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Chapter

Folliculogenesis, Fertility and Biotechnology in Dairy Cattle

Marcelo M. Seneda, Sofia B. Delchiaro, Amanda F. Zangirolamo, Amauri A. Alfieri and Fabio Morotti

Abstract

The ovarian follicle population is formed by thousands of follicles, preantral and antral, where oocytes are included. During fetal life, the first follicles produced are preantral, and, as they undergo the development process, they reach the final stage of antral follicles, where a cavity/or antrum is developed. All this growth phase is called folliculogenesis, and this chapter will abord the most important aspects of this process. Moreover, not all follicles reach the preovulatory phase and can be fertilized, so we will discuss how reproductive biotechniques can positively influence the fertility of bovine females. We will also discuss the possibility of antral follicle count to influence reproductive performance and the correlation to biotechniques. Finally, we present alternatives on how to improve fertility and productive efficiency in dairy herds.

Keywords: dairy cattle, folliculogenesis, antral follicle count, fertility, embryo production

1. Introduction

Dairy farming plays an essential role in the global socioeconomic scenario, being one of the most traditional rural activities and fundamental for agribusiness. Milk is one of the most complete and most consumed food globally, in addition to providing a social function, generating thousands of direct and indirect jobs throughout the production chain. The development of new technologies should add more efficiency to the milk production chain, a constant challenge for several sectors involved in the segment.

The use of animal reproduction biotechnologies has contributed to the increase in animal productivity and has been one of the main responsible for the increase in milk production. The current scenario is the search for a model capable of providing high production efficiency concerning animal welfare without harming the environment and with the most advanced reproductive techniques for obtaining pregnancies and genetic improvement. In this way, ovarian physiology is a key aspect to contribute to the efficiency of dairy production.

The ovarian follicular population is characterized by the total amount of follicles present in the ovary. Each follicle contains an oocyte, so it is known that there are a great number of oocytes in the ovary. However, only a small portion of the ovarian follicles undergo ovulation. Therefore, the ovarian follicular reserve is an important

indicator of fertility in cattle, which may influence the applicability of reproductive biotechniques.

This chapter addresses the mechanism of folliculogenesis and the most recent research. It also brings discussions on how reproductive biotechniques can influence fertility in dairy cattle.

2. Oogenesis and folliculogenesis

The origin of the female reproductive system is still in embryonic life in the sublumbar region located caudally to the kidneys. The primordial germ cells, which will give rise to the germline formation, originate in the proximal epiblast and then move from the yolk sac to the gonadal ridges through the mesentery, around day 30 of germinal development [1]. At this moment, the Müller and Wolff ducts are still present, which will give rise to the female and male reproductive tract, respectively.

After the colonization of the ridges, around 35 days of gestation, the differentiation process begins by specifying the somatic cells of the ridge, where Sertoli cells will originate from the XY chromosome, and the granulosa cells will originate from the XX chromosome. There is an involution of Wolff's duct (or mesonephros) and development of Müller's duct (or paramesonephric) in the escarpments. In males, as they inherit the testicles determining factor (TDF) from the Y chromosome, Sertoli cells release the anti-müllerian hormone (AMH) and inhibit the development of Müller's ducts [2, 3]. After this process, they are formed as oogonia that through mitotic and meiotic divisions form a nest of oogonia in a tubular shape, and then a process of differentiation into oocytes begins [4].

Gonadal structures called germline cysts are elevated in the ovigerous cords and surrounded by a basement membrane shortly after colonization of the gonadal ridges by primordial germ cells. Meiotic divisions are initiated until the process is stopped in meiosis prophase I when primary oocytes are already formed. The primary oocytes are surrounded by a layer of undifferentiated pregranulosa cells [5]. The interruption of meiosis can last for years until a given follicle enters the growth process, resuming meiosis and continuing the follicular development through the primordial follicle until its final stage in the antral follicle [6].

It is known that folliculogenesis depends on interactions between the somatic cells of the follicle and the oocyte, so the communication between the granulosa and theca cells with the oocyte is essential for follicular development and growth to occur [7, 8]. The passage from the primordial follicle to the primary follicle is a transition phase and is characterized by the action of specific growth factors for each stage of folliculogenesis [9]. In bovine species, the so-called follicular growth waves correspond to a stimulus for the recruitment of preantral follicles.

Once the primordial follicle is recruited, whose granulosa cells are flat, it becomes the primary follicle and there is a transition between the flat cells to the cuboidal-shaped granulosa cells [9]. At this stage, the zona pellucida appears, which will remain around the oocyte throughout the follicle's development. Continuing to grow, the secondary follicle is constituted when the granulosa cells multiply and form two layers of cubic morphology, in addition to the emergence of the first theca cells [10].

The growth of these secondary follicles (when they reach approximately 4 mm in diameter) is regulated by the follicle-stimulating hormone (FSH), which has its receptors in the granulosa cells. When they reach a larger size (approximately 7–9 mm in diameter), they start to be controlled by luteinizing hormone (LH). At this stage, the follicle is already characterized as tertiary and has LH receptors in the theca cells that are already entirely organized, and the formation of the follicular antrum can be observed [11, 12]. The phases of follicular development are shown in **Figure 1**.

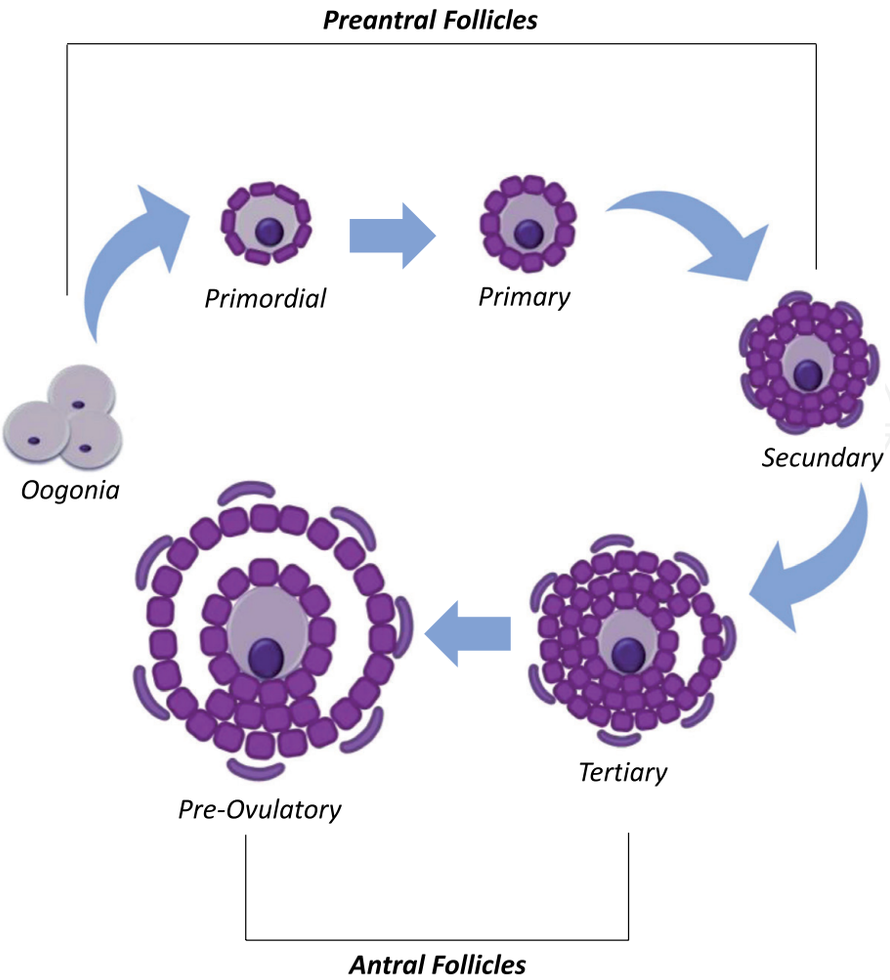


Figure 1.
Schematic sequence of complete follicular development.

3. Ovarian follicular population

The follicular population may vary between individuals, and some factors such as genetics, breed, age, species, and hormone levels can influence the number of follicles present in the ovaries [13]. For bovine females, it is estimated that the number of follicles at birth is about 235,000 [14].

An increase in the number of antral follicles present in the ovary and stimulated by gonadotrophin secretion is influenced by body development in heifers [15]. Endocrine activity at first seems to be controlled by suppressing negative feedback mechanisms until the heifer has a good body condition to initiate the estrous cycle and reproduction activities [16–18].

The first ovulation in heifers is marked by a gradual increase in LH secretion, which leads to the development of the antral follicles and the secretion of estrogen. At birth, antral follicles are not typically present in the ovaries, and the number of follicles increases when heifers reach 2 months of age. After that, the number of follicles declines at 5 months of age, and some changes continue to occur throughout the productive life of the female [19–21].

4. Antral follicle count and fertility in dairy cattle

Ovarian follicular population is highly variable among species, a concept that is well established [20–22] and has been already reported in cattle [23]. In recent

years, numerous studies have focused on the ovarian follicular population and its influence on reproductive activities, as well as on animal reproduction biotechniques [22, 24]. The antral follicle count (AFC) is a strategy to identify different profiles of cows, performing transrectal B-mode ultrasonography and counting all follicles larger than 3 mm [25]. The total number of follicles counted in the pair of ovaries is added up, and the cow is classified as low, medium, or high AFC. A feature in cattle is the high variability of AFC between animals, but it is known that there is high repeatability in the same individual [19, 26, 27]. The appearance of the ovary on ultrasound examination of cows with high and low AFC is presented in **Figure 2**.

Furthermore, according to [28], it has already been established that the concentrations of anti-müllerian hormone (AMH), which is released by the granulosa cells of growing ovarian follicles, are positively related to the entry into puberty of bovine females. In other words, the higher serum concentrations of this hormone are, the higher AFC will be [27]. AMH is a glycoprotein that belongs to the TGF-β growth factor family [29] and it is correlated with follicular growth [30]. AMH is in the granulosa cells and it is responsible for the growth of preantral and antral follicles [31] and follicular growth modulator through the control of ovarian follicular reserve depletion [32].

The intrafollicular AMH expression increases until the follicle reaches 5 mm in cows and then decreases as the follicle reaches the antral stage and increases in size [31]. The positive correlation of AMH with the ovarian follicular population has already been described in previous studies [33, 34]. Thus, the measurement of AMH can be a method of predicting AFC [35]. In *Bos taurus taurus*, *Bos taurus indicus*, and *taurus* × *indicus* crosses, animals with a high plasma concentration of AMH present a greater number of antral follicles than those with a low concentration of this hormone [36].

Reproductive biotechniques, such as embryo transfer (ET) and IVEP, depends on the population of antral follicles present in the ovary of donor females to succeed. Among other factors that interfere with ET and IVEP, it is important to mention genetics, breed, and age [37, 38]. High AFC bovine females have been described to have a greater number of viable embryos produced *in vivo* per animal [39–41]. Similarly, in IVEP—ovum pick-up (OPU) procedures, high AFC animals resulted in a higher rate of blastocyst production than low AFC females [24, 42].

In contrast, a high conception rate was observed after the use of TAI in low AFC females *Bos taurus indicus* compared to high AFC animals [43, 44]. Additionally,

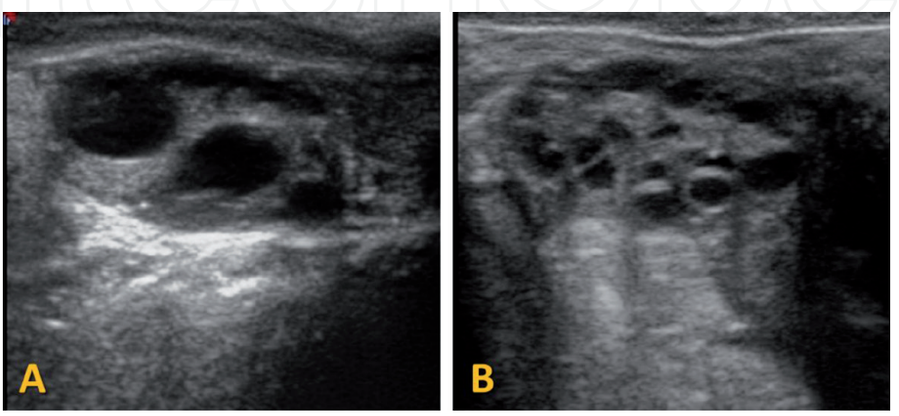


Figure 2.
Aspect of the ovary on ultrasound examination of cows with different counts of antral follicles (AFC). On the right (A) cow with low AFC, and the left (B) cow with high AFC. Images were generated via the transrectal route with the equipment model S8v (SonoScape®) with a frequency of 8.6 MHz and a linear transducer of 5–10.0 MHz.

other authors [45] observed that high AFC *Bos taurus taurus* females had lower fertility and shorter reproductive life than females with low AFC, but contrasting data have been reported [46].

Donors with a high number of antral follicles have been selected, mainly for OPU-*in vitro* fertilization (IVF) procedures, due to the quantitative advantages for producing *in vitro* embryos. Because of the high number of calves generated from IVF, there is an increasing interest in studying the relationship between AFC and reproductive characteristics.

In summary, several studies have tried to verify the influence and the correlation of AFC in the reproductive performance of bovine females. So far, it is not possible to establish the role of AFC in fertility parameters due to the controversial results. Although it is quite predictable that AFC may be related to reproductive efficiency, a better understanding of the subject is necessary. Furthermore, considering basic research, it is necessary to elucidate some aspects of follicular physiology that remain unknown [47].

5. Reproductive biotechniques and fertility in dairy cattle

Increasing the productive efficiency of a herd is one of the great challenges for dairy cattle farming. In the past, genetic selection programs sought essential characteristics for increasing milk production, with effective gains in milk quantity and quality, but reproductive efficiency was disregarded. In recent years, several works have been presented to increase milk production and increase reproductive performance, a key association for efficient dairy farming.

Considering the importance of a sustainable, intensive, and economically viable production system, achieving the reproductive efficiency of the dairy herd is crucial for the effects on profitability by the number of offspring produced, genetic progress, and the shorter interval between lactations. This is a great challenge, as there is low heritability between production and reproduction traits. Therefore, the crucial importance of precisive reproductive assistance is highlighted, providing maximum production efficiency in the smallest possible area and respecting the aspects of animal comfort.

5.1 *In vitro* embryo production (IVEP)

Despite the rapid development of the technique since its emergence in the late 1980s, until recently, IVEP was used only as a last resource when traditional techniques failed. However, the high genetic gains provided to the herds, obtaining a greater number of pregnancies concerning *in vivo* production, and lower costs due to high productivity have contributed to making IVEP the first choice in many dairy farms [48].

Holstein cows typically have lower oocyte production when subjected to IVEP. However, it is possible to obtain good results by performing a pre-selection of females with a high number of antral follicles using ultrasound. It is important to highlight that non-lactating females often have a higher number of follicles and oocytes.

Until a few years ago, some obstacles prevented the large-scale use of IVEP in dairy cattle. One of them was the large number of calves born from unwanted sex (male), which significantly increased the production cost. Another difficulty was the distance, often thousands of kilometers, between the laboratories and the properties where the recipient cows were located. The inefficiency of cryopreservation techniques for IVP embryos, especially when dealing with *Bos indicus* embryos,

limited their production and transfer connection. Thus, discarding untransferred embryos was a common practice.

These two major obstacles have now been overcome, making large-scale *in vitro* embryo production a reality. Some researchers [49] reported an IVEP program in which over 20,000 dairy embryos were produced with sexed semen (female). Embryos were transported through two or three days during the *in vitro* culture period using portable incubators. In just over a year, 8000 female calves were produced, with an average pregnancy rate of 39%.

In addition, some alternatives can be employed to improve the methods that assist in the recovery of better-quality oocytes and a higher competence in OPU to obtain more interesting results in embryo production. In this context, the follicular wave synchronization before OPU and consider the influence of the antral follicle population seem to be good alternatives [50].

5.2 IVEP and artificial insemination (AI)

Dairy European breeds, such as Holstein and Jersey, suffer great discomfort under high temperatures and high humidity conditions. Therefore, failures in cyclicity and the demonstration of estrus occur, making management difficult and compromising AI results. Furthermore, it is known that embryos are naturally more resistant to heat stress than gametes, which can suffer degeneration and further reduce pregnancy rates in the summer [51]. The transfer of embryos 7 days after fertilization avoids the harmful effects before this period, providing more advantageous rates than AI [52–54]. For dairy cattle, therefore, the use of transferred embryos seems to be the most viable option, especially in periods of excessive heat [55].

In AI, the number of descendants of genetically selected bulls is multiplied. IVEP, in turn, also generates descendants of females of high genetic merit, causing an even more significant impact on the improvement of a herd. Greater genetic gain is achieved in each generation with the transfer of embryos produced *in vitro* than with AI [48].

In some dairy farms, cows with better genetic potential are used as embryo donors and recipients. Thus, an efficient genetic selection from animals in the herd becomes possible. As for the economic aspect, with the number of pregnancies in IVEP, it is possible to produce embryos at affordable costs, making the embryo commercially attractive compared to semen [56]. Another advantage is the better use of high-value semen due to the possibility of fertilizing ten or more cows with a single dose.

5.3 Use of sexed semen in AI and timed artificial insemination (TAI)

The use of conventional semen, both in AI and *in vitro* fertilization, requires twice as many recipients compared to sexed semen [57]. By ensuring that almost all embryos are of the desired sex—female—the use of sexed semen significantly reduces the cost of production [58].

The most used technique for semen sexing is flow cytometry, which offers an accuracy of 85–95% [59]. However, during the sexing process, the sperm may be damaged, which might compromise their viability, reducing the fertilization potential and embryonic development [60]. The sexing process reduces sperm motility, compromising AI indices [61]. Related that the mean conception rate after AI between 2012 and 2016 was 56.9% with conventional semen and 47.3% using sexed semen. In IVEP, however, the method allows obtaining very satisfactory rates of blastocysts, with quality similar to those produced with conventional semen, since this technique requires fewer viable spermatozoa [62, 63]. Generally, the conception

rate obtained with sexed semen is 50–60% of the rates obtained with conventional semen in cows and 70–90% of conventional semen in heifers [64].

As there is a reduction in fertility using this semen, some strategies are currently suggested to improve conception rates in insemination programs that use sexed semen. First, it is recommended to use this semen in heifers and most in the first three services due to greater fertility. In AI programs with heat observation, the highest conception rates were achieved, with AI being performed between 16 and 24 h after the onset of heat [65]. Finally, in TAI programs, the best rates were achieved with semen deposition 60 h after removing the progesterone source [66].

5.4 Ovum pick up/IVEP *vs.* superovulation/embryo transfer (ET)

In the *in vivo* production of embryos, it is necessary to administer hormones so that superovulation (SOV) occurs and, subsequently, the transfer of the embryos. In Ovum Pick Up (OPU)/IVEP, however, obtaining oocytes and producing embryos do not require hormonal use. Furthermore, it is known that in *Bos indicus* animals, the number of embryos produced per aspiration session is higher than that of superovulation [67].

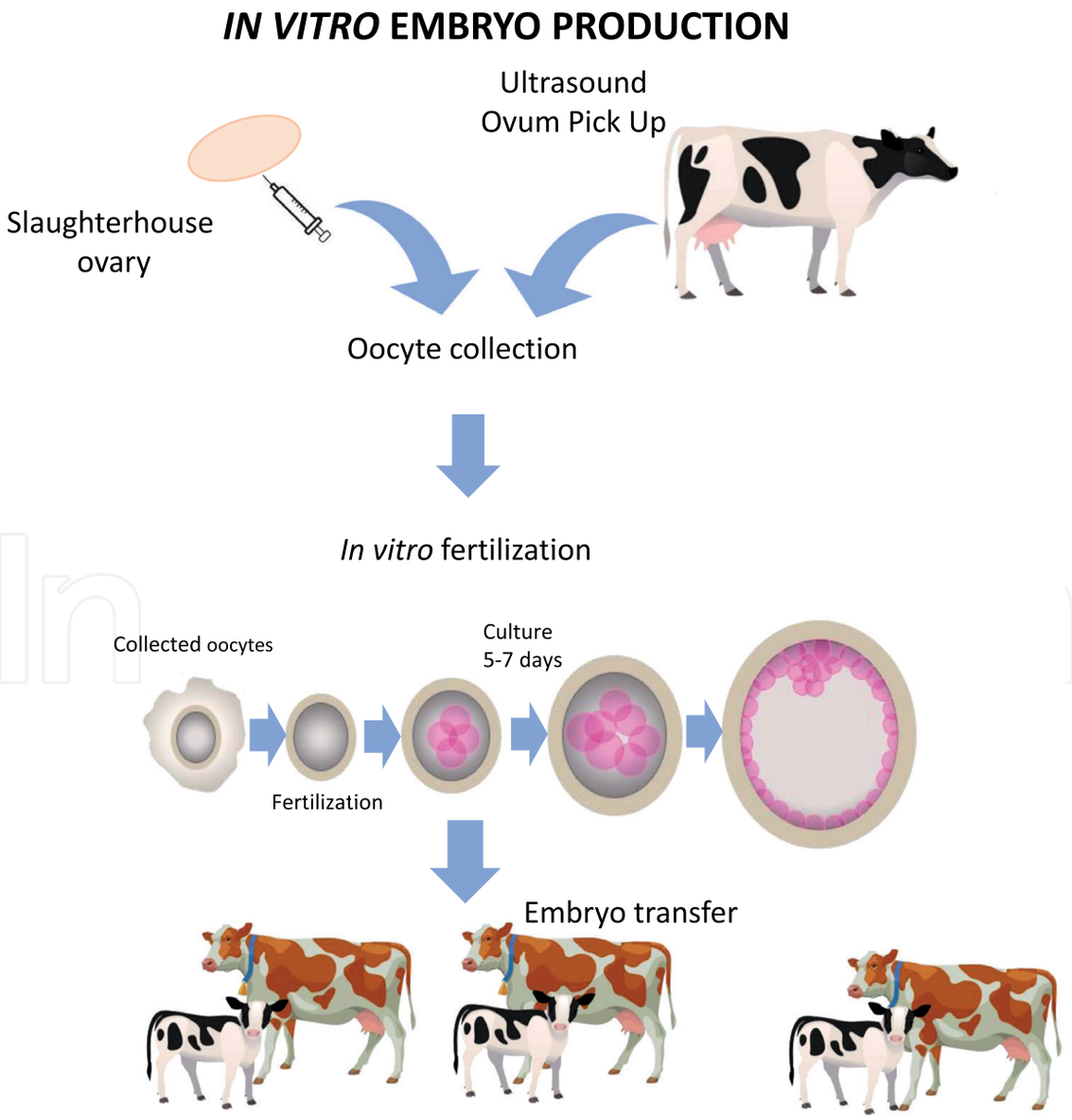


Figure 3.
Schematic sequence of steps in the in vitro *embryo production process (IVEP).*

The *in vitro* technique also allows for less spaced collections of oocytes from donors. In general, the minimum interval is 15–30 days, and there is no limit to the number of aspirations performed on the same cow [68]. On the other hand, SOV requires intervals of 40–60 days and should only be performed three or four times before a period of several months apart [69].

The production of embryos by SOV also does not allow pregnant cows, while in IVEP, this is possible. Follicular aspiration can be performed as long as the ovaries can be manipulated without being subjected to excessive traction. The process flow of *in vitro* and *in vivo* embryo production is shown in **Figures 3** and **4**, respectively.

5.5 Cryopreservation of *in vitro* produced embryos

The cryopreservation of bovine embryos generated *in vivo* has protocols very well established and effective through a freezing process. However, despite the benefits obtained and the advantages of IVF already reported in previous topics, cryopreservation represents a challenge. The low cryotolerance of IVP embryos is a limiting factor for using the cryopreservation process associated with this process. IVP embryos are more susceptible to damage caused by cryopreservation when compared to those produced *in vivo*, as they present differences in morphological, metabolic, and chromosomal aspects of their structure [70].

The greater sensitivity of these embryos to low temperatures is mainly due to the greater accumulation of lipids in the cytoplasm [71]. Lipids, made up mostly of triacylglycerols, directly affect the survival of embryos during cooling, as they can undergo irreversible changes and severely compromise development. An alternative method to promote chemical delipidation of embryos and increase cryotolerance by decreasing lipid accumulation has been related [72]. Forskolin, for example, a compost derived from the Indian plant *Coleus forskohlii*, is able to promote intracellular lipolysis in swine [72] and bovine [73] embryos. When added to the medium at strategic periods of *in vitro* culture, this substance raises embryonic tolerance to levels that provide good pregnancy rates, even in *Bos indicus* embryos [73].

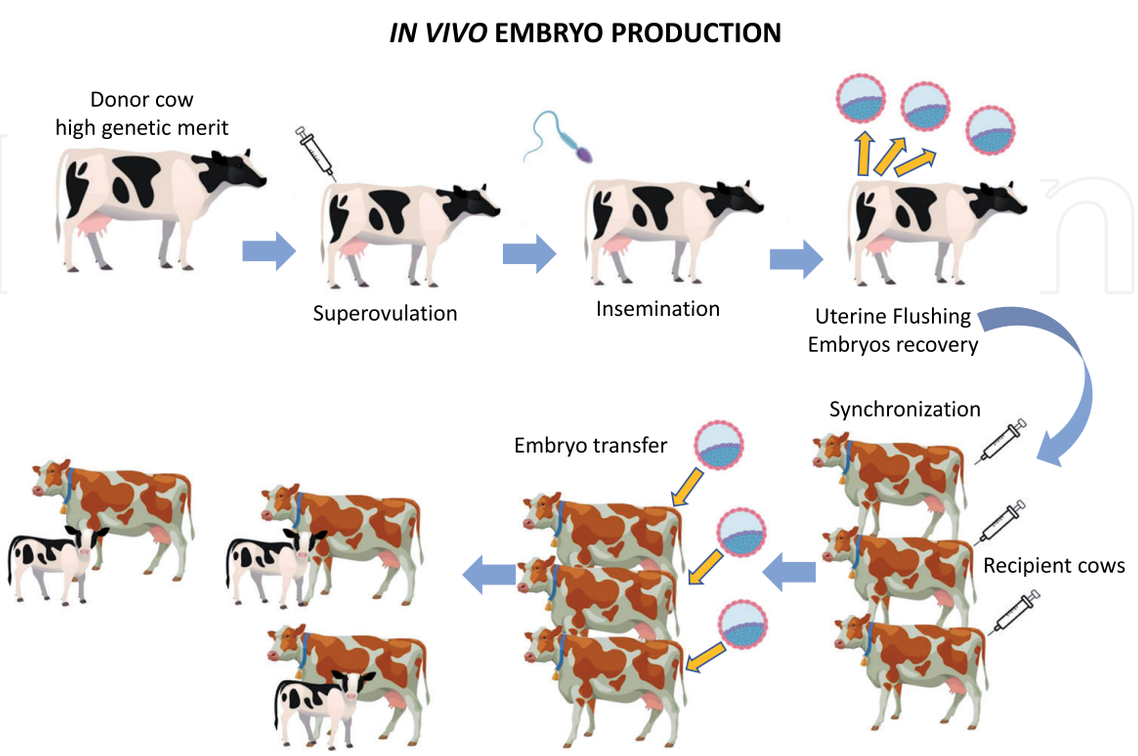


Figure 4.
Schematic sequence of steps in the in vivo *embryo production process (SOV/ET).*

Among the cryopreservation methods, vitrification is the most used technique worldwide due to the speed of the process and its low cost [74]. On the other hand, direct transfer (DT), a technique used to simplify the *in vivo* post-thawing rehydration step of embryos, has its main advantage the low concentration of cryoprotectants reducing embryotoxicity [75]. Also, DT eliminates the evaluation process before the transfer, thus becoming a more practical way than vitrification [76].

6. Challenges of *in vitro* production embryos

The *in vitro* production technique comprises a greater number of steps than those necessary *in vivo*. Thus, skilled labor is necessary so that it is possible to obtain efficient results under controlled laboratory conditions. Due to the fixed costs of laboratory equipment, materials, and professionals, the number of embryos produced determines the commercial viability of the technique [77].

Due to the metabolic and morphological differences compared to those produced *in vivo*, the pregnancy rates are lower in the *in vitro* production of embryos. Furthermore, cryopreservation and rewarming processes are more critical for IVP embryos. Therefore, the use of cryopreserved *in vitro* embryos must be very judicious. Genetic growth must be considered together with the need for an adequate herd pregnancy rate to ensure milk production on the property. The most advantageous aspect of *in vitro* produced embryos refers to the wide success of using sexed semen in this biotechnique. In the current context, the efficiency of sexed semen in *in vivo* production is unsatisfactory.

Thus, if the proposal is to associate embryo transfer and sexed semen, the best strategy at the moment is the *in vitro* production technique. The use of cryopreserved or female sexed IVEP embryos has a precise indication for donor replacement and herd genetic improvement. AI with sexed semen and embryos produced *in vivo* is equally interesting biotechniques, and there may be an association between them all to ensure milk production, reproductive efficiency, and genetic improvement.

7. Challenges of dairy farming and the contribution of reproduction to increase productive efficiency

In order to minimize the effects of early embryonic loss, the Doppler ultrasound technique has been included in reproductive programs. This non-invasive and real-time biotechnology allows the characterization of blood perfusion of reproductive organs and tissues throughout the estrous cycle and pregnancy in cattle. One of its purposes is to accurately estimate the corpus luteum (CL) functionality for the selection of recipients and for the early diagnosis of pregnancy in TAI and TETF (Fixed Time Embryo Transfer) programs.

In addition to allowing for greater accuracy in the evaluation of the recipient, another feature of the Doppler is the diagnosis of pregnancy at 20–22 days, which is early compared to the conventional system performed at 30 days after insemination. Super-early resynchronization programs developed in heifers and cows are being introduced in dairy herds, as the reduction in the interval between two TAIs promotes gains in reproductive efficiency. Despite the correct evaluation being dependent on the experience and knowledge of the operator and the correct configuration of the equipment, the popularization of the technique is consolidated every day and presents good prospects for the future.

The current scenario of reproduction biotechnology demonstrates great potential for a sustainable increase in milk production, mainly due to the increase in reproductive and productive efficiency. Furthermore, the growth in the use of reproductive biotechniques is associated with the parallel development of a support network such as veterinarians, the pharmaceutical industry, disposable materials, equipment, and service providers. The generation of employment and the need to train human resources to meet the demand for activities are intended to provide social growth.

With the possibility of obtaining an accelerated genetic gain through the shortening of the generation interval, the use of prepubertal females, mainly in the production of embryos, has aroused great commercial interest and investment in research. The genetic potential of the female must first be evaluated in advance, that is, before total production. This is feasible thanks to progress in research with genetic markers for accurate prediction of the females that will be more efficient in milk production. It is also important to consider improving equipment for OPU (oocyte recovery by Ovum pick-up). There are currently fully adapted transducers for use in very young females. Despite the good number of aspirated follicles, a challenge in this category is the low blastocyst rate, promoting limited results in IVF.

Thus, to be viable for the use of these females, the next step is to develop protocols that improve the competence of the retrieved oocyte. Gonadotropin stimulus to increase the proportion (and size) of large follicles and synchronization of follicular waves before OPU to decrease immature oocytes have been investigated. A revolution in dairy farming that has become increasingly accessible is genomic selection which has significantly altered the global dairy industry. The reduction in the generation interval from 7 to 2.5 years and the reduction of costs with progeny tests were only the first benefits presented by the gene-editing biotechnique.

Silencing, altering or replacing genes that cause problems are effective strategies to increase the productive efficiency of the herd, selecting and breeding genetically superior animals. The generation gap is likely to narrow further as assessments gain wide acceptance, as genetic gains are cumulative across generations. Genetic progress is expected as continued genetic selection is implemented. Since 2009, more than one million animals have received genetic evaluations. Although these tests are carried out primarily on male animals, genotyping costs are currently economically viable. Currently, genomic selection programs are investing more in health traits (resistance to disease), reproduction, and selection for environmentally sustainable production, including reducing waste production and gas emissions.

This change of concept, which seeks longevity and animal welfare, is because, in recent years, there has been a decline in fertility and resistance in several populations, leading to a decrease in the profitability of the herds. The increase in slaughter rates, veterinary expenses, replacement costs, and reduced milk sales were just some of the consequences of the negative impact of years of selection focusing only on milk production and animal appearance. Furthermore, the adoption of a selection index, such as evaluating the quality and viability of embryos before the transfer, increases the efficiency of the process.

An example of this has been in North America, where the implementation of a genetic-based selection program for reproductive disorders is actively researched. A high and positive genetic correlation between retained placenta and metritis is being observed, implying selection of genes to improve one trait reflecting positively on the other. This demonstrates that the increased need for genomic traits for these traits contributes to the reproductive efficiency of dairy herds.

Other characteristics that have been valued in genomic tests are identifying biomarkers considered for genetic improvement, highly correlated with reproductive

performance, such as anti-müllerian hormone (AMH), and identifying relevant genes to reduce pregnancy losses. Identifying genetic markers related to the development and anticipation of the embryo and their selection to avoid embryonic losses can minimize economic damage. Another issue to be further elucidated shortly is whether genes relevant to embryonic development are positively associated with fertility traits. Estimates of the heritability of conventional reproductive traits are generally low. Even so, the progressive inclusion of genomic tests, as a routine in the field, has great potential for identifying superior animals. In the medium and long term, one perspective is that genetic improvement programs will bring consistent profitability for the dairy industry.

Genomic testing still faces challenges because a decisive outcome in the short term is unlikely. Genetic variation for economic characteristics is maintained by increased frequency of rare alleles, new mutations and changes in goals, and no selection management. Moreover, although genomic selection is being well applied at rates of genetic gain, we still know very little about the genetic structure that promotes this variation. The most relevant future challenge will probably be the incorporation of new characteristics in the selection index in breeding programs, overcoming a measurement difficulty or low heritability of them. Added to this, it is still uncertain whether traits produced over several generations emerged included in routine genomics, as gene frequencies change over time.

It is already known that the selection of some genes can directly or indirectly influence other aspects. The concern with creations called "ecologically correct" remains controversial. The inclusion of characteristics such as lower gas emissions can compromise herd productivity. It should be remembered that the increase in milk production per animal reduces the total production of residues in the atmosphere. In other words, it is something broader than simply a genetic alteration to favor an environmental issue narrowly.

Genomic testing positively changes productivity dynamics, but attention is needed to the consequences of these genetic manipulations. The pioneering application of genomic selection in cattle will lead to a series of unanticipated discoveries that could affect animals and society. An accidental finding was recently published in highly relevant research. It was discovered that two cloned bulls whose cell lineage had undergone gene editing, aiming at the characteristic of not having horns, were transgenic. The animals contained in their genome the genetic material of the bacterium used as a vector in gene editing. The Food and Drugs Administration (FDA) guarantees that intentional genomic alterations are safe for animals and anyone who consumes foods derived from them. However, there is still no universally accepted verification method for genomic editing.

Finally, with all the technological changes, the dairy herd has its premises, but the consumer market has also increased its requirements. Producers face the challenge that today there are claims for harmonic milk in ingestion (A2A2) [78], welfare for female producers, and respect for the environmental preservation area. People worldwide are looking for information about the products daily and are no longer limited to the final part of the milk production chain.

The increase in reproductive efficiency is a proposal fully adjusted to environmental sustainability. More productive herds require less area to generate more feed. Furthermore, the use of genetically improved animals according to the climatic conditions of each region prevents land competition with agriculture. As for differentiated milk production, the inclusion of bulls genotyped for the A2 allele of beta-casein accelerates aggregation of A2A2 animals in the herd.

Another critical aspect is the mandatory link between reproductive biotechnology and animal welfare. More productive animals only respond to greater reproductive efficiency if they have all vital requirements well met. Technological

innovations such as robotic milking, with the cow's autonomy about milking, signals a prospect of increased milk production with the same number of animals. A new change in concept which, adding welfare to the creation of dairy cattle will reflect positively on the profitability of producers.

8. Conclusion

All aspects of folliculogenesis remain a vast area to be studied, despite the notable progress made with previous research. It is not possible to determine the complete influence of AFC on female bovine fertility. The use of AFC as a tool to produce embryos *in vitro* and *in vivo* seems to be evident. However, further investigations need to be carried out for TAI and fertility. Despite the significant challenges of dairy farming, the development of reproductive biotechnologies, associated with the establishment of genomic analysis, has been used as a potential tool to increase dairy productivity, meet world demand, and meet the demands of the present consumer market.

Overcoming the main limitations of IVEP, together with the good results and its high applicability, has contributed to the use of biotechnique on a large scale. Thus, IVEP is no longer limited to elite animals or animals that do not respond to super-ovulation but actively contributes to the production, improvement, and profitability of dairy production.

The transfer of IVP embryos is a great strategy to reduce the cost of high genetic value semen, and it seems to be the most viable option in periods or regions of high temperatures. Thus, IVEP has benefited dairy farms of all sizes and animals of different breeds, whether *Bos taurus taurus* or *Bos taurus indicus.* However, factors such as nutrition and management must be considered before implementing this technique, as they directly influence reproductive efficiency.

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