RESEARCH ARTICLE

Effect of Sperm Preparation Methods on *In-Vitro* Fertilization of Goat Oocytes

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Abstract

The present study was undertaken to evaluate the effect of different sperm preparation methods*, viz.,* Percoll density gradient, Swim up and Simple Washing by centrifugation, on *in-vitro* fertilization (IVF) of goat oocytes. Ovaries obtained from slaughtered goats were used to recover cumulus–oocyte complexes (COCs) by the slicing method, and oocytes were graded based on cumulus cell layers and cytoplasmic characteristics. Grade A and B oocytes were selected for *in-vitro* maturation (IVM) and then subjected to IVF using spermatozoa processed through the three preparation methods. Fertilization efficiency was assessed after *in-vitro* culture. Among the treatments, percoll density gradient yielded the highest cleavage rate (51.11 ± 3.15%), followed by swim up (45.47 ± 4.27%) and simple washing (38.56 ± 3.98%) ($p<0.05$). These findings indicate that sperm preparation technique significantly influences IVF outcome in goats, emphasizing the importance of sperm functional quality over numerical sperm recovery.

Key words: Goat oocytes, *In-vitro* Fertilization, Percoll, Swim up, Sperm preparation.

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Introduction

Assisted reproductive technologies (ART) have greatly improved breeding efficiency and the preservation of genetic resources. However, in goat's reproductive performance is often limited by poor nutrition, reproductive disorders, difficulties in estrus detection, and restricted access to superior males. *In-vitro* fertilization (IVF) offers an effective approach to address these constraints (Luo *et al.,* 2019). *In-vitro* embryo production (IVEP) involves oocyte recovery, maturation, sperm preparation, fertilization, and embryo culture, with sperm preparation being a critical determinant of IVF success. Efficient preparation removes seminal plasma, dead sperm, and debris, ensuring a population of viable and motile spermatozoa suitable for fertilization (Rho *et al*., 2001; Henkel and Schill, 2003). It also helps counteract cryopreservation-induced damage, which can reduce motility, disrupt membranes, and impair fertilizing ability (Watson, 2000).

Several sperm selection techniques are available, and the choice among them depends not only on the number of motile sperm recovered but also on factors such as technical difficulty, required equipment, and time involved (Mortimer, 1994). Commonly used methods include swim up, Percoll density gradient, and simple washing by centrifugation. The swimup method selects motile sperm through upward migration, though recovery is often low. Percoll density gradient separation provides a higher proportion of morphologically normal, motile sperm and has shown improved fertilization outcomes in several species. Simple washing is rapid and practical but offers limited selection capability (Henkel and Schill, 2003). Despite progress in caprine IVEP, inconsistent fertilization and cleavage rates persist, partly due to variation in sperm quality obtained from different preparation methods (Souza-Fabjan *et al*., 2023). Therefore, the present study aimed to compare the effectiveness of Percoll density gradient, swim up, and simple washing techniques on IVF outcomes in goats.

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Materials and Methods

The study was conducted in the Department of Animal Reproduction, Gynaecology and Obstetrics (ARGO), Veterinary College, Bidar, KVAFSU, Karnataka (India). The WASH media, IVM media, IVF media, IVC media, H and PHE, Percoll Conventional and Percoll Diluent purchased from Vitrogen (Brazil), Mineral oil (R045) from HiMedia Laboratories (Mumbai) and all chemicals required were procured from Sigma-Aldrich chemical company.

Oocyte Collection and *In-Vitro* Maturation

Goat ovaries were collected from a local abattoir and transported to the laboratory in warm normal saline (0.9% NaCl; 35-37°C) containing 50 µg/mL gentamicin. In the laboratory, ovaries were rinsed 4-5 times with warm saline supplemented with antibiotics. Cumulus–oocyte complexes (COCs) were recovered by slicing the ovarian tissue in oocyte collection medium under sterile conditions and transferred to WASH media (Vitrogen) droplets. Recovered COCs were examined under a stereo-zoom microscope and graded based on cumulus cell layers and homogeneity of ooplasm as described by Kharche *et al.* (2008). Grade A and B oocytes, having compact multi-layered cumulus and evenly granulated cytoplasm, were selected for IVM, whereas grade C and grade D oocytes were discarded.

Prior to IVM procedure, the IVM medium (Vitrogen) was equilibrated overnight in a CO_2 incubator (5% CO_2, 95% relative humidity, 38.5°C). The 50 µL droplets of maturation medium were prepared in 35 mm sterile petri-dishes and covered with sterile mineral oil. Selected COCs were washed 4-5 times in WASH medium, followed by two washes in IVM medium, and placed into the equilibrated maturation droplets. Oocytes were matured in a humidified CO_2 incubator (5% CO_2, 38.5°C) for 27 h. After 27 h of IVM, the oocytes were assessed for maturation by the degree of cumulus cell mass expansion and extrusion of the 1st polar body into the perivitelline space.

In-Vitro Fertilization

Frozen buck semen with proven *in vivo* fertility purchased from BAIF Development Research Foundation (Pune) was used for this study; two 0.25 mL straws each for swim up, Percoll density gradient and simple washing by centrifugation procedures were used.

On the day before *in-vitro* fertilization, *in-vitro* fertilization media (IVF) (Vitrogen) was prepared by adding 220 µL of PHE aliquot (Vitrogen) and 55 µL of H aliquot (Vitrogen) to 4725 µL of IVF media, making a total of 5 mL. To prepare the *in-vitro* fertilization dish, 100 µL droplets of prepared IVF media were added to a 35 mm sterile petri dish and covered with sterile mineral oil. The dish was then kept for equilibration in CO_2 incubator (5% CO_2 in air, 95% RH, and 38.5°C) overnight.

Percoll density gradient centrifugation was performed as described in the Vitrogen Manual. Frozen-thawed buck semen was processed for IVF using a Percoll density gradient. Thawed semen was layered over a two-step gradient [400 µL of percoll (Vitrogen, Brazil) + 400 µL of diluted percoll (200 µL sperm (Vitrogen, Brazil) + 200 µL percoll) as bottom and top layers, respectively] and centrifuged at 600 g for 6 min at 37°C. The supernatant was discarded, and the remaining 100 µL pellet was resuspended in 400 µL IVF medium and centrifuged again at 150 g for 3 min. After removing the supernatant, the final sperm pellet containing motile spermatozoa was obtained.

Swim up procedure was performed as per Palomo *et al*. (1999) and Singh *et al*. (2016) with some modification. Two 0.25 mL semen straws were emptied into 1 mL Sp-TALP supplemented with BSA (6 mg/mL), sodium pyruvate (1 mM), gentamicin (50 µg/mL) and HEPES (10 mM), and centrifuged at 300 g for 5 min. The supernatant was removed, and the sperm pellet was washed repeatedly with Sp-TALP. After the final wash in fertilization medium, the sperm fraction was placed at the bottom of a conical tube containing IVF medium and incubated at 38.5°C for 1 h to allow motile sperm to swim upward. The upper layer containing actively motile spermatozoa was collected and used for insemination.

Simple washing by centrifugation procedure was prepared as described by Mara *et al*. (2013) with some modification. Two 0.25 mL semen straws were emptied into 1 mL Sp-TALP and centrifuged at 300 g for 5 min. The supernatant was removed, and the sperm pellet was washed repeatedly with Sp-TALP. After the final wash, the sperm suspension was diluted in heparin-containing medium and incubated for 15 min at 38.5°C in a CO_2 incubator to allow capacitation (Palomo *et al*., 1999).

Matured oocytes were washed 3-4 times in WASH media and twice in IVF medium and co-incubated with processed spermatozoa in equilibrated IVF droplets under mineral oil in a CO_2 incubator at 38.5°C. Fertilization was assessed after 18–24 h based on extrusion of the second polar body into the perivitelline space and early cleavage.

After 18-24 h of gamete co-incubation, presumptive zygotes were transferred to WASH medium and mechanically denuded of cumulus cells. The zygotes were washed 3-4 times in WASH and twice in IVC media (Vitrogen), then placed into 100 µL droplets of equilibrated IVC medium overlaid with mineral oil. Culture was carried out at 38.5°C in 5% CO_2 for 5 days post insemination, after which cleavage was recorded.

Statistical Analysis

The statistical analysis was carried out by using one-way ANOVA followed by Duncan's multiple comparisons test using SPSS software.

Results and Discussion

Determination of an Effective Sperm Separation Method for IVF of Matured Oocytes

The effect of sperm preparation methods on the cleavage rate of presumptive zygotes is presented in Table 1.

The fertilization outcome, expressed as the number of presumptive zygotes kept for *in-vitro* culture (IVC), varied significantly among the three sperm preparation methods. In the Percoll density gradient group, 87 oocytes were subjected to IVC and 44 cleaved, yielding a mean cleavage rate of 51.11 ± 3.15%. In the swim up group, 31 of 72 cultured oocytes cleaved, with a mean rate of 45.47 ± 4.27%. The simple washing by centrifugation method with 23 cleavages from 60 oocytes, yielded the lowest mean cleavage rate of 38.56 ± 5.56%. Statistical analysis revealed a significant difference ($p<0.05$) between the Percoll density gradient and simple washing groups, while the swim up group did not differ significantly from either Percoll or simple washing.

In this study, we compared a simple, rapid, and cost-effective simple washing by centrifugation method with other techniques, such as Percoll density gradient and swim up. Although several studies have evaluated these methods individually, very few have examined all three approaches together in relation to the fertilization outcome. The present findings of cleavage rate agreed with Jaakma *et al.* (1997), who found no significant difference between swim up (69.1%) and centrifugation (66.1%), and Palomo *et al.* (1999), who reported cleavage rates for swim up (34.4%) and Percoll (35.9%) with no significant difference. Atalla *et al.* (2019) observed cleavage rates between swim up (79.2%) and Percoll (76.1%) with no significant difference. Rho *et al.* (2001) further demonstrated the advantage of Percoll in goats, with cleavage significantly higher in Percoll (62.4%) than swim up (49.6%) technique ($p<0.05$). In pigs, Matas *et al.* (2003) observed higher cleavage in Percoll (43.5 ± 2.4%) than washing by centrifugation (26.6 ± 2.1%). Similarly, Dode *et al.* (2002) also reported no significant difference among swim up (56.7%), Percoll (51.5%), and washing (47.0%) in the study of cattle.

Rahman *et al.* (2020) found that Percoll yielded significantly higher cleavage (62.3%) than centrifugation (51.6%), and was comparable with swim up (60.8%). Conversely, Mehmood *et al.* (2009) in buffalo found cleavage rates of 66.8% for the swim up method, which showed a significant difference when compared to 55.6% for Percoll ($p<0.001$). Likewise, Ben *et al.* (2013) in yak reported cleavage rates of 73.17 ± 2.10% for bull 1 and 75.83 ± 1.52% for bull 2 for the swim up method, which showed a significant difference when compared to 62.14 ± 2.66% and 67.10 ± 2.20% for Percoll ($p<0.05$).

Table 1: Effect of sperm preparation methods on *in-vitro* fertilization of goat oocytes

Method	Oocytes for IVC	Cleaved	Cleavage rate (%±SE)
Percoll density gradient (n=6)	87	44	51.11 ± 3.15^{b}
Swimup (n=6)	72	31	45.47 ± 4.27^{ab}
Simple Washing by centrifugation (n=5)	60	23	38.56 ± 3.98^{a}

* n=number of replicates. Values bearing different superscripts within the column (a, b) differ significantly ($p<0.05$).

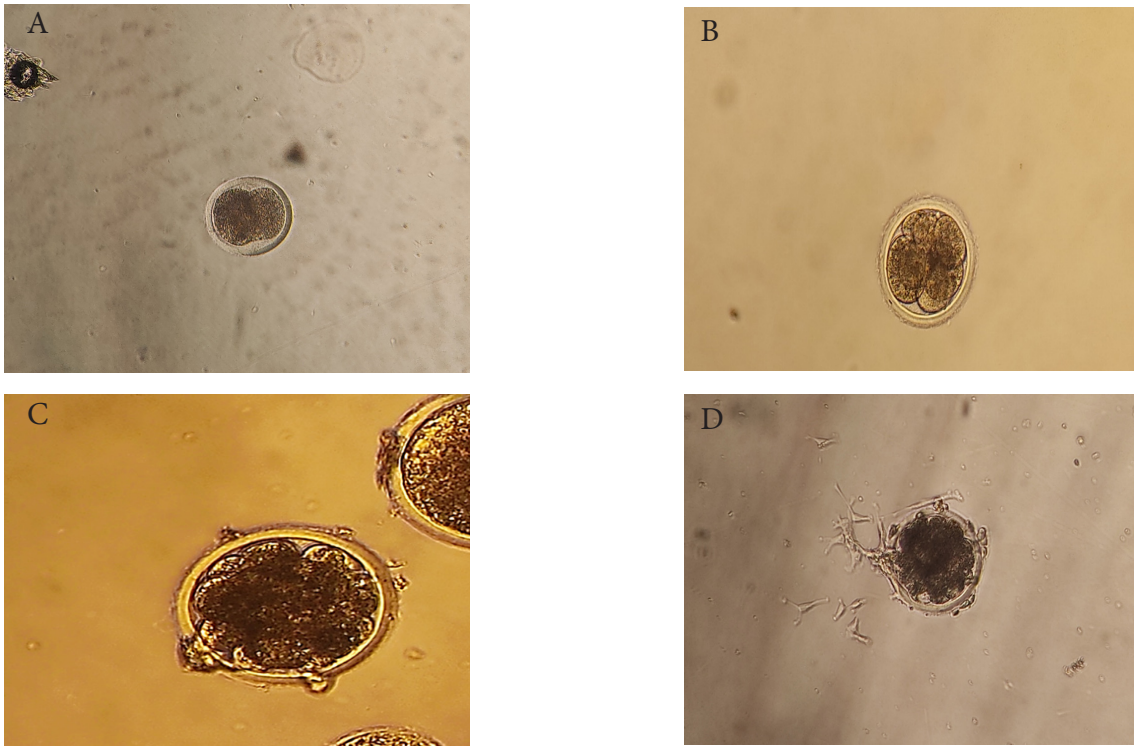

Fig. 1: Cleavage-stage embryos produced after *in-vitro* fertilization showing: (A) 2-cell, (B) 4-8 cell, (C) 8-16 cell and (D) 16-32 cell stages.

The cleavage rate after IVF is considered a reliable indicator of sperm fertilizing ability and is commonly used to validate sperm-viability assays (Ward *et al*., 2003). Successful fertilization depends on multiple functional attributes of spermatozoa, including an intact plasma membrane essential for metabolic activity, survival, and fertilizing potential (Anzar *et al.,* 1997), and preservation of acrosome integrity until zona binding, which ensures timely acrosome reaction and enzyme release required for oocyte penetration (Graham and Moce, 2005). Percoll density gradient separates sperm based on density and morphology, effectively concentrating motile sperm with intact membranes and acrosomes while eliminating immotile and abnormal cells (Rho *et al*., 2001). However, simple washing retains both motile and non-motile sperm (Mehta and Sigman, 2014), while centrifugation may expose functional spermatozoa to defective ones, leading to excessive ROS-mediated membrane damage and compromised fertilization ability (Henkel and Schill, 2003).

Differences in oocyte competence can also account for variation between studies, as less competent oocytes fail to reveal the benefits of sperm preparation methods, whereas competent oocytes are able to translate sperm-quality improvements into higher fertilization and cleavage outcomes (Khatun *et al*., 2011).

Conclusion

From the present findings, it is concluded that, Percoll density gradient selects sperm with higher motility, normal morphology, and functionality, resulting in improved post-thaw sperm quality and fertilization potential. While swim up is a good alternative for sperm preparation although sperm recovery rate is lower than Percoll density gradient, sperm parameters are not affected. In contrast, simple washing recovered more sperm in number, but their lower functional quality reduced IVF efficiency. These findings highlight that sperm quality, rather than quantity, is the key determinant for successful embryo development.

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References

Anzar, M., Graham, E.F., & Iqbal, N. (1997). Post-thaw plasma membrane integrity of bull spermatozoa separated with a Sephadex ion-exchange column. *Theriogenology*, *47,* 845-856.

Atalla, H., Demir, K., Yagcioglu, S., Arici, R., Ersoy, N., Eser, A., Coskun, N., Armutak, E.I., Uvez, A., Evecen, M., Ak, K., Birler, S., & Pabuccuoglu, S. (2019). The effect of swim up and Percoll gradient separation on ram sperm parameters, DNA integrity and embryo development. *Rev Méd Vét*, *170*(4-6), 87-94.

Ben, L., Yan, C., & Si-jiu, Y. (2013). Effect of swim up and percoll treatment on sperm quality and *in vitro* embryo development in yak. *Journal of Integrated Agriculture*, *12*(12), 2235-2242.

Dode, M.A.N., Rodovalho, N.C., Ueno, V.T., & Fernandes, C.E. (2002). The effect of sperm preparation and co-incubation time on *in vitro* fertilization of *Bos indicus* oocytes. *Animal Reproduction Science, 69,* 15-23.

Graham, J.K., & Moce, E. (2005). Fertility evaluation of frozen-thawed semen. *Theriogenology*, *64,* 492-504.

Henkel, R.R., & Schill, W.B. (2003). Sperm preparation for ART. *Reproductive Biology & Endocrinology, 1,* 108.

Jaakma, U., Zhang, B.R., Larsson, B., Niwa, K., & Rodriguez-Martinez, H. (1997). Effects of sperm treatments on the *in vitro* development of bovine oocytes in semi defined and defined media. *Theriogenology*, *48*(5), 711-720.

Kharche, S.D., Yadav, E.N., Goel, A.K., Jindal, S.K., & Sinha, N.K. (2008). Influence of culture media on *in vitro* fertilization of goat oocytes. *Indian Journal of Animal Sciences, 78,* 1075-1077.

Khatun, M., Bhuiyan, M.M.U., Ahmed, J.U., Haque, A., Rahman, M.B., & Shamsuddin, M. (2011). *In vitro* maturation and fertilization of prepubertal and pubertal black Bengal goat oocytes. *Journal of Veterinary Science*, *12*(1), 75-82.

Luo, J., Wang, W., & Sun, S. (2019). Research advances in reproduction for dairy goats. *Asian-Australasian Journal of Animal Science, 32*(8), 1284-1295.

Mara, L., Sanna, D., Casu, S., Dattena, M., & Mayorga Munoz, I.M. (2013). Blastocyst rate of *in vitro* embryo production in sheep is affected by season. *Zygote*, 22, 366-371.

Matas, C., Coy, P., Romar, R., Marco, M., Gadea, J., & Ruiz, S. (2003). Effect of sperm preparation method on *in vitro* fertilization in pigs. *Reproduction*, *125*(1), 133-141.

Mehmood, A., Anwar, M., & Saqlan, S.M. (2009). Motility, acrosome integrity, membrane integrity and oocyte cleavage rate of sperm separated by swim up or Percoll gradient method from frozen-thawed buffalo semen. *Animal Reproduction Science, 111,* 141-148.

Mehta, A., & Sigman, M. (2014). Identification and preparation of sperm for ART. *Urology Clinics of North America, 41,* 169-180.

Mortimer, D. (1994). Sperm recovery techniques to maximize fertilizing capacity. *Reproduction Fertilization & Development, 6,* 25-31.

Palomo, M.J., Mogas, T., & Paramio. M.T. (1999). Effect of semen preparation on IVF of pre-pubertal goat oocytes. *Theriogenology, 51,* 927-940.

Rahman, M.M., Rahman, M.M., Juyena, N.S., & Bhuiyan, M.M.U. (2020). Optimization of *invitro* fertilization technique for oocytes of indigenous zebu cows. *Journal of Animal Reproduction & Biotechnology*, *35,* 142-148.

Rho, G.J., Hahnel, A.C., & Betteridge, K.J. (2001). Comparisons of oocyte maturation times and of three methods of sperm preparation for their effects on the production of goat embryos *in vitro*. *Theriogenology, 56,* 503-516.

Singh, A.P., Kumar, D., Gopalakrishna, R., Ranjan, R.S., Pandey, S.K., & Sarkhel, B C. (2016). Comparison of culture media for their effects on development of caprine IVF embryos using fresh and cryopreserved semen. *Indian Journal of Animal Research, 50*(6), 846-850.

Souza-Fabjan, J.M.G., Leal, G.R., Monteiro, C.A.S., Batista, R.I.T.P., Barbosa, N.O., & Freitas, V.J.F. (2023). *In vitro* embryo production in small ruminants: what is still missing? *Animal Reproduction, 20*(3), e20230055.

Ward, F., Rizos, D., Boland, M.P., & Lonergan, P. (2003). Effect of reducing sperm concentration during IVF on the abilityto distinguish between bulls of high and low field fertility: work in progress. T*heriogenology, 59,* 1575-1584.

Watson, P.F. (2000). The causes of reduced fertility with cryopreserved semen. *Animal Reproduction Science, 60-61*, 481-492.