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Embryonic mortality in sheep: a review

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Abstract: Despite extensive research on the reproductive health of domestic animals, embryonic mortality continues to be a problem that brings about significant losses for both commercial breeding and scientific research. This review paper discusses the genetic and environmental factors that affect embryo mortality rates in sheep along with the different techniques developed to overcome this issue. Additionally, one of the objectives of this study is to emphasize the importance of the use of time-lapse cinematography and color Doppler ultrasonography as quick and reliable methods for early detection of pregnancy and embryonic death.

Key words: Embryo, mortality, sheep, time-lapse cinematography, color Doppler ultrasonography

1. Introduction

Among all mammals, embryonic death is a persisting problem. More often than not, the reasons for the occurrence of this phenomenon are unknown, as it occurs in very early stages of pregnancy or during the preimplantation period. The most common reasons are usually genetic mutations or abnormalities leading to poor embryo quality, hormonal imbalances in the mother, or environmental causes. In humans, extensive research has been done to attempt to tackle this issue; however, in sheep, our knowledge is limited. Apart from sheep breeding for meeting commercial requirements, it is also of great relevance as a model for humans and for other ruminants, and hence it is necessary to further our understanding in this field. Therefore, this review covers the latest developments in detection, control, and prevention of embryo mortality in sheep.

2. Gametogenesis and its importance with respect to embryo mortality

Primordial germ cells (PGCs) in male sheep undergo several mitotic divisions in the developing testes and remain in the quiescent state until puberty. After puberty, these germ cells begin to rapidly divide and multiply mitotically. At this stage, they are known as primary spermatocytes and are diploid in nature. This is then followed by meiotic division, which results in haploid secondary spermatocytes (Figure 1). Sertoli cells present in the seminiferous tubules then proceed to nourish and promote the growth of the spermatozoa prior to spermiation, which is the release of immature sperm into the lumen of the seminiferous tubules. From this location they are then transported to the epididymis and further [1].

On the other hand, oogenesis begins with mitotic division followed by dormancy in the prenatal stage. At birth, the oogonia proceed into their first meiotic division. Interestingly, the oogonia do not fully complete their meiotic division at birth; instead, at the stage of prophase, they enter into a dictyate phase (resting phase) until puberty. It is only after puberty, with the help of follicle-stimulating hormone (FSH) and gonadotropic hormones, that the process of meiosis is resumed. This finally results in the formation of a large haploid secondary oocyte and a smaller haploid polar body (Figure 2). This larger secondary oocyte then remains in metaphase until the penetration of a spermatozoon [2].

Gametogenesis in both males and females is quite complex in nature. Often, during the stages of mitotic and meiotic phases, due to improper separation of chromosomes or distribution of genes, abnormalities tend to occur, which later have adverse effects on embryo mortality after fertilization. Common issues are problems in gene expression and regulation, along with chromosomal aberrations such as aneuploidy, deletions, or insertions, which lead to structural imperfections in chromosomes as well [3–5].

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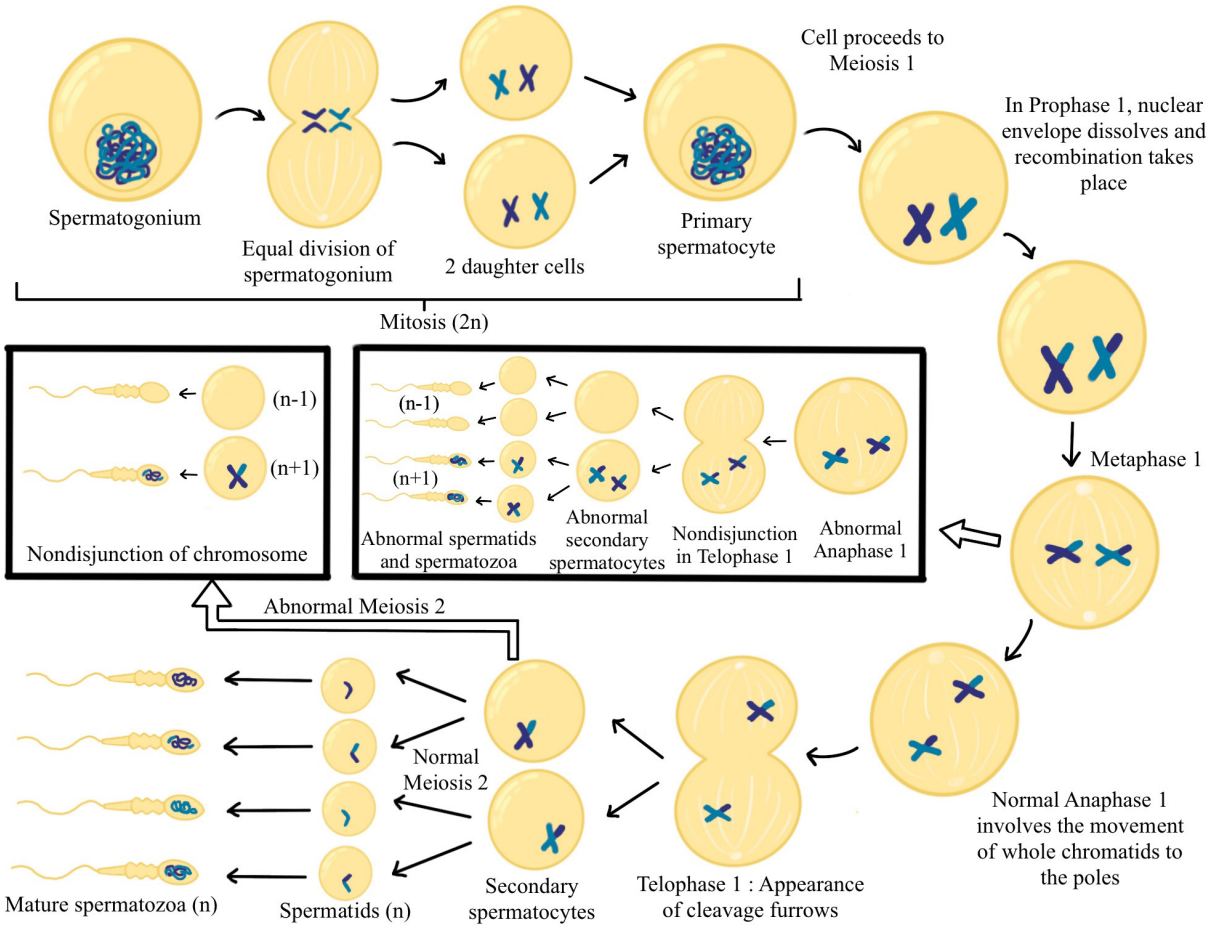

Figure 1. Normal and abnormal spermatogenesis.

3. Embryonic mortality factors

There are several genetic failures and chromosomal abnormalities that lead to embryonic death, and these rates are found to be far higher when any part of the procedure or the entire procedure is conducted in vitro [6]. It was found by Bolet [7] that embryonic mortality in sheep is usually around 30% and embryonic death most commonly occurs prior to implantation. Chromosomal abnormalities like centric fusions or reciprocal translocations are some of the reasons for this embryonic mortality. This was supported by further studies on chromosomal mosaicism by Dupont et al. [8], which showed that mosaicism occurred due to blastomere degeneration and that abnormalities were more frequent when there were hormonal imbalances in the ovaries or in the presence of poor in vitro environments. Overall, it was found that 18.2% of embryos studied were abnormal and 31.2% of these were mosaic embryos.

Coppola et al. [9] conducted a study aimed at using the ZOO-FISH model to detect such chromosomal abnormalities and to analyze the extent of chromosome deviation both in vitro and in vivo in sheep embryos. It was found that deviation from chromosomal normalcy and ploidy was far greater in the embryos produced in vitro than in the in vivo-produced ones, primarily due to mosaicism (Figure 3). Tetraploidy was found to be the most common occurrence, although polyploidy in general was found to range from 3n to 8n.

With respect to nutrition, overnourished ewes were found to have experienced twice as much embryonic loss and undernourished ewes experienced thrice as much embryonic loss than normal after the 45th day of pregnancy It was found that control ewes lost only 18% of their ova as compared to the 39% lost by the overnourished ewes and 54% lost by the undernourished ewes [10]. In another experiment by Grazul-Bilska et al. [11], maternal nutrition was tested on the quality of IVF embryos where ewes were divided into three groups: underfed, overfed, and control. It was observed that the number of cleaved embryos proceeding into the morula and subsequent stages following IVF was less among the underfed and overfed embryos in comparison to the control group. This study showed that, overall, underfeeding the ewes affected the cleavage of embryos and was found to increase the serum estradiol 17-β (E2) concentration, while overfeeding

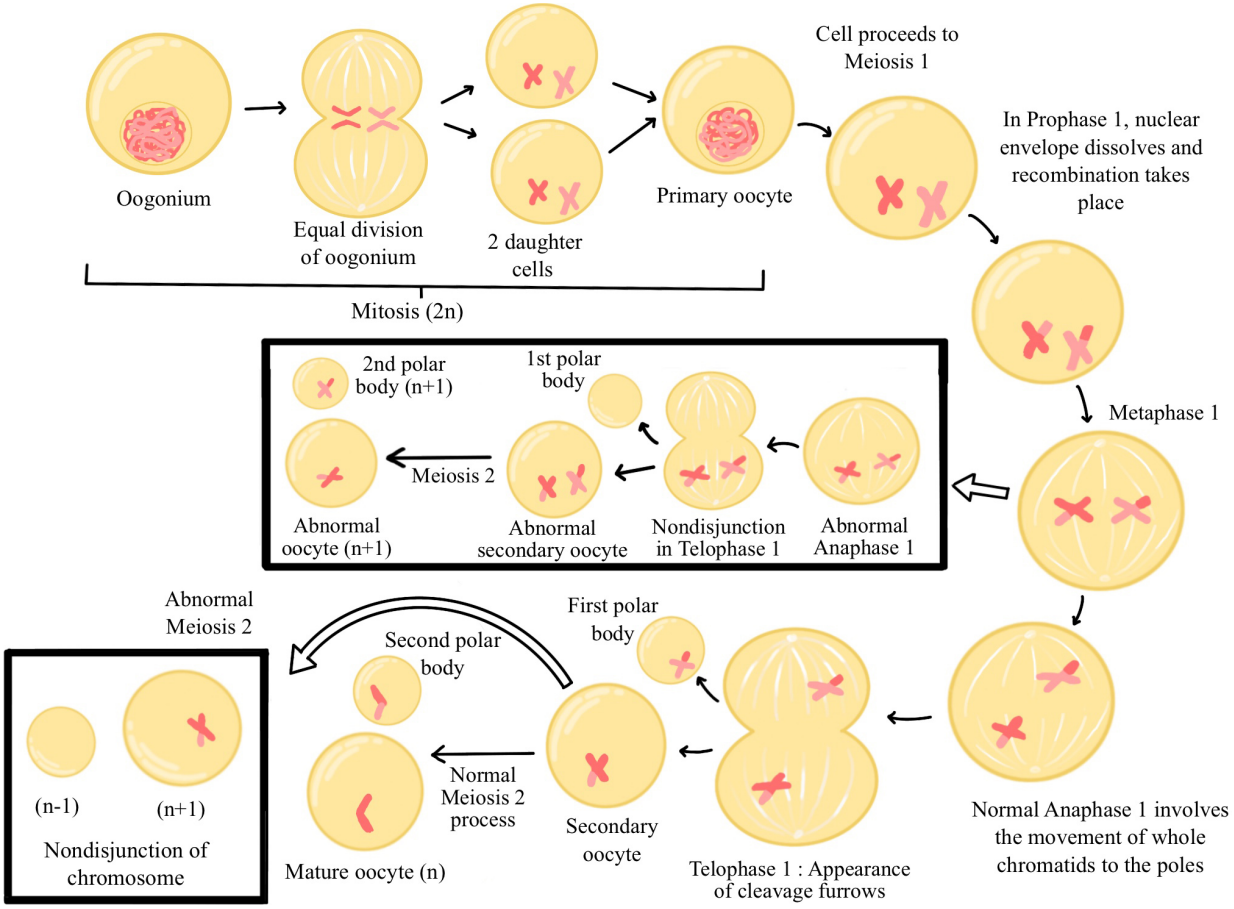

Figure 2. Normal and abnormal oogenesis.

increased serum insulin levels. Additionally, nutritional supplements along with management and handling of the sheep can affect the rates of embryo mortality. Viñoles et al. [12] found that supplementing ewes with 500 g of lupin grain, which is usually composed of high amounts of protein, fiber, and starch and some amounts of lipids, for 15 days after mating resulted in lowered progesterone concentrations and increased metabolic hormone concentrations, which promoted embryo survival. Most lupins are known to have a high concentration of alkaloids, which are not recommended for consumption. This is specifically due to the presence of toxic quinolizidene alkaloids, which can cause neurological paralysis and liver damage in sheep [13]. Care must be taken to supplement sheep with a variety that is low in these toxic alkaloids to prevent these diseases.

Primiparous ewes and ewes that have had more than five previous pregnancies were found to be less fertile and suffered more embryonic losses due to climate factors affecting maternal health. Also, it was found that heat stress 2 days prior to artificial insemination had the greatest effect on embryonic mortality [14]. External factors such as the uterine environment, maternal age, diet, and stresses due to heat, among others, also contribute greatly to the rates of embryo mortality [15,16]. Although these stresses do not show their effects in the beginning, in later stages these stresses can affect the embryonic development.

4. Methods for assessment of embryonic mortality

At a fundamental level, it is important to analyze the morphology of early embryos to be informed of their quality and the chances of embryonic death. This analysis indicates the early cleavage rates of the embryo, zona pellucida thickness, and the uniformity and proportionality of the cell division during blastomere formation [17]. However, this analysis is subjective in nature. Hence, Sugimura et al. [18] used time-lapse cinematography (TLC) imaging with microwell culture dish and oxygen consumption analysis to study the division of bovine embryos up to the blastocyst stage to understand and classify healthy and unhealthy embryos. Healthy embryos have certain characteristics during the preimplantation period that render them different from unhealthy embryos. One such characteristic is early cleavage. Late or slow cleavage in embryos has been

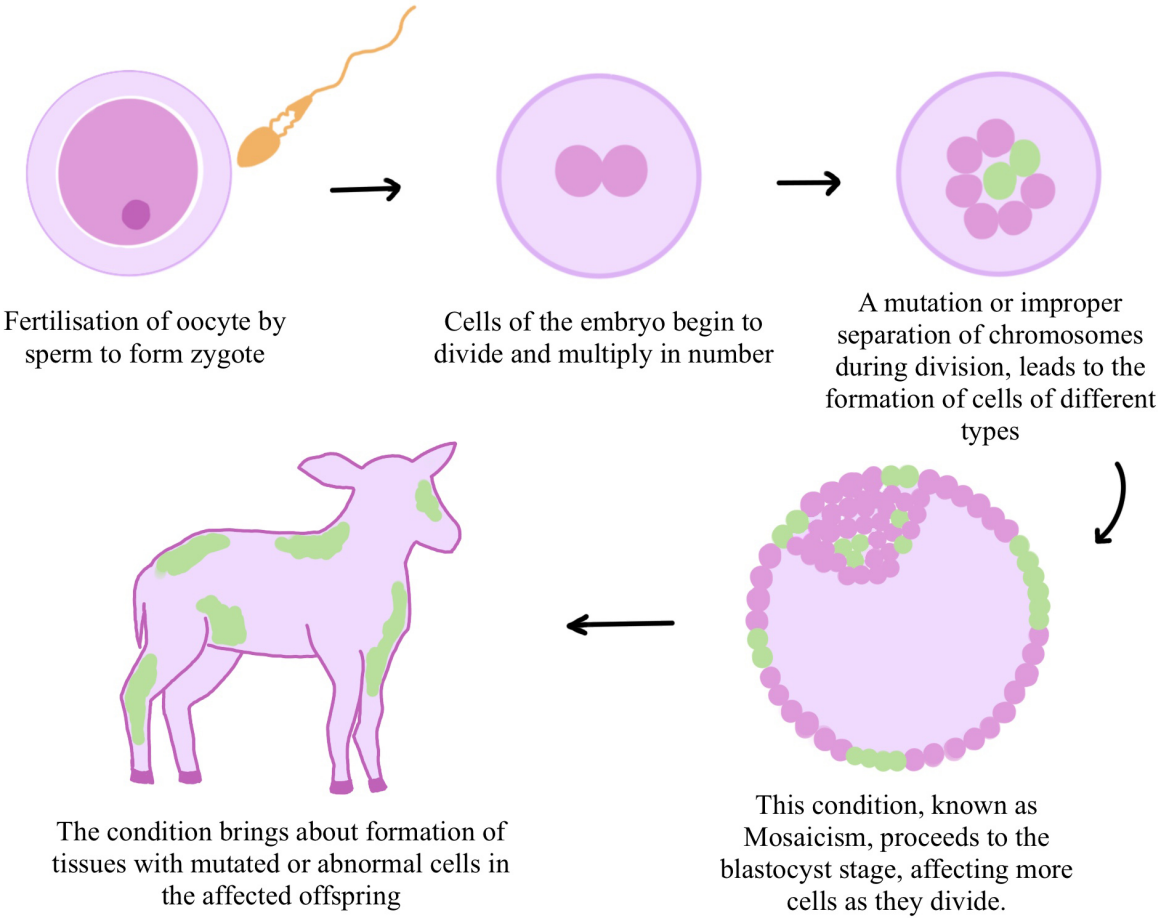

Figure 3. Mosaicism leading to different types of cells in the offspring.

found to not lead to successful pregnancies and can also have chromosomal abnormalities like mixoploidy [19,20]. It was also found that uneven numbers of blastomeres and fragmentation were a result of these chromosomal abnormalities. Additionally, Sugimura et al. [18] analyzed the oxygen consumption of dividing embryos and found that a low oxygen consumption rate led to less cleavage and lower hatchability. This method of TLC imaging can also be used in a similar manner to study ovine embryos as it is most useful in understanding the preimplantation events in the embryo.

Another very useful way of detecting the chances of embryo mortality is studying the metabolism of the embryo before implantation. The rates of nutrient uptake and metabolite uptake are the most commonly monitored parameters when studying embryo mortality [21]. Similarly, the events that occur during compaction are of significance as the embryo begins to transition from carboxylic acid uptake to glucose uptake [22]. In fact, high concentrations of glucose were found to be harmful for dividing embryos. Instead, compounds such as lactate and pyruvate were found to be favorable.

One of the most popular and fast evolving methods for studying embryonic mortality and fetal losses is ultrasonographic analysis. With the invention of B-mode or brightness-mode ultrasonography, we are able to noninvasively obtain a real-time view of the events leading up to implantation and events during the pregnancy itself. In this technique, high frequencies greater than 10 MHz, although not penetrating deeply, give us images with better resolution [23]. Typically, pregnancy in ewes is confirmed by the presence of early placentomes, amniotic sacs, embryos, or fetuses [24]. Indications of ovine embryonic death were observed via ultrasonography by Barbagianni et al. [25] when there was shrinkage of the embryo, absence of fetal heartbeat and movements later followed by a lack of anechoic fluids, and disappearance of previously observed placentomes. Moraes et al. [26] found 10% embryonic loss from among the 160 Santa Ines ewes studied using ultrasonographic analysis. Also noteworthy is that the mortality rates were significantly lower in single pregnancies as compared to multiple pregnancies. Also, Yotov [27] found upon studying three breeds of sheep with ultrasonography that late embryonic death

could be identified with the greatest ease using a single ultrasonogram between the 20th and 40th gestational days. Fetal mortality was lower during this period, but it increased after the 40th gestational day. Schrick and Inskeep [28] extensively studied the use of transrectal ultrasonography on ewes by performing a daily analysis from day 0 to day 25 and then again on days 30, 35, and 40 after insemination. They were able to identify early embryonic and fetal losses and also were able to study those embryos that were carried to term. Likewise, Dixon et al. [29] studied the timing of late embryonic loss in ewes through ultrasonographic analysis. They found that nearly 20% of the studied sheep experienced embryonic deaths and fetal losses. Interestingly, their studies indicated that the embryonic losses observed did not have any correlation with temperature and humidity.

In addition to ultrasonography, color Doppler ultrasonography can be used for early pregnancy prediction and detection. In principle, Doppler ultrasonography works by the reflection of sound waves on moving erythrocytes. Hence, those erythrocytes that move towards the probe are found to emit reflected waves of a higher frequency and can be identified as arteries, while those that emit a lower frequency of reflected waves can be identified as veins. These are represented in red and blue colors, respectively [30]. Therefore, with this technique, several studies have been conducted to get a better perspective on the development of the fetus throughout the pregnancy. Arashiro et al. [31] successfully utilized color Doppler ultrasonography to study luteal vascularization as a means of predicting the chances of pregnancy. Results achieved by this method were effective and real-time. It was based on the concept that increased progesterone levels after fertilization promotes the process of luteal vascularization. Conversely, low levels of progesterone have been found to cause significant decreases in luteal vascularization, leading to luteolysis and consequently failed conception [32], thereby enabling the prediction of pregnancy. Color Doppler sonography proved to be an important tool in the experiment by Cosentino et al. [33], where hormonal treatments with progestagens and equine chorionic gonadotrophin (eCG) were used for resynchronization of ovulation. Color Doppler helped detect the embryos and pregnancy at an early stage, thereby eliminating the need for a second attempt at insemination and hormonal treatments where unnecessary.

5. Relevant advances for the control of embryo mortality rates and improvement of embryo quality

Studies have indicated the importance of two oocyte-derived growth factors from the transforming growth factor-β (TGF-β) superfamily, bone morphogenetic protein-15 (BMP-15) and growth and differentiation factor-9 (GDF-9), for normal functioning of ovaries in ewes. Mutations in these genes lead to increased infertility [34]. Ewes observed as homozygous for mutations in the *BMP-15* and *GDF-9* genes were found to be anovulatory, while those heterozygous were found to have significantly high rates of normal ovulation [35]. Ptak et al. [36] studied the effect of DNA methyltransferase 1 (DNMT1) expression on the placental development of in vitro-produced (IVP) embryos in sheep. DNMT1 is known to control the methylation of the genes during cell division and this is of great epigenetic significance [37]. Reduced expression of DNMT1 was found in placentas from certain IVP embryos, which halted their growth and led to the subsequent death of these embryos. It was also observed that the embryos that managed to survive past the early placental stage had a greater expression of DNMT1. However, low DNMT1 expression proved to be lethal only up to day 24 after implantation, after which embryos survived nevertheless.

Apart from these, the effect of L-carnitine on preimplantation embryo development was studied by Mishra et al. [38]. Separate studies were conducted by using L-carnitine as an in vitro maturation medium and as a postfertilization development medium. Mortality rates of the embryos were found to be lower in embryos that received L-carnitine in the maturation medium as compared to its use on embryos after fertilization.

Embryo quality is another very important factor to be considered, especially in in vitro fertilization. Several studies have been conducted to improve the quality of embryos obtained. One such study by Bugliolo et al. [39] analyzed the effect of high hydrostatic pressure on IVP ovine oocytes. It was found that the embryos treated at 40 MPa had a higher hatching rate than those treated at 60 MPa. Blastocysts indicated higher inner cell mass and trophectoderm number along with a low percentage of pyknosis at 40 MPa. Another experiment conducted by Waldenström et al. [40] involved studying the effect of oxygen concentration on embryo quality. It was found that low oxygen concentration of about 5% was more favorable towards producing viable and good-quality embryos that successfully developed into blastocysts as compared to high oxygen concentrations. Also, in a recent review by Abecia et al. [41], the effect of melatonin was discussed as a factor that affects embryo mortality rates. Treatment of ewes with melatonin was found to improve the rates of pregnancy, indicating embryo survival by improved luteal function lowering the embryo mortality.

Besides these factors, nutrition plays a major role in embryo quality. Meza-Hererra et al. [42] supplemented ewes with high amounts of protein in the periconceptual period. This led to increased pregnancy loss and retarded embryo growth in the ewes. The few surviving embryos later suffered from reduced weight, which could impact the

fetus consequently. Hence, it is noteworthy that nutrition has a crucial role in embryo quality and viability, and undernutrition or overnutrition often leads to hormonal imbalances causing infertility [43].

6. Conclusion

Controlling the rates of embryo mortality in sheep still remains an area with scope for improvement. Identifying embryos with genetic and chromosomal abnormalities continues to be one of the greatest problems, and the internal and external factors leading to these abnormalities need to be regularly investigated. Proper nutrition has been highlighted several times as the most important control parameter for ensuring quality embryos that have a high chance of healthy development. Apart from these, hormonal control and genetic factors also contribute to the ovulation and preimplantation events and hence are also considered to be of significance for embryonic health. To support the aforementioned factors, one of the most important techniques for studying embryo viability today is via Doppler ultrasonography, which gives us rapid, real-time, and accurate results. However, this technique has not been employed enough on sheep models. Hence, this is as an opportunity for further research.

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