Chapter

Bovine Embryonic Development to Implantation

Loide Valadão, Helena Moreira da Silva and Fernando Moreira da Silva

Abstract

Throughout this chapter, we will express the embryonic development from fertilization, commonly called conception, to the implantation. It is well documented that preimplantation is considered a critical period for embryo development in ruminants, in which high pregnancy loss occurs; in fact, several authors point out that 50–75% of blastocysts fail to implant. The high rate of implantation failure is one reason why pregnancy typically requires on average two ovulation cycles to achieve. Events involved in the embryo growth and survival are directly or indirectly related to cytokines, steroids, metabolites, and growth factors. When one of these compounds fails, it normally leads to the death of the embryo or fetus. As known, the period required for full development of a fetus in utero is referred to as gestation, and it is commonly subdivided into two distinct periods. The first 2 weeks of prenatal development are referred to as the pre-embryonic stage. By the end of the embryonic period, all of the organ systems are structured in rudimentary form, and the embryo shifts to the fetus from the ninth week of gestation until birth.

Keywords: bovine, oocyte, fertilization, embryonic development implantation

1. Introduction

The reproductive system of the bovine female includes several organs such as ovaries, genital pathways or tubular portion that surround the oviducts, uterus, vagina, and vulva, the attached glands, embryonic vestiges, blood vessels, and nerves. The ovaries have two differentiated portions: a medullary zone formed by connective tissue, fibroelastic and vessels (arteries, veins, and lymphatics), and nerves, all together responsible for the conservation and nutrition of the organ. The other portion is the cortical zone, which is surrounded by the germinal epithelium and the tunica albuginea within it. The follicles and corpora lutea in their different stages of development and regression are located in this last portion. In adult females, this structure of the cortical zone undergoes cyclical changes according to the regulation of the sexual cycle or of pregnancy. The female sexual organs include two functions essential to the reproduction of females, a gametogenic function, composed by folliculogenesis and oogenesis, and an endocrine function in which the main produced hormones are estrogen, progesterone, and relaxin. The ovary of the cow or heifer, relatively to its weight is small when compared to other species, has an ovoid shape and varies in size and contour at each cycle due to the projection of follicles and corpus lutea on its surface (**Figure 1**).

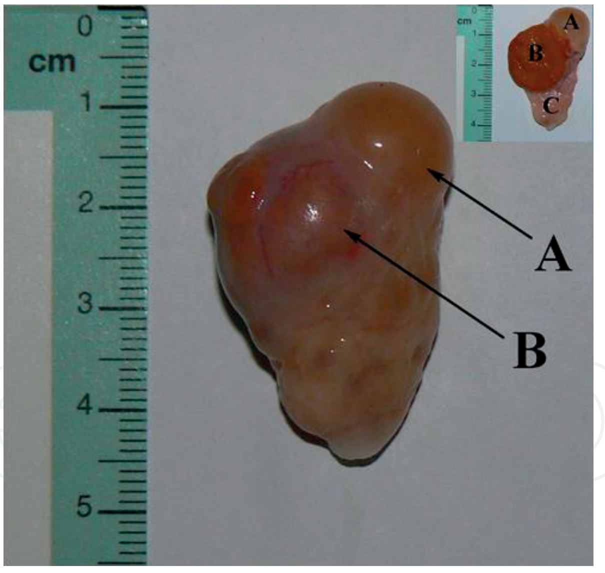

Figure 1.
The ovary where the following can be observed: A follicle (A) and a corpus luteum (B). In the upper right corner, these ovarian structures are emphasized.

Its weight ranges from 3 to 20 g and can reach 37 mm in length. In the cortical area of the ovaries, structures called follicles are present, which cyclically enter the growth phase. On average, if cow or heifer is not pregnant, a follicle grows and ovulates every 21 days. When follicles reach maturation, they rupture and release the oocyte within. The primordial follicle is an oocyte surrounded by a single layer of low follicular cells. When these cells multiply by mitosis, this is called the secondary follicle. When it reaches its maximum development, it is called the vesicular, mature, or antrum follicle. Follicular development is observed in the fetal period, in prepubescent heifers, heifers, and cyclic cows and during gestation. The oocytes are conceived from germ cells that originate the female sex cells, which contain in their cytoplasm ribosomes, mitochondria, glycogen granules, large lipid droplets and endoplasmic reticulum, and poorly developed Golgi complex. During oocyte growth, changes in the distribution, number, and size of cytoplasmic organelles occur [1]. The oocyte is surrounded by the zona pellucida (**Figure 2**), which is a dense membrane with multiple functions, being primordial for the normal development of the follicle [2].

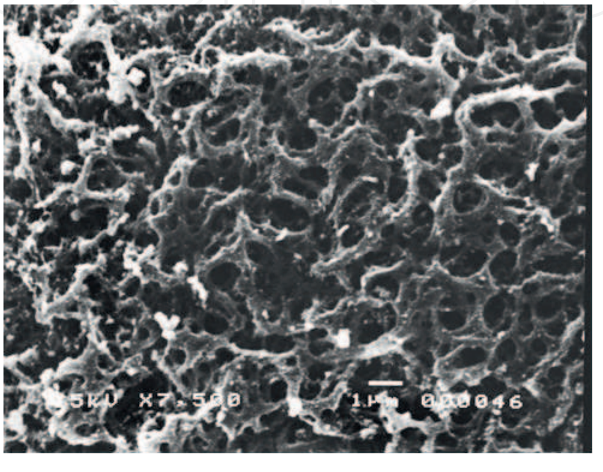

Figure 2.
Fine detail of a bovine oocyte zone pellucida captured by transmission electron microscopy. The bar represents 1 μm.

2. Oocyte maturation and ovulation

During the evolution of the oocyte, the nucleus that had entered the prophase of the first meiotic division will support the reductive divisions. Two daughter cells appear that contain half of the chromosome load in the first division, where each of the cells gets a large part of the cytoplasm, called the secondary oocyte. The smaller one is called the first polar body. Throughout the second meiotic division, the secondary oocyte divides into two (ovoid and second polar body). The corpus luteum is an endocrine gland that occurs by cycles in the ovary of females and has a short secretory activity during the sexual cycle. In the bovine species, it has an ovoid or spherical shape. Its main function is the production of progesterone, which is responsible for the preparation of the endometrium and the blastocyst for implantation. According to Gordon [3], it takes 11 days for the corpus luteum to develop and reach a 4 g weight in a female of beef cattle; in a female of milk breed, its weight is higher. The fast growth of the yellow body in the first phase of the ovulatory cycle occurs until the tenth day.

The infundibulum is a funnel-shaped tube that encloses the ovary during the ovulation. It serves to capture oocytes and channels them to the oviduct, where fertilization occurs in the presence of viable spermatozoa [4]. Finally, the uterus is a muscular, cavitary, pelvic abdominal organ and with great capacity of dilation and displacement to welcome the development of the embryo. This organ is divided into three parts, uterine horns and posteriorly, through the cranial orifice of the cervical canal, cervix which is the caudal portion of the uterus with a well-individualized structure due to its thick wall, constricted light and full of protrusions and recesses, the cervical rings [1]. The body of the cow's uterus is short and undeveloped. Its size varies with age and number of deliveries and can reach 5 cm in length. It has several functions like assisting the transport of the spermatozoa to the oviduct and helping in the expulsion of the newborn. In this organ the placenta that will allow nutrition and protection to the fetus also develops [4]. The cervix is a unique structure within the reproductive apparatus of the cow. It has thick walls and attaches the vagina to the uterus. Its main function is to protect the uterus from the external environment.

The vagina is a copulatory organ that has a thin, elastic wall that allows its distension during mating and delivery. It serves as a free passage for the calf at the time of its expulsion.

Folliculogenesis begins with the formation of primordial follicles, progressing to primary, secondary, tertiary, and preovulatory, and ends with the ovulation of a mature oocyte (**Figure 3**).

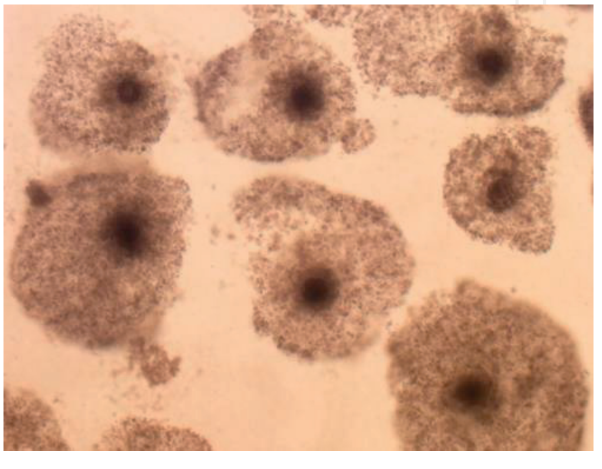

Figure 3.
Bovine typical oocyte's aspect after maturation.

That is a process of follicular formation, growth, and maturation involving the proliferation and differentiation of cells [2]. These cycles start when heifer attains puberty, but the development of oocytes and follicles begins in the mother's uterus before the calving. Primordial germ cells proliferate by mitosis to form primary oocytes; the first meiotic prophase starts between days 75 and 80 of pregnancy [5]. At the diplotene stage of meiosis (around day 170), a primordial follicle forms; the oocyte is delimited by a single layer of 4–8 pre-granulosa cells. Then, these oocytes remain in the resting phase until they are stimulated to grow [6] until ovulation or became an atresic follicle. Factors regulating formation of primordial follicles are not well known [7]. Russe [8] postulated that primordial, primary, and secondary follicles appear in the fetal ovary on days 90, 140, and 210, respectively. A secondary follicle is characterized by the addition of a second layer of granulosa cells [9], the initial deposition of zona pellucida material, formation of cortical granules within the oocyte cytoplasm, onset of oocyte RNA synthesis [10], and gonadotrophin responsiveness [7]. The transition to the tertiary follicle includes development of the theca interna and externa, the basal lamina, and cumulus cells, as well as the formation of a fluid-filled antral cavity [9]. At this stage, follicles can attain a tremendous size being impaired only by the availability of FSH, as at this time they are dependent on. The oocyte reaches the stage of metaphase II, just before ovulation. Only if the oocyte reaches this meiosis stage it can be able to be fertilized.

3. Oocyte fertilization

Fertilization is a complex sequence of events that begins with the contact of a spermatozoid with an oocyte and culminates with the mixture of the maternal and paternal chromosomes in the metaphase of the first mitotic division of the embryo. The union of the male gametes with the female gametes involves several phases. Firstly, the passage of the sperm through the radiata corona that surrounds the zona pellucida of the oocyte [11]. The movements of the spermatozoa tail are important for its penetration into the radiata corona. The most important phase of the initiation of fertilization involves the penetration of the surrounding zona pellucida to the oocyte. Then, the fusion of the plasma membranes of the oocyte and the spermatozoid occurs in which the head and tail of the same penetrate the cytoplasm of the oocyte, leaving the plasma membrane behind. After entering the spermatozoa, the oocyte that was in the metaphase of the second meiotic division completes this division, and completing this division forms a mature oocyte and a second polar body. Within the cytoplasm of the oocyte, the nucleus of the sperm increases in size, forming the male pronucleus [12]. The membranes of the pronucleus dissolve and the chromosomes condense and prepare to mitotic cell division, ending up to 24 h after ovulation [13]. Pronuclear fusion and mitosis are most easily seen with transparent eggs, with low-power microscopic magnification that the originally eccentric pronucleus moves to the center of the egg at about 20–30 min after fertilization and that the nuclear envelope disappears as the egg enters late prophase.

The first provided a description of bovine ovulated oocytes and two-cell stage embryos which was made by Hartmen and collaborators in 1931 [14], but only 15 years later, a more detailed description of developmental stages, from the unfertilized oocyte to the blastocyst, was reported by Hamilton and Laing [15]. Concerning the activation of embryonic genome, the zygote and early cleavage-stage embryo are thought to be controlled maternally hereditary by mRNA molecules until genomic activation occurs. The transition from oogenetic to embryonic genomic activation (EGA) is called the maternal-to-embryonic transition (MET) [16] and allows further embryogenesis to become dependent on the expression of the embryonic genome [17, 18]. In the bovine, the onset of MET occurs at the 8- to 16-cell stage. However, it was suggested that the onset of MET may be controlled temporally (i.e., at a time after fertilization) rather than at a developmental stage, as minor transcriptional activity was detected as early as the pronuclear stage after in vitro fertilization (reviewed by [19]).

In cattle, the gestation has a duration of approximately 282 days, being divided in three stages. In a first phase, the formation of the zygote occurs, and the implantation of the embryo begins. Then, in the second stage, the onset of trophoectodermal adhesion to the endometrium occurs, and the culmination of the embryonic differentiation period occurs when the onset of fetal bone mineralization occurs. The last stage is called the fetal phase that is between the beginning of fetal bone mineralization and moment of the expulsion of the fetus.

4. Embryo development

In the oviduct, after fertilization, while the one-cell embryos are projected toward the uterus by peristalsis and beating cilia, the zygotes undergo five or six rapid mitotic cell divisions, not increasing, however, the total volume of the conceptus. The cleavage of the zygote is defined as being repeated mitotic divisions of the zygote, which leads to a rapid increase in the number of cells (blastomeres). These are decreasing in size with each division of the cleavage. The zygote first divides into two blastomeres, and then these two cells divide into four blastomeres, eight blastomeres, and so on (**Figure 4**). This division occurs about 30 h after fertilization, followed by other divisions, forming progressively smaller blastomeres [20]. Up to the eight-cell stage, these form a cluster. After the third cleavage, the blastomeres maximize their contact with each other, giving rise to a compact cluster of cells, called compaction. Three days after the fertilization approximately, the cells of the compacted embryonic structure divide again to form 16 cells (morula). As morula embryo continues growing, these blastomeres will divide into two kinds of cells. The inner cell mass, that is, the inner cells of the morula, which will give birth to the embryo tissues, and the surrounding cells create the external cell mass that will contribute to the formation of the placenta [13]. Then once inside the uterus (about 4–5 days after fertilization), the conceptus floats freely for several more days, creating a ball of approximately 100 cells and consuming nutritive endometrial secretions called uterine milk while the uterine lining thickens, and the conceptus is referred to as a blastocyst.

Within this structure, a small amount of cells forms an inner cell mass, which will become the embryo and then the fetus. The other cells form the outer shell are called trophoblasts (trophe = "to feed" or "to nourish") and then will develop into the chorionic sac and the fetal portion of the placenta (the organ of nutrient, waste,

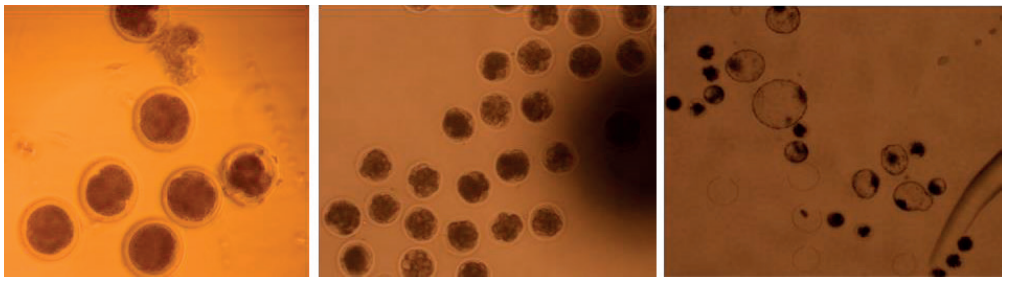

Figure 4.
Bovine oocytes after fertilization: on day 2 between two and four cells (on the left side), on day 6 as the morula stage (on the center), and on day 9 as blastocysts (on the right side).

and gas exchange between mother and the developing offspring). This mother/embryo dialog induces dynamic changes in the uterine epithelia, tightly regulated by steroid hormones, cytokines, and growth factors, which establish uterine receptivity toward the developing conceptus. The inner mass of embryonic cells is totipotent during this stage, meaning that each cell has the potential to differentiate into any cell type in the body. In a process called "hatching," the conceptus breaks free of the zona pellucida and the implantation begins. The blastocyst typically implants in the fundus of the uterus or on the posterior wall. At this time the trophoblast secretes pregnancy serum protein B (PSPB or PAG), a hormone that directs the corpus luteum to survive, enlarge, and continue producing progesterone and estrogen to suppress menses, as well as to create an environment suitable for the developing embryo. Studies developed in our department clearly showed that PAG/PSPB increases from the beginning to the end of pregnancy, reaching its maximum in the calving day [21, 22].

The cells of the inner cell mass are now an embryoblast, which are in a pole, and the cells of the outer cell mass are called trophoblast, which flatten and form the epithelial wall of the blastocyst. At this stage the embryo separates from the zona pellucida allowing the beginning of the implantation. In bovine, although the blastocyst is formed several days after fertilization, placentation starts on day 21, beginning then the implantation. The uterus upon implantation is in the secretory phase; the blastocyst is implanted in the endometrium along the anterior or posterior wall [23]. The trophoblast differentiates into a single nucleus of mitotically active cells, called cytotrophoblast, and a rapidly expanding multinucleated mass, syncytiotrophoblast, which causes erosion of maternal tissues. On the ninth day, gaps are formed in the syncytiotrophoblast. Subsequently, the maternal sinusoids are eroded by the syncytiotrophoblast, the mother's blood passes into the lacunar network, and at the end of the second week, the primitive uteroplacental circulation begins. During this time, the blastocyst is perfectly implanted and consolidated. The embryoblast is differentiated into epiblast and hypoblast, which form the bilaminar disk. Amnioblasts are lining the amniotic cavity superiorly to the epiblast layer. In turn, the hypoblast cells are continuous with the exocoelomic membrane, and together they surround the primitive yolk sac. The amniotic cavity and the yolk sac are formed from the primitive extra-embryonic mesoderm with the onset of somatopleure and splanchnopleure.

During the third week, gastrulation occurs which is the process by which the bilaminar embryonic disk is converted into a trilaminar embryonic disk (beginning of morphogenesis). Gastrulation begins with the appearance of the primitive line where the primitive node is at its cephalic end. Epiblast cells in the knot and primitive line are invaginated to form new leaflets (endoderm and mesoderm). At the end of the third week, the three basic germ leaflets in the cephalic region (ectoderm, mesoderm, and endoderm) are already demonstrated [13]. The ectoderm gives rise to organs and structures that maintain contact with the exterior, central nervous system, peripheral nervous system, pituitary gland, mammary glands, sweat glands, and tooth enamel. By the end of the fourth week, there is the production of these germ leaflets in the more caudal areas of the embryo. Differentiation of tissues, extra-embryonic membranes, and organs begins (**Figure 5**).

The trophoblast progresses rapidly. The primary villi obtain a mesenchymal core, and the small capillaries originate. When these villous capillaries come in contact with capillaries on the chorionic plaque and the attachment pedicle, the villous system is ready to provide the embryo with nutrients and oxygen [11]. During this time, embryo-maternal crosstalk remains one of the most challenging subjects in reproductive biology. The decoding of embryo-maternal interactions may allow the development of new therapeutic strategies to enhance embryonic survival,

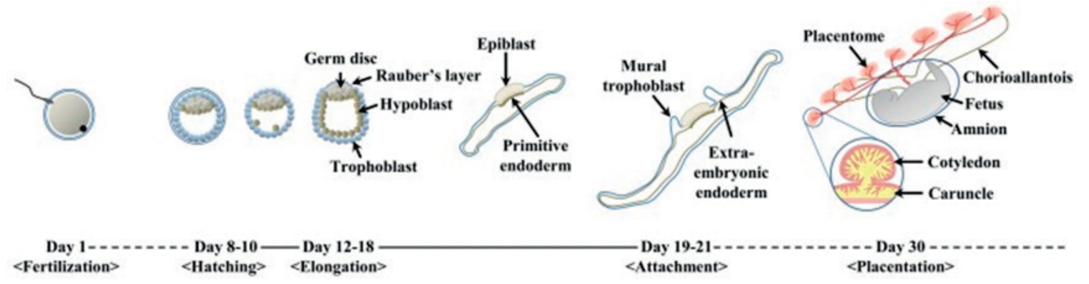

Figure 5.
Schematic development of a bovine embryo from fertilization to day 30 (adapted from [23]).

which would have a major impact in cattle reproductive efficiency and profitability of modern cattle industry providing relevant advancements in our knowledge of the determinants of normal and abnormal deviations of health.

5. Strategies to improve embryo survival

Several strategies have been designed to enhance embryo survival. Due to its unequivocal role in pregnancy establishment and maintenance, P4-based strategies have received great attention from both researchers and practitioners. Strategies designed to increase post-ovulatory peripheral concentrations of P4 include increasing peripheral levels of P4 or manipulating nutrition either to decrease plasma concentrations of E2 or inhibiting the PGF2α-synthesizing enzymatic machinery in the endometrium during the critical period [24, 25]. Hormonal manipulations to increase P4 include direct P4 supplementation [26] and administration of gonadotrophin-releasing hormone (GnRH; [27]), bovine somatotropin (BST; [28]), equine chorionic gonadotrophin (eCG; [29]), and human chorionic gonadotrophin (hCG; [30]).

6. Fetal period

The fetal period begins 9 weeks after fertilization and ends at birth. It is characterized by being a period of rapid body growth and maturation of organs and systems. At first, the fetus increases its length more rapidly than it gains weight. In the third trimester of gestation, the length increases slowly increasing rapidly in weight. The energy requirements of the fetus increase from the third trimester of gestation. Fetus size varies according to genetic factors, such as race, fetus phenotype, and other environmental factors, such as the mother's age, nutrition, and management.

7. Conclusion

Embryonic development has fascinated scientists and philosophers from ancient culture to the present day. Once fertilized, the zygote travels down the fallopian tube and mitotically divides many times to form a droplet of cells called a blastocyst. The blastocyst consists of an inner mass that develops into the embryo, while the outer layer develops into tissue that nourishes and protects the embryo. The blastocyst attaches onto the wall of the uterus and receives nourishment through the mother's blood. The major systems structures of the calf develop during the embryonic period in a process called differentiation. During this stage, kidney, brain, spinal cord, nerve, heart, and blood cells start to develop, and the gastrointestinal tract begins to form. Despite years of dedicated research, much still remains to be discovered on the formation of gametes (the sex cells), fertilization, and the subsequent development of the embryo.

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Conflict of interest

The authors declare, for all legal purposes, the absence of any conflict of interest related to this paper.

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