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Research Article

Embryo Biotechnologies in Sheep and Goats: Current Advances, Limitations, and Integration with Genomic Selection

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Abstract

Sheep (Ovis aries) and goats (Capra hircus) collectively support the livelihoods of hundreds of millions of people in the developing world, providing meat, milk, fibre, and hides in agro-ecosystems that are often too arid or marginal for other livestock species. Despite their global importance, the application of reproductive biotechnologies to accelerate genetic improvement in small ruminants has lagged substantially behind progress in dairy cattle, constrained by unique anatomical barriers (the tortuous caprine and ovine cervix), marked reproductive seasonality, smaller commercial scale, and limited investment in research infrastructure relative to the bovine industry. This review comprehensively examines the current status and technical constraints of the major embryo biotechnologies applicable to sheep and goats: superovulation and embryo transfer (MOET), ovum pick-up-based in vitro production (OPU-IVP), embryo cryopreservation, sexed semen application, somatic cell nuclear transfer, and the emerging integration of embryo technology with genomic selection (OPU-IVF-GS). For each technology, the biological and logistical factors influencing performance are critically evaluated, empirical performance benchmarks are synthesised, and practical implementation guidance for both research and field settings is provided. The review also evaluates the evidence for using melatonin supplementation, out-of-season breeding, and laparoscopic approaches to overcome the major constraints of seasonality and cervical anatomy. Finally, the potential for combining OPU-IVF with genomic embryo selection to accelerate genetic gain in small ruminant breeds is assessed in the context of recent pilot implementations.

Keywords: *Sheep, Goat, Multiple ovulation and embryo transfer, OPU-IVF, Superovulation, Embryo cryopreservation, Vitrification, Genomic selection, Laparoscopic AI, Seasonality*

1. Introduction

The global small ruminant population is estimated at approximately 1.21 billion sheep and 1.00 billion goats (FAO, 2016), with the largest populations concentrated in Asia (China, India, Pakistan, Bangladesh), sub-Saharan Africa, and the Middle East. In these regions, small ruminants constitute a primary source of animal protein and play a critical role in household nutrition and economic resilience. The dairy goat industries of France (Saanen, Alpine), Spain (Murciano-Granadina), Italy (Garganica), and the Canary Islands produce high-value artisan cheeses, while Cashmere and Merino wool industries in Central Asia and Australia respectively contribute billions of dollars to their national economies.

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Despite this diversity and scale, the rate of genetic improvement in most small ruminant populations is very slow by comparison with dairy cattle, reflecting the limited adoption of artificial insemination, embryo transfer, and related biotechnologies.

The primary anatomical constraint limiting reproductive biotechnology in small ruminants is the structure of the cervix. In both sheep and goats, the cervix contains multiple interlocking annular folds that create a tortuous canal, making transcervical embryo collection and deposition practically impossible without specialised instrumentation in most animals and most reproductive cycle stages. This contrasts sharply with cattle, where transcervical embryo transfer is a routine non-surgical field procedure. As a consequence, embryo transfer in small ruminants has historically required laparotomy (midventral surgical approach) or laparoscopy under general anaesthesia, substantially increasing cost, limiting throughput, and restricting implementation to veterinary clinic settings.

Reproductive seasonality constitutes the second major constraint. Both sheep and goats are short-day breeders in temperate latitudes, concentrating reproductive activity in autumn and winter when photoperiod is decreasing. Superovulatory responses and IVF blastocyst rates are significantly higher during the breeding season than during anoestrus, limiting year-round application of embryo biotechnologies and complicating integration with commercial production calendars. The use of exogenous melatonin, ram/buck effect, and artificial light manipulation to induce out-of-season cyclicity has been explored as a countermeasure, with variable but generally positive results.

2. Superovulation and Embryo Transfer (MOET)

2.1 Superovulatory Protocols

Superovulation in sheep and goats is induced by administration of FSH preparations (pFSH, oFSH, or eCG) in conjunction with progesterone-based synchronisation (intravaginal sponges containing medroxyprogesterone acetate or FGA for sheep; CIDR devices for goats). The standard FSH protocol consists of twice-daily decreasing doses over three to four days, with sponge removal at the beginning of the FSH treatment period and GnRH or LH administration at the time of laparoscopic AI. The superovulatory response — typically measured as the number of corpora lutea (CL) per donor — is highly variable among individuals, with coefficients of variation commonly exceeding 60%.

Factors contributing to superovulatory response variability include breed (Ile-de-France and Lacaune sheep typically respond better than Merino; Alpine goats better than most indigenous breeds), age and parity (mature multiparous donors generally outperform ewe lambs), body condition score (BCS 2.5–3.5 on a 1–5 scale is optimal), season (breeding season > anoestrus), FSH source and batch (biological variability between batches of pFSH is significant), and the interval from previous superovulation (minimum three months is recommended to avoid ovarian refractoriness). Despite extensive investigation, no reliable non-invasive predictor of superovulatory response has been identified, making donor selection largely empirical.

2.2 Embryo Collection, Evaluation, and Transfer

In sheep, embryo collection is performed by retrograde flushing of the uterine horns through a midventral laparotomy or laparoscopy seven days after AI (morula to early blastocyst stage). Typical embryo yields range from 5–10 per donor in well-managed programmes, but with substantial variability. Embryos are evaluated under a stereomicroscope using IETS criteria (Grades 1–4), with only Grades 1 and 2 (excellent/good and good) being suitable for transfer or cryopreservation. Surplus ova and degenerate embryos typically represent 20–40% of flushed structures. Table 1 summarises performance benchmarks for the major embryo biotechnologies in sheep and goats.

Table 1. Performance benchmarks for major reproductive biotechnologies in sheep and goats compiled from published literature

Technology	Species	Embryo Yield/Session	Pregnancy Rate (%)	Key Reference
Conventional MOET	Sheep	5–10 embryos	50–65	Evans & Maxwell, 1987
Laparoscopic AI (frozen semen)	Goat	—	55–75	Paulenz et al., 2005
Laparoscopic AI (fresh semen)	Sheep	—	65–80	Morrell et al., 2012
OPU-IVF (in vivo matured)	Sheep	4–7 oocytes	35–50	Cognie et al., 2003
OPU-IVF (follicular)	Goat	6–10 oocytes	30–45	Paramio & Izquierdo, 2014
Vitrification (in vivo embryos)	Both	—	45–60	Baril et al., 2001
Sexed semen + ET	Sheep	3–5 embryos	30–45	Morton et al., 2010
SCNT (somatic cloning)	Goat	—	5–15	Vajta & Gjerris, 2006

MOET = Multiple ovulation and embryo transfer; OPU = Ovum pick-up; IVF = In vitro fertilisation; SCNT = Somatic cell nuclear transfer; AI = Artificial insemination.

3. Ovum Pick-Up and In Vitro Production (OPU-IVP)

3.1 Oocyte Collection

Ovum pick-up (OPU) combined with in vitro production (IVP) circumvents the superovulation variability problem by directly aspirating oocytes from antral follicles under ultrasound guidance. Transvaginal OPU, standard in cattle, is technically feasible in goats and larger-framed sheep breeds using a 5.0–7.5 MHz transvaginal probe with a dedicated aspiration needle guide (inner diameter 0.75 mm, vacuum pressure 40–50 mmHg). Laparoscopic OPU, developed by Cognie et al. (2003) in sheep, provides

better visualisation and follicle access but requires anaesthesia and surgical skill. OPU sessions can be repeated every 3–7 days in non-stimulated animals, generating a continuous supply of oocytes without the limitations imposed by breeding season.

3.2 In Vitro Maturation, Fertilisation, and Culture

Oocytes are classified immediately after aspiration based on cumulus morphology and cytoplasm homogeneity. Grade I and II oocytes (compact cumulus with ≥3 layers, homogeneous cytoplasm) are selected for IVM in SOF medium supplemented with gonadotrophins (FSH + LH, 0.1 IU/mL each), oestradiol-17β, EGF, and 10% FCS or defined protein supplement at 38.5–39°C under 5% CO2, 5% O2, 90% N2 for 21–24 hours. IVF is performed using capacitated spermatozoa (swim-up or density gradient centrifugation from frozen-thawed semen) in TALP medium supplemented with heparin (2 µg/mL) for 18–20 hours.

Table 2 summarises the critical IVP parameters and their effects on blastocyst development rates, providing practical guidance for laboratory optimisation.

Table 2. Critical parameters affecting blastocyst development rates in small ruminant in vitro production systems

Factor	Optimal Range	Effect on Blastocyst	Practical Notes
Maturation medium (IVM)	SOF + 10% FCS or EGF	Positive (+15–20%)	EGF reduces need for feeder cells
Maturation duration	21–24 h (goat), 22–24 h (sheep)	Critical	Shorter = immature; longer = aged oocyte
Temperature	38.5–39°C	Optimal	Avoid fluctuation; use calibrated incubator
O2 tension during IVC	5% O2 vs 20% air	+10–15% blastocysts	5% O2 strongly recommended
CO2 tension	5–6%	pH 7.2–7.4	Bicarbonate-buffered media; monitor pH
Sperm preparation	Swim-up or DGC	Reduces DNA damage	DGC preferred for frozen semen
Culture density	Group culture (10–25 embryos)	Paracrine benefit	SOF + 0.4% BSA; semi-defined

IVM = In vitro maturation; IVC = In vitro culture; SOF = Synthetic oviduct fluid; FCS = Fetal calf serum; EGF = Epidermal growth factor; DGC = Density gradient centrifugation.

4. Embryo Cryopreservation

The ability to cryopreserve small ruminant embryos for storage, transport, and genetic resource banking is essential for the practical utility of MOET and OPU-IVP programmes. Two approaches have been validated: conventional slow cooling (equilibrium) and vitrification (ultra-rapid, non-equilibrium cooling). Table 3 presents the major cryoprotectant protocols and their performance characteristics.

Table 3. Cryoprotectant protocols and survival rates for small ruminant embryo cryopreservation

Cryoprotectant	Method	Survival Rate (%)	Comments
Glycerol (7.2%) + sucrose	Slow cooling	65–80	Standard for in vivo ovine embryos
EG (1.5M) + DMSO (1M)	Vitrification (Cryotop)	75–88	Preferred for IVF-produced embryos
Propylene glycol (1.5M)	Slow cooling	60–72	Caprine in vivo embryos; field applicable
EG (30%) + sucrose (0.5M)	Open pulled straw (OPS)	70–82	High cooling rate; simple equipment

Donor preparation (synchronisation ± FSH priming) → OPU (transvaginal/laparoscopic, Day 0, 3, 6) → Oocyte grading (GI/GII selection) → IVM (SOF+EGF, 22h, 39°C, 5% O2/5% CO2) → IVF (TALP+heparin, swim-up sperm, 18h) → IVC (SOFaaci+BSA, 7 days, 5% O2) → Blastocyst evaluation → Vitrification (Cryotop, EG+DMSO) or Direct transfer (laparoscopic ET)

Figure 1. Integrated workflow for OPU-IVF based embryo production in small ruminants, from donor preparation through blastocyst vitrification or direct transfer.

In vivo-derived embryos generally tolerate both slow cooling and vitrification well, achieving post-thaw survival and pregnancy rates of 50–75% when Grades 1–2 are used. In vitro-produced embryos are significantly more sensitive to cryoinjury due to their higher lipid content and altered membrane composition compared with in vivo counterparts. Vitrification using the Cryotop or open pulled straw

(OPS) devices with ethylene glycol-based solutions currently provides the best results for IVF-produced caprine and ovine embryos, with survival rates of 70–88% and pregnancy rates of 40–60%. Delipidation of IVF embryos by centrifugation prior to vitrification has been explored as a strategy to reduce cryoinjury in small ruminant embryos, with encouraging initial results in goats.

5. Integration with Genomic Selection

The integration of OPU-IVF with genomic selection (OPU-IVF-GS), analogous to the juvenile genomic selection concept proposed by Sonesson and Meuwissen (2009) for aquaculture, offers a powerful strategy for accelerating genetic gain in small ruminants. In this approach, OPU is performed on genotyped females of high GEBV, their oocytes are fertilised with semen from genotyped elite males, and the resulting blastocysts are biopsied (trophectoderm biopsy, TE biopsy) to extract DNA for SNP genotyping before transfer. Only embryos with the highest GEBV — across both the selection index traits and health/robustness traits — are transferred, maximising selection intensity at the embryo stage.

The feasibility of TE biopsy in small ruminant embryos has been demonstrated in sheep (Chitwood et al., 2013) and goats, with DNA yields from 5–10 TE cells sufficient for amplification and SNP genotyping using whole-genome amplification (WGA) followed by low-density chip genotyping and imputation to higher density. Biopsy survival rates of 80–90% and blastocyst re-expansion rates of 75–85% post-biopsy have been reported with optimised protocols, suggesting minimal negative impact on viability. Pilot implementations in Lacaune dairy sheep populations in France have demonstrated that combining OPU-IVF-GS with an accelerated paternal pathway (genomically selected young rams with short generation intervals) can more than double the rate of genetic gain relative to conventional MOET-based programmes.

6. Conclusions

Embryo biotechnologies in small ruminants have advanced substantially, though significant practical barriers — particularly cervical anatomy, seasonality, and lower blastocyst yields compared to cattle — continue to limit widespread commercial adoption. The combination of OPU-IVF with genomic selection and trophectoderm biopsy represents the most promising strategy for accelerating genetic progress in elite small ruminant breeds.

Continued investment in non-surgical embryo collection and transfer methods, optimisation of serum-free IVP media, and development of affordable genotyping solutions for small ruminant reference populations are the priority research areas that will determine the pace of progress over the coming decade.

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