THE SCIENCE WORLD
A Monthly e Magazine
ISSN:2583-2212
Popular Article
April, 2025 Vol.5(4), 6722-6725

Invitro Embryo Production in Goats: Strategies and Future Prospects

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DOI:10.5281/ScienceWorld.15268519

Goat is a versatile domestic species that can thrive well with limited inputs. They have multiple economic traits and ever demanding market that makes goat a most preferred choice for the farmers especially with small land holding and is called known as “poor man’s cow or “moving ATM”. Despite high demand there has been a constant genetic wash as result of indiscriminate slaughtering with elite goats as most preferred choice for slaughter. This trending scenario for years has limited the availability of elite breeding animal in country. Today, Indian goat husbandry is featured with poor growth rate, low fecundity and poor milk yield. So, with limited genetically superior goats available it becomes important to conserve and propagate the superior germplasm of goat.

Artificial insemination (AI) and *In vitro* embryo production has been most widely used assisted reproductive technology for genetic improvement programme. Although artificial insemination is being considered most accepted technology for genetic improvement programme, it preferably focuses on dissemination of male germplasm whereas the *In vitro* embryo production gives an upper hand over AI in utilizing female germplasm for dissemination and conservation. This reproductive technology known as IVF and ET has revolutionized animal breeding, and goats are no exception. Goat *In vitro* embryo production has exciting opportunities to improve genetic diffusion, livestock production and provide embryo for commercial applications of transgenic and cloning technologies. *In vitro* embryo production is a multi-step methodology comprising the following procedures: (i) *In vitro* Maturation (IVM) of oocytes recovered directly from the follicles (ii) *In vitro* fertilization (IVF) of oocytes with capacitated spermatozoa and (iii) *In vitro* culture (IVC) of

zygotes up to the blastocyst stage. This article provides basic understanding of IVF in goats and strategies involved.

What is Invitro Embryo Production

In vitro embryo production is a technology that involves extraction of oocytes directly from the ovary of a stimulated donor female using laparoscopy or even from dead or slaughtered female and fertilizing them in artificial medium which are later cultured to blastocyst stage *in vitro* prior to its transfer in recipient animal. The process involves oocytes collection form live animal or slicing of collected ovary from slaughter to collect the oocyte which are segregated under stereo zoom microscope, washed, matured, fertilised and later cultured in suitable media for 7-8 days to reached up to blastocyst stage and either implanted in a healthy female or cryopreserved for future use in embryo bank.

Steps involved in Invitro Embryo Production

1. ***Oocyte Collection*:** First, the oocyte (egg cell) from donor is harvested. These are often extracted by ultrasound guided follicle aspiration (laparoscopic method) or recovered from ovaries of dead or slaughtered female. The superior genetic traits of the donor animal such as high milk yield, illness resistance, or meat production are the basis of selection.
2. ***Maturation and Fertilisation:*** *In vitro* maturation is the most critical step in whole process of *In vitro* embryo production. For successful IVM, oocytes must undergo synchronically nuclear and cytoplasmic maturation. The most widely used media is TCM-199 medium, bicarbonate buffered and containing minerals, carbon and energy sources as well as vitamins and amino acid supplemented with hormones (FSH, LH,17β-E2). Conventionally, goat oocytes are *in vitro* matured in groups and incubated at 38-39°C in humidified atmosphere of 5% CO_2 in air for 24-27hr.
3. ***Invitro Fertilization*:** For successful IVF, depends upon appropriate oocyte maturation, sperm selection, sperm capacitation and IVF media.

   First step is to select the most motile and viable spermatozoa from the fresh ejaculate or the frozen-thawed sperm. The most common method used for separating the sample into motile and non-motile fractions is the swim up technique or percoll gradient. For frozen-thawed semen, preferably motile spermatozoa are obtained by centrifugation on a discontinuous percoll gradient. Once the most viable and motile spermatozoa are selected, sperm capacitation is carried out in vitro. Sperm and oocytes are coincubated in IVF media (synthetic oviduct fluid) for 16-24 hr at 38-39℃ at humidified atmosphere of 5% CO_2 in air.

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Published 23/04/2025

4. ***In Vitro Embryo Culture:*** The last step of in vitro embryo production is the culture of presumptive zygote up to blastocyst stage at 6-7 days after IVF in goats. Different culture media are used based on experimental condition of labs, mostly SOF medium plus serum is used.
5. ***Embryo transfer or Cryopreservation:*** This step specify the utility of developed *Invitro* embryo. Either the embryo developed are transfer in the recipient goat instantly or are cryopreservation and store for future use, enabling breeders to access genetic resources across generations or even transport them across border.

Advances related to Invitro embryo production

Recent advancements in goat IVF focuses on improving oocyte retrieval, fertilisation, and embryo development, with techniques adapted for both genetic improvement and conversation efforts.

1. ***Ovum Pick-Up (OPU):*** Modern method involve ultrasound-guided OPU to harvest oocyte efficiently. In this method oocytes recovered directly from the follicle in hormonally stimulated or non-stimulated females by laparoscopic ovum pick up. Hormonal protocols can be followed for better results. This method ensures collection of ova efficiently with minimal invasion.
2. ***Intracytoplasmic Sperm Injection:*** ICSI has emerged as an alternative to traditional IVF, especially in cases where fertilisation rates are low or sperm quality is poor. Although widely used in other species, ongoing research is focused on adapting this method in goats.
3. ***Vitrification of Embryo:*** It is an alternative method for cryopreservation that offers promising results for goat embryos. It eliminates the need for gradual dehydration by utilising rapid freezing, which is more economical. In vitro produced embryos have shown encouraging post-thaw survival rates under these conditions.
4. ***Advanced Embryo Culture:*** Researchers have refined culture media to support embryonic development, minimizing the accumulation of toxic metabolic byproducts that can impair development. This has improved success rates for blastocyst formation.
5. ***Genomic and Biotechnological Applications:*** Genomic screening and embryo biopsy enable the selection of embryos with specific traits such as disease resistance or high production potential. This integration supports precision breeding and genetic improvement.

Future prospects

- ✓ ***Genetic Improvement*** – IVF makes it possible to produce large number of offsprings from an genetically superior goats with desired characteristics. Breeders can even enhance herd quality by selecting for particular traits like disease resistance, high milk output or improved meat quality.

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- ✓ ***Preservation of Biodiversity***-IVF is helpful in protecting unique or endangered goat breeds. Scientists can save biodiversity by ensuring long term survival of these animals through the cryopreservation of oocytes, sperm, embryos.
- ✓ ***Gene Editing and Invitro embryo production*** - With the advent of CRISPR and gene-editing technologies, IVF in goats could soon take on a new dimension. Gene editing allows for the precise modification of goat DNA, potentially leading goats with enhanced traits or immunity to disease.
- ✓ ***Cloning and Invitro embryo production*** - Cloning technology when combined with IVF, can further increase the efficiency of reproducing elite animals. Cloning allows for the replication of genetically superior goats, while IVF accelerates the process of producing large number of these animals.
- ✓ ***Global Food Security-*** As the globe struggles with food security, goat IVF may prove to be crucial instrument for raising animal productivity. As for meat, milk, and fibre, goats are essential in many poor nations. It will give farmers more stable incomes dependable food supply.

Conclusion

Invitro embryo production in goats is a growing field with immense potential. It offers significant advancements in livestock production and genetic improvement, playing a critical role in modern breeding strategies. The step-by-step process of oocyte collection, maturation, fertilisation, and embryo culture has not only improved the efficiency of reproduction but has also paved the way for preserving biodiversity and integrated new biotechnologies such as gene editing and cloning. This evolving field could play a significant role in addressing global challenges related to food security, sustainable agriculture, and animal conservation.

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Published 23/04/2025