprennent la microfluidique, l'impression tridimensionnelle de matériaux de culture cellulaire, la culture d'organoïdes, l'imagerie de cellules vivantes, les nouvelles avancées en matière de cryoconservation et l'intelligence artificielle. Objectif: Cette étude vise à faire le point sur les technologies de reproduction assistée au Cameroun axées sur les bovins. Résultats: Au Cameroun, plusieurs méthodes ont été utilisées pour synchroniser l'œstrus chez les bovins, utilisant des progestagénes (PRID<sup>ND</sup> Delta et CIDR-B), des prostaglandines (PGF) et de la GnRH. L'ajout de progestérone au protocole CoSynch améliore la fertilité des vaches locales. Depuis la première insémination artificielle réalisée en 1942 avec de la semence fraîche, de nombreux projets d'amélioration génétique utilisant l'insémination artificielle à temps fixe avec de la semence congelée ont amélioré la génétique des races locales, avec un taux de gestation global de 49,5% (32,8-57). Les principales contraintes pour l'insemination artificielle au Cameroun sont la disponibilité et le coût de l'azote liquide pour le transport de la semence, notamment en zones rurales. Plusieurs études ont été menées sur le potentiel ovarien des bovins de races locales pour la production d'embryons in vitro, et une grande partie de ces recherches a été réalisée sur des ovaires d'abattoir présentant un potentiel de 55 à 60 % d'ovocytes sélectionnables pour la production d'embryons in vitro (grades I et II) par la technique de decoupage en tranches. Conclusions: L'utilisation des procédés de collecte d'ovocytes guidée par ultrasons à partir de vaches de haute valeur génétique fertiles et infertiles, la MIV, la FIV, la CIV et d'autres techniques de reproduction assistée (production d'embryons in vivo, transfert d'embryons, division d'embryons, clonage, production d'animaux transgéniques et technologies émergentes) ne sont pas encore popularisées au Cameroun. Il est urgent que les parties prenantes au Cameroun élaborent et mettent à jour des politiques et des lignes directrices pour aider à répondre aux préoccupations éthiques concernant les techniques de reproduction assistée.

*Mots-clés* : bovin, maîtrise de la reproduction, synchronisation de l'œstrus, insémination artificielle, transfert embryonnaire, Cameroun

### Resumen

Kouamo J. Técnicas de reproducción asistida en Camerún

Contexto: Las técnicas de reproducción asistida comprenden: inseminación artificial; transferencia embrionaria de ovulación múltiple; sincronización de celos y superovulación; extracción de ovocitos por laparoscopia, maduración in vitro (MIV), fecundación in vitro (FIV) y cultivo in vitro (CIV), colectivamente llamados producción de embriones in vitro (PEIV); inyección intracitoplásmica de espermatozoides, crioconservación de espermatozoides, crioconservación de ovocitos y de embriones, sexado de espermatozoides y bisección de embriones; división embrionaria, clonación de embriones, transferencia nuclear, transferencia de genes y selección asistida por marcadores. Las tecnologías emergentes comprenden: microfluídica, impresión tridimensional de materiales de cultivo celular, cultivo de organoides, imaginería de células vivas, nuevos avances en materia de crioconservación e inteligencia artificial. Objetivo: Este estudio tiene como objetivo proporcionar información actualizada sobre las tecnologías de reproducción asistida centradas en los bovinos del Camerún. Resultados: En el Camerún se utilizaron varios métodos para sincronizar el estro en los bovinos: mediante progestágenos (PRID<sup>ND</sup> Delta y CIDR-B), prostaglandinas (PGF) y GnRH. Añadir progesterona al protocolo CoSynch mejora la fertilidad de las vacas locales. Desde la primera inseminación artificial realizada el 1942 con esperma fresco, numerosos proyectos de mejora genética mediante inseminación artificial en tiempo fijo con esperma congelado mejoraron la genética de las razas locales, con una tasa de gestación global del 49,5 % (32,8-57). Las principales limitaciones para la inseminación artificial en el Camerún son la disponibilidad y el coste del nitrógeno líquido para el transporte del esperma, especialmente en las zonas rurales. Se llevaron a cabo varios estudios sobre el potencial ovárico de los bovinos de razas locales para la producción de embriones in vitro, y una gran parte de estas investigaciones se realizó en ovarios de matadero que presentaban un potencial del 55 al 60 % de ovocitos seleccionables para la producción de embriones in vitro (grados I y II) con la técnica de corte en láminas. Conclusiones: El uso de procedimientos de extracción de ovocitos guiada por ultrasonidos a partir de vacas de elevado valor genético fértiles y estériles, MIV, FIV, CIV y otras técnicas de reproducción asistida (producción de embriones in vivo, transferencia de embriones, división de embriones, clonación, producción de animales transgénicos y tecnologías emergentes), todavía no se han popularizado en el Camerún. Es urgente que las partes interesadas en el Camerún elaboren y actualicen las políticas y líneas conductoras para ayudar a responder a las preocupaciones éticas que afectan a las técnicas de reproducción asistida.

**Palabras clave**: ganado bovino, reproducción dirigida, sincronización del celo, inseminación artificial, transferencia de embriones, Camerún