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Problems and Solutions in the Applications of Assisted Reproductive Technologies in Mammalian Livestock

ABSTRACT

Sustainability in farm animals is based on reproduction, and in this respect, the use of technologies in the field of reproductive biology is very important. Achieving production goals can be realized through the effective management and optimization of the reproductive processes in animals. In this regard, in recent years, assisted reproductive technologies have developing rapidly and in parallel with this development, it is reflected in practical applications. Although reproductive technologies are quite diverse, the current study focused on in vitro maturation, in vitro fertilization, in vitro embryo production and embryo transfer, and also considered species such as cattle, sheep, and goats as mammalian farm animals. The processes involved in in vitro embryo development and embryo transfer applications are extremely complex and intricate. These procedures involve stages such as in vitro oocyte maturation, in vitro sperm capacitation, the cryopreservation of gametes and embryos, and the selection and preparation of donor and recipient animals. Various problems can be encountered in each of the mentioned processes. In this study, it is aimed to include some common problems in assisted reproductive technologies and their solutions.

Keywords: Embryo transfer, sperm, gamete, reproduction, assisted reproductive technologies

Memeli Çiftlik Hayvanlarında Üremeye Yardımcı Teknolojiler Uygulamalarında Sorunlar ve Çözümler

ÖZ

Çiftlik hayvanlarında sürdürülebilirlik üremeye dayalıdır ve bu yönüyle üreme biyolojisi alanındaki teknolojilerin kullanımı oldukça önemlidir. Üretim hedeflerine ulaşılabilmesi, hayvanların üreme süreçlerinin etkin bir şekilde yönetilmesi ve optimize edilmesi ile sağlanabilir. Bu bağlamda, son yıllarda üremeye yardımcı teknolojiler hızla gelişmekte ve bu gelişime paralel olarak da pratik uygulamalara yansımaktadır. Üremeye yardımcı teknolojiler oldukça çeşitli olmakla birlikte, mevcut çalışmada in vitro maturasyon, in vitro fertilizasyon, in vitro embriyo üretimi ve embriyo transferi konularına odaklanılmış olup aynı zamanda memeli çiftlik hayvanları olarak sığır, koyun, keçi gibi türlerde yapılan çalışmalar esas alınmıştır. In vitro embriyo üretimi ve embriyo transferi uygulamalarında yer alan süreçler son derece karmaşık ve detaylıdır. Bu prosedürler, in vitro oosit olgunlaştırma, in vitro sperm kapasitasyonu, gametlerin ve embriyoların dondurulması ve donör-alıcı hayvanların seçimi ve hazırlanması gibi aşamaları içerir. Bahsedilen süreçlerin her birinde çeşitli sorunlarla karşılaşılabilir. Bu çalışmada, üremeye yardımcı teknolojilerde sık karşılaşılan bazı sorunlar ve bunların çözüm önerilerine yer verilmesi amaçlanmıştır.

Anahtar Kelimeler: Embriyo transferi, sperm, gamet, üreme, üremeye yardımcı teknolojiler.





INTRODUCTION

The general aim of animal science activity is to increase the benefit of animal husbandry (Düzgüneş et al., 2003). At the same time, the sustainability of the existing herd is possible with reproduction (Kaymakçı, 2006). One of the most essential features that characterize living things is the ability to reproduce, and by this means, living things can produce individuals similar to themselves (Fusco and Minelli, 2019). Reproductive activities in farm animals can generally be managed with *in vivo* and/or *in vitro* applications.

In vivo studies consist of applications such as artificial insemination, superovulation, and exogenous hormone use and these can lead to changes in animal physiology and behavior. Such as, it has been reported that melatonin implants cause changes in both hormone concentration and sexual behavior (Tölü et al. 2024).

In vitro reproduction studies encompass applications such as *in vitro* maturation (IVM), *in vitro* fertilization (IVF), and *in vitro* embryo production (IVEP). Moreover, IVEP represents an effective method for enhancing the reproductive potential of farm animals, as it is a reproductive technology that can be applied using oocytes obtained from both slaughtered and live animals (Yusuf, 2024).

Assisted reproductive technologies provide various benefits in terms of livestock, and each technique has different aspects that can cause problems compared to other technologies. In the current study, some problems and difficulties encountered on IVF, IVEP and embryo transfer (ET) applications, mainly in terms of mammalian farm animals, and certain solutions are emphasized.

Assisted Reproductive Technologies In Mammalian Livestock

Assisted reproductive technologies (ART), in addition to artificial insemination, include applications such as *in vivo* and *in vitro* embryo production, and they also have the potential to contribute to genetic advancement by shortening generation interval of farm animals (Amiridis and Cseh, 2012). To guarantee the success of genetic improvement in farm animals, reproductive technologies like artificial insemination, superovulation, IVF, and ET have been employed. It has been highlighted that these procedures are advantageous not only for regulating reproduction but also for halting the spread of diseases (Chakravarthi and Balaji, 2010). The most endangered species are the ones most likely to benefit from ART, but they are also the species least likely to be accessible for research, so ART is very important for endangered species (Herrick, 2019). There are many reproductive techniques such as IVM, IVF, IVEP, and ET that can be applied in the reproductive processes of mammalian farm animals, and these techniques also with each other. Technology for IVF also offers opportunities for other technologies in the cattle breeding field such as sex determination, cloning and ET (Oguejiofor, 2019). Although ARTs have been used in cattle for a long time, there are limiting factors such as low pregnancy rates and increased early embryo loss, and it has been reported that problems during *in vitro* embryo development may also affect reproductive performance (Siqueira et al., 2020).

In Vitro Fertilization, In Vitro Embryo Production and Embryo Transfer

The IVF process can be expressed as the realization of the fertilization process in a laboratory environment by providing artificial conditions.

IVEP covers the processes of obtaining oocytes from antral follicles, IVM of these supplied oocytes, fertilization of mature oocytes and capacity sperm, and culturing the embryo until blastocyst formation (Cognié et al., 2004).

ARTs, the general procedure of which is shown in Figure 1, are very detailed and each step of these procedures requires extensive knowledge, experience and expertise.

On *in vitro* applications by various researchers, incubation is carried out at %5 O₂ and %5 CO₂ for the incubation environment (Yang et al., 2020; Aoto et al., 2011). On IVF applications, the superovulation protocol allows multiple oocytes to be provided and the best embryos to be transferred, and excess embryos can be frozen and stored for future embryo transfers (Goldberg et al., 2007). Embryo transfer aims to transfer embryos without trauma to the specific uterine region where implantation is at the highest level; and conditions such as damage to the endometrium with the catheter, induction of uterine contractions, damage to the embryos during the transfer process or accumulation in the non-optimal region are the reasons for failure of embryo transfer (Mains and Van Voorhis, 2010).

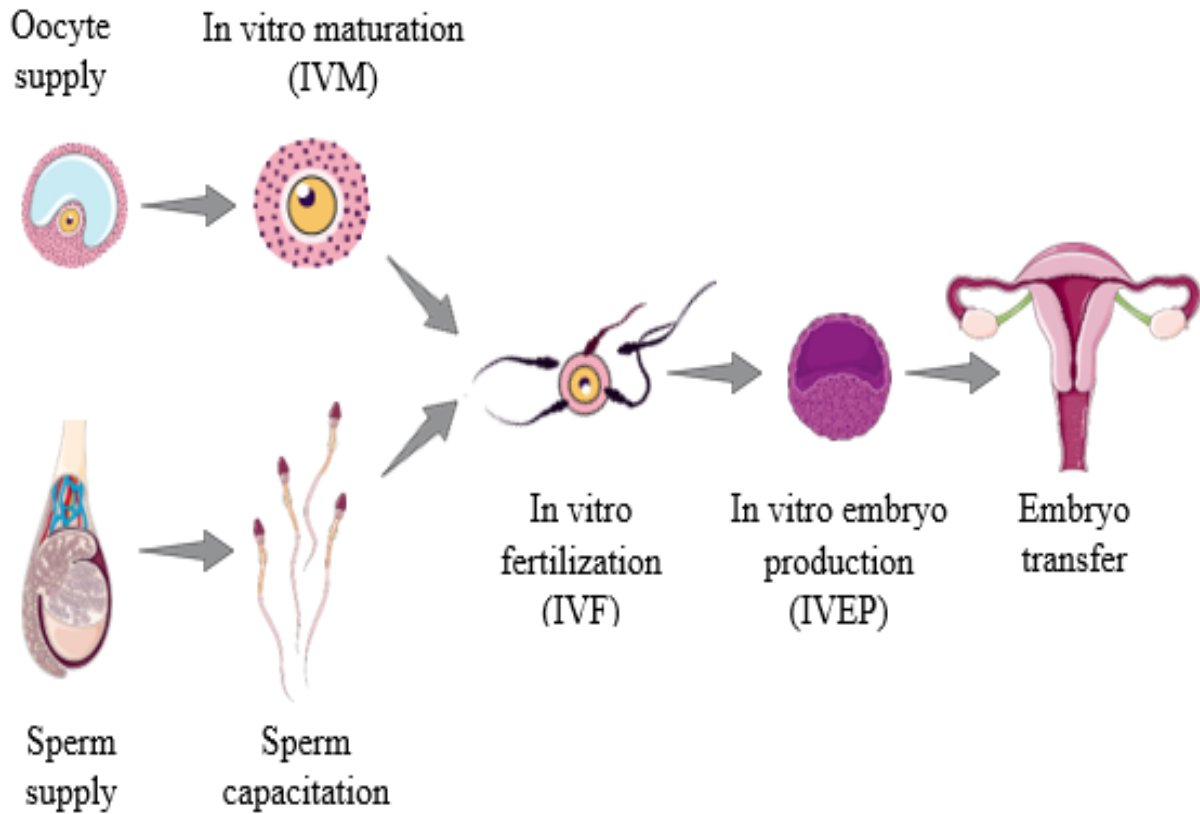


Figure 1. General procedure of in vitro embryo production and embryo transfer

Şekil 1. İn vitro embriyo üretimi ve embriyo transferi genel prosedürü

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Problems and Solutions On In Vitro Fertilization and Embryo Production

The stages of assisted reproductive procedures include gamete and embryo freezing, sperm and oocyte supply, oocyte maturation, sperm capacitation, and in vitro fertilization. Each of these phases is rather specific on its own. The general problems that may occur in these processes and their solution suggestions are summarized below.

Sperm-related conditions

In assisted reproductive technologies, there are various methods used in sperm retrieval, and research in this field continues (Başar and Soysal, 2019).

The addition of epidermal growth factor (EGF) during sperm cryopreservation has been reported to preserve sperm motility and viability after thawing, and to enhance the sperm's resistance to cryoinjury (Abdelnour et al., 2025). These and similar approaches may be explored to preserve sperm quality parameters.

Proximal droplet status should also be considered in the evaluation of sperm. It has been reported that sperm containing proximal droplets are functionally inadequate and their zona attachment rates are low (Thundathil et al., 2001).

In addition, the sperm separation protocol has an impact on the division rate and embryo production, as the Percoll sperm separation method led to a higher division rate and embryo production compared to the bovine serum albumin and washing sperm separation methods (Mendes Jr. et al., 2003).

It can be thought that studies aimed at improving sperm parameters such as motility, vitality, and abnormal morphology will also indirectly affect fertilization and subsequent processes. In this context, it is important to first identify the sperm-related problem and then go for a solution.



Aged donor oocyte

Oocytes are taken from aged animals by assisted reproductive technologies; Due to their high commercial value, they can be used for reasons such as prolonging the reproductive process of aged females, protecting the genetic resources of endangered and rare farm animals, and investigating the processes related to the problems of oocytes from old donors will also be beneficial to overcome infertility (Armstrong, 2001). In a study conducted within the framework of in vitro fertilization in cattle, it was stated that the follicular fluid of aged cows contains high concentrations of advanced glycation end-products (AGE) and that AGE reduces the developmental capability of bovine oocytes, and that AGE also induces the formation of reactive oxygen species (ROS) (Takeo et al., 2016). It has been reported that adding resveratrol to in vitro media improves IVF of oocytes from both age groups. Older cow oocytes had a lower total cell number in the blastocyte stage and a higher abnormal fertilization rate than oocytes from young cows (Takeo et al., 2013). It can be thought that paying attention to the age of the animal that is the source of the ovary, especially in ovarian-based studies taken from the abattoir, will contribute to more optimized in vitro studies.

Genetic and epigenetic factors

If the gametogenesis and early embryonic development processes are disrupted, these processes are changed or performed in vitro, the accompanying epigenetic processes may also be impaired (Shufaro and Laufer, 2013). Similarly, it has been reported by Sirard (2017) that assisted reproductive technologies have the potential to lead to aberrant epigenetic gene regulation by creating suboptimal environments for the development of gametes and embryos. In vitro culture affects gene expression and methylation, but adding growth broth to the culture medium may be advantageous (Canovas et al., 2017).

Polyspermy

In a study examining the effect of sperm concentration and maturation times of oocyte and spermatozoa on polyspermy, it was reported that polyspermy increased with the increase in sperm concentration and incubation times of oocyte - spermatozoa; in this direction, polyspermy can be reduced by decreasing sperm concentration and incubation times of oocyte - spermatozoa (Long et al., 1994). Furthermore, heat stress during fertilization tended to increase the rate of polyspermy, according to a study that looked at how temperature affected polyspermy at the fertilization stage in vitro (Sakatani et al., 2015).

Freezing and thawing gametes

Although new technological studies are constantly being added to the freezing of oocyte and sperm, it is an important point that the viability and development potential of the oocyte or sperm after thawing is at the optimum level. The process of freezing both oocytes and embryos is a very important application, oocytes and embryos are sensitive to freezing processes and may be damaged morphologically and functionally (Pereira and Marques, 2008). When the effect of freezing the ovarian tissue on follicular morphology was examined, it was reported that vitrification preserved the follicular morphology better than slow freezing (Labrune et al., 2020). The cryoprotectants used in oocyte freezing and their concentration also have an effect on oocyte maturation (Yamada et al., 2007). Also, cryoprotectants used in sperm freezing affect post-thaw results; In fact, they protect sperm better than glycerol, ethylene glycol and dimethyl formamide and give better results in aspects such as motile sperm and mitochondrial function (Forero-Gonzalez et al., 2012). In this direction, it will be beneficial to plan the subjects such as the freezing method, cryoprotectants and cryoprotectant concentrations to be used before the freezing process.

Zona pellucida thickness

The zona pellucida has functions in the species-specific processes of fertilization, division, and hatching of the blastocyst (Hafez and Hafez, 2000). Abnormalities of the zona pellucida impair the IVM and developmental ability of oocytes (Fang et al., 2024). Similarly, it has been reported that oocytes with abnormal zona pellucida have lower rates of high-quality embryos and blastocysts compared to those with a normal zona pellucida (Wang et al., 2025).

There are applications for zona pellucida manipulations such as partial zona dissection (PZD), acid / chemical zona thinning, laser assisted hatching and piezo technology (Hammadeh et al., 2011). The methods of perforating the zona and dissection of the zona are similar and a path is created for the passage of sperm in these



methods, the sperm must still be motile (Keefer, 1990). Research has examined how zona pellucida dissolves in many mammals, and it has been found that the pronase enzyme effectively dissolves the zona (Gwatkin, 1964). Furthermore, in situations of sperm-induced inadequacy where sperm motility, morphology, and other issues are observed, micromanipulations of the zona pellucida may be employed (Palermo and Bedford, 2000).

Cultural media content

While creating the culture media, various media and protein sources, vitamins, antioxidants, etc. added to these media for the study. can be used (Ali and Sirard, 2002; Olson and Seidel, 2000; De Matos et al., 2002). Looking at various examples, the epidermal growth factor positively affects IVEP (Ahmed et al., 2023). Similarly, insulin-like growth factor (IGF) supports oocyte maturation and blastocyte production (Yang et al., 2019). Furthermore, it was noted that the penetration rate rose noticeably when heparin at different concentrations was added to caffeine; in the presence of heparin alone, the penetration rate was 35%, whereas in the presence of heparin+caffeine, it was 68% (Niwa and Ohgoda, 1988).

If the ingredients used and their combinations are to be evaluated, hundreds of results can be encountered. As a matter of fact, there are no fixed procedures for media content and the necessary content for each study can be designed by the researcher. When creating medium content, the biological material to be used, laboratory conditions, devices, etc. should be evaluated and planned in general.

Laboratory environment and personnel competence

In the in vitro culture (IVC) process of bovine embryos, it has been reported that the use of a carbon-activated air filtration system (CODA) in the incubator improves pregnancy rates after transferring these embryos to recipient cattle by embryo transfer method (Merton et al., 2007). Appropriate air filtration systems for the safe execution of contamination sensitive applications will provide contamination control in the laboratory, and there are air filter systems such as "central heating, ventilation and air conditioning filtration", "compact filtration systems", "portable filtration systems" in this regard (Sadir et al., 2017).

In terms of personnel competency, it is recommended to provide a comparison system in terms of parameters such as grading of oocyte maturity and fertilization rates, grading of embryo quality, and monitoring the results of applications such as fresh embryo transfer and cryopreservation at certain periods (Keck et al., 2005).

Problems and Solutions in Embryo Transfer

Similar to gamete manipulation, ET can have certain issues. These include topics like embryo freezing and thawing, ET techniques, and the selection and preparation of donor and recipient animals. So, it is aimed to give brief information about these subjects.

Freezing and thawing embryos

Cryopreservation of in vitro embryos is very important for both the production and preservation of high breeding value animals, and there are two methods in this field, slow freezing and vitrification (Ferré et al., 2020). Embryos frozen by vitrification have less cryodamage and higher survival than slow-frozen embryos (Arshad, 2021). The developmental stage of the embryos during freezing also affects the viability rates after thawing, as the viability rate of the zygotes is highest, followed by the 2nd and 3rd day embryos (Salumets et al., 2003). In addition, in recent studies, it has been reported that the addition of an antioxidant, glutathione, to the post-thaw recovery culture of embryos increased the total antioxidant capacity of the embryo and decreased the rate of apoptotic cells, thus reducing the harmful effects of freezing (Olexiková et al., 2023).

Transfer method

The conditions that impact the pregnancy rate in ET applications include donor-recipient estrus synchronization, embryo quality, embryo development stage, and similar factors (Hasler et al., 1987). It is important to pay attention to the early detection of difficult cases because implantation and pregnancy rates are lower in difficult ET than in easy ones. Some challenges that may arise during the ET process include using force when inserting the catheter and manipulating the uterus (Ghaffari et al., 2013). When the studies on the use of soft and hard catheters in embryo transfer are evaluated, it is reported that the use of soft catheters performs better in pregnancy formation (Abou-Setta et al., 2005). In addition to these, it is a feature of difficult embryo



transfer that the catheter treated in embryo transfer is contaminated with blood, and it has been reported that when the embryo transfer catheter is contaminated with blood, the pregnancy rate is lower than when the catheter is clean (Awonuga et al., 1998).

Donor and recipient

The developmental adequacy of oocytes was found to decline with maternal age in a study comparing young and old cows under the framework of ET used following artificial insemination (Malhi et al., 2007). Considering the relationship between age in cows and embryos subject to embryo transfer, it has been reported that the age of the donor cow affects the average number of embryos that can be transferred and the average number of total embryos, and that the total number of embryos is less in young cows (2-3 years old) (Hızlı et al., 2011). A study examining the relationship between the donor cow and the season was also carried out on embryo transfer applications, it was reported that the season has an effect on the rate of transferable and non-transferable embryos in donor cows, and the rate of transferable embryos is lower in summer months (Ayaşan et al., 2011). When cows and heifers are evaluated together, it has been reported that the number of superovulation affects the number of transferable quality embryos, the number of non-transferable quality embryos and the total number of embryos, and the response to the number of repeated superovulation is different in cows and heifers (Hızlı et al., 2012).

Additionally, it is critical to limit stress, which is a significant component for recipient animals, and to guarantee the wellbeing of all animals involved in ET procedures (Gordon, 2004)

CONCLUSION

The manipulation of reproductive processes, the preservation of genetic resources, and the storage, preservation, transfer, and transportation of embryos having superior animal genetic material are only a few advantages of assisted reproductive techniques in mammalian farm animals. However, a number of challenges are also faced at different phases, including cryopreservation, ET, IVM and IVF.

It is evident that each problem requires a unique solution. However, in general terms, it can be stated that the selection of morphologically normal and healthy gametes contributes positively to IVF outcomes. Moreover, with regard to gamete and embryo cryopreservation, vitrification appears to be more preferable compared to slow freezing. Additionally, given the higher number of human studies compared to those conducted on farm animals, the benefits of zona pellucida micromanipulations observed in humans are expected to yield similar advantages in livestock species.

ART protocols are not standardized. Various factors such as exposure duration, temperature, and medium can significantly influence the outcomes. This variability makes it challenging to compare results across different studies. Indeed, although the use of different techniques and various chemicals such as hormones, antioxidants and cryoprotectants in eliminating or minimizing some of the problems encountered in assisted reproductive technologies has been researched, there are still many issues that need to be improved today.

It is thought that conducting various studies to eliminate or minimize the existing problems in both in vitro studies and ET applications, and examining studies that focus on human and laboratory animals in mammalian farm animal models will increase the success in IVF and ET studies.

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