RESEARCH ARTICLE

Improved *In Vitro* Maturation of Goat Oocytes using Cysteamine and Epidermal Growth Factor-Enriched Media

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Abstract

The current study was performed to see the effect of cysteamine and epidermal growth factor (EGF) on *in vitro* maturation of goat oocytes (n=8516). The four maturation media used in the study were: Control (TCM-199 + FSH 1 µg/mL + 20 IU/mL hCG + 1 µg/mL Estradiol + Gentamicin 50 µg/mL + 10% EGS); Group I (Control + 100 µM/mL Cysteamine); Group II (Control + 20 ng/mL EGF) and Group III (Control + 100 µM/mL Cysteamine and 20 ng/mL EGF), involving 1591, 1252, 1242, and 1214 oocytes, respectively, for 50, 33, 31, and 30 trials. Later the oocytes were incubated in CO_2 incubator for 27 h at 38.5°C, 5% CO_2, and 90-95% relative humidity. The *in vitro* maturation of goat oocytes, assessed based on cumulus expansion (77.02±2.24 to 77.56±1.57 vs 52.63±1.84%) and first polar body extrusion (55.01±3.00 to 64.11 ±1.93 vs 31.64±2.68%) was significantly higher ($p \leq 0.05$) in Group I, II and III compared to Control group. In summary, the addition of cysteamine and epidermal growth factor alone or in combination in the maturation media had a beneficial effect on maturation rate in goat oocytes.

Key words: Cysteamine, Epidermal growth factor, *In vitro* maturation, Goat.

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Introduction

Assisted reproductive technologies (ART) play a vital role in accelerating genetic improvement across animal species. Among these, *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) techniques are gaining increasing attention in small ruminants such as goats and sheep, primarily due to their shorter reproductive cycles, higher fertility, and lower maintenance costs compared to larger livestock (Kharche *et al.,* 2009). The *in vitro* maturation environment is a key determinant of successful fertilization and embryo development. Selecting a suitable oocyte culture medium - typically enriched with serum and hormones - is essential for optimizing IVM and IVF outcomes in goats (Pawshe *et al*., 1996). This medium must support both nuclear and cytoplasmic maturation of oocytes. Additionally, the type and concentration of energy substrates included in the culture system significantly influence the efficiency of the maturation process. Previous studies have shown that supplementing the maturation medium with proteins, follicular fluid, hormones, antioxidants, and growth factors can enhance oocyte competence (Borah *et al.,* 2018). Despite these advancements, limited research exists on the comparative and synergistic effects of combining antioxidants and growth factors in goat oocyte maturation. Therefore, the present study was aimed to evaluate the influence of cysteamine (an antioxidant) and epidermal growth factor (EGF) on the *in vitro* maturation of goat oocytes.

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Materials and Methods

The current study was performed in the Department of Animal Reproduction Gynaecology & Obstetrics, Veterinary College, Bidar, KVAFSU, Karnataka (India) following approval of the protocols by the Institutional Animal Ethics Committee. All chemicals and medias used in the study were procured from Sigma Aldrich. All the maturation media were sterilized by 0.22 µm syringe filters before the procedure.

Ovaries from goats, with an unspecified stage in their reproductive cycle, were sourced from local slaughterhouses in and around the Bidar district (Karnataka). Goat ovaries (n=1330) were collected in a saline solution with gentamicin 50 µg/mL. They were kept at 30-32°C and taken to the lab within 2-3 h after slaughter.

Recovery of Oocytes and Grading

By sterile forceps, the ovaries were securely placed in a sterile petri dish with 5 mL of oocyte collecting media (OCM). The entire ovarian surface was incised with a sterile surgical scalpel blade and cut into thin sections. The oocytes were allowed to settle for 3-5 min before being inspected for the presence of oocytes under a stereo-zoom microscope.

Based on layer of cumulus cells and the characteristics of the ooplasm, the oocytes were selected and categorised into the four groups as described by Kharche *et al.* (2008), *i.e.,* Excellent (Grade A), Good (Grade B), Fair (Grade C), and Poor (Grade D).

In Vitro Maturation

Oocytes of culturable quality (Grade A and B) were matured in four different maturation medium, keeping the TCM 199 as a base media. Control group containing TCM-199 + FSH 1 µg/mL + 20 IU/mL hCG + 1 µg/mL Estradiol + Gentamicin 50 µg/mL + 10% EGS; Group I supplemented with Control + 100 µM/mL Cysteamine; Group II containing Control + 20 ng/mL Epidermal growth factor, and Group III administered with Control + 100 µM/mL Cysteamine and 20 ng/mL Epidermal growth factor.

The maturation media was sterilized by 0.22 µm syringe filter and 50 µL droplets were prepared in a petri dish, covered with nontoxic sterile mineral oil until the drops were submerged, and then incubated in a CO_2 incubator overnight at 38.5°C, 5% CO_2, and 90-95% RH. Grade A and B oocytes were washed 3-4 times in maturation media. Following washing, 6-10 oocytes were cultured in pre-incubated droplets and then kept in a CO_2 incubator for 27 h at 38.5°C, 5% CO_2, and 90-95% RH. Following 27 h of incubation period in the maturation media, the degree of oocyte maturation was assessed microscopically by cumulus cell expansion (cytoplasmic maturation) and first polar body extrusion (nuclear maturation).

Cumulus Expansion (Cytoplasmic Maturation)

Oocyte maturation was evaluated based on the extent of cumulus expansion following 27 h of maturation (Kobayashi *et al*., 1994): Degree 0- No expansion; Degree 1 - Clustered cells were still visible and cumulus cells were not uniformly distributed and Degree 2- Clustered cells disappeared and cumulus cells were uniformly distributed.

Only degree 1 and 2 cumulus expanded oocytes were considered as matured one. The maturation rate was determined by dividing the total number of matured oocytes having degree 1 and 2 by the total number of oocytes used for maturation by specific medium.

First Polar Body Extrusion (Nuclear Maturation)

Following oocyte evaluation based on cumulus cell expansion, cumulus cells were removed by repeated pipetting using a pipette. The existence of the first polar body in the perivitelline space was then recorded by examining each oocyte under a stereo-zoom microscope (Lv *et al*., 2010). The nuclear maturation rate was determined by dividing the total number of oocytes extruded first polar body by total number of oocytes kept for maturation.

Statistical Analysis

The results of *in vitro* maturation of oocytes were presented as mean percentages and analysed using General Linear Model SPSS software followed by Tukey's test.

Results and Discussion

Oocyte Recovery using the Slicing Method

A total 1330 ovaries were used in the study to harvest the oocytes by slicing technique and total oocytes recovered were 8516, thus the average oocytes recovered per ovary was 7.88±0.45. The average yield of oocytes recovered per ovary of excellent, good, fair and poor quality was 19.78%, 42.43%; 18.12 % and 19.65%, respectively (Table 1). It was observed that the mean number of pooled cultured grade (A+B) of oocytes (4.88 ± 0.27) per ovary obtained by slicing method was more as compared to fair and poor quality of oocytes (2.99 ± 0.22).

Similar findings were observed by Mahesh *et al.* (2014), who reported 7.88 ± 0.54 oocytes per ovary in buffalo. Also, Naik *et al*. (2023) noticed 8.55 ± 0.47 oocytes per ovary in goats. In contrast to present findings, Pawshe *et al.* (1994) recorded mean oocyte recovery rate per ovary as 2.40 ± 0.21. Ambili (2010) reported a lower yield of 4.44 ± 0.06 oocytes in

Table 1: Oocyte recovery from goat ovaries by slicing method (Mean ± SE values, n=50 trials or replicates)

Total no. of ovaries	Total no of oocytes recovered	Average oocytes recovered per ovary (Mean ± SE)					Recovery rate of oocytes per ovary (Mean ±SE)
		Grade A- Excellent (1685)	Grade B- Good (3614)	Grade C- Fair (1543)	Grade D- Poor (1674)	Grade A + B (5299)	
1330	8516	1.55 ± 0.11 (19.78 %)	3.33 ± 0.21 (42.43%)	1.42 ± 0.10 (18.12%)	1.58±0.12 (19.65 %)	4.88 ± 0.27	7.88±0.45

goats, whereas Parmar *et al*. (2022) observed a lower recovery of 4.12 ± 0.34 in Jaffarabadi buffalo. Kale *et al.* (2020) found 10.79 ± 1.00 oocytes per ovary in abattoir collected goat ovaries, which was higher than the current findings. Wani *et al*. (2000) recorded good quality of oocytes per ovary (5.2±0.23). Mahesh *et al*. (2014) found culturable grade of oocyte per ovary as 4.06±0.21, with a pool of good and fair quality of oocyte per ovary as 5.40 ± 0.29, and poor quality of oocytes as 2.48 ± 0.17. Conversely, Pawshe *et al.* (1994) recorded very low mean oocyte recovery of good, fair and poor quality (0.91 ±0.06, 0.80±0.07 and 0.69±0.04, respectively).

In vitro embryo development requires a sufficient number of high-quality oocytes per ovary. The major goal of the recovery method is to increase the number of oocytes per ovary, which is necessary for *in vitro* maturation (Rajendra, 2022). Therefore, slicing technique was performed in this study. The higher yield of cumulus–oocyte complexes (COCs) per ovary using the slicing method can be attributed to its ability to release oocytes not only from surface follicles but also from those embedded within the ovarian cortex. In contrast, the aspiration technique primarily targets only superficial follicles (Pawshe *et al*., 1994; Bohlooli *et al.,* 2015).

Additionally, follicular aspiration may result in the loss of some oocytes, a limitation that slicing helps to overcome (Wani *et al*., 2000). Although slicing enables the collection of oocytes from both surface and cortical area, but it also generates a significant amount of cellular debris due to mechanical dissection of ovarian tissue which potentially hinders the oocyte identification and causes damage during the process (Akter *et al.,* 2022). Moreover, oocytes those are firmly attached within small to medium follicles - especially before cumulus expansion begins - are more readily retrieved through slicing, as they may not be effectively collected via aspiration.

Effect of Maturation Media on Maturation Rate in Goat Oocytes

A total of 5299 culturable grade (A+B) oocytes were utilized for *in vitro* maturation (IVM). The impact of various supplements added to the IVM medium (Control, CYST, EGF, and CYST + EGF in 50, 33, 31 & 30 trials or replicates, respectively) on the maturation percentage of goat oocytes is summarized in Table 2. Maturation was evaluated by observing the extent of cumulus cell expansion (Fig. 1), following the criteria described by Kobayashi *et al.* (1994) and extrusion of first polar body (Fig. 2) as per Lv *et al*. (2010).

The maturation rate of goat oocytes, based on cumulus expansion and first polar body extrusion for the Control, Group I (CYST), Group II (EGF) and Group III (CYST + EGF) was 52.63 ± 1.84 vs 31.64 ± 2.68, 77.56 ± 1.57 vs 64.11 ± 1.93, 77.51 ± 1.34 vs 61.66 ± 3.07 and 77.02 ± 2.24 vs 55.01 ± 3.00, respectively. There was a significant ($p \leq 0.05$) difference in the maturation rate (cytoplasmic and nuclear maturation) of Group I (CYST), II (EGF) and III (CYST + EGF) occytes compared to Control group. However, there was no significant ($p \geq 0.05$) difference among the Group I, II and III (Table 2).

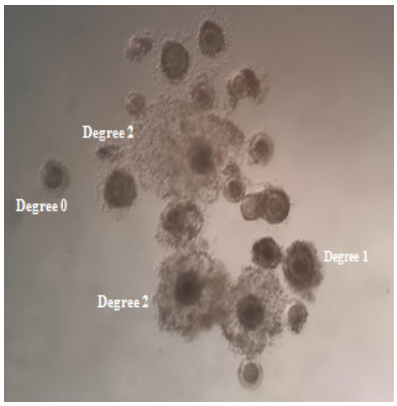

Fig. 1: Cumulus cell expansion

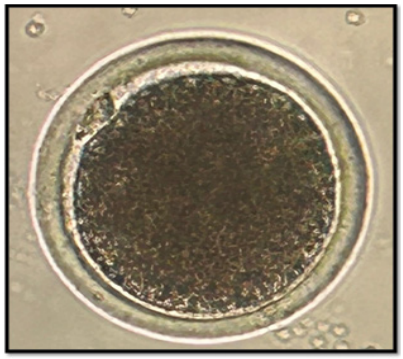

Fig. 2: Polar body extrusion in perivitelline space

The results from the present study are in accordance with Mardenil and Aryan (2014), who observed the maturation rate by cumulus cell expansion as 80.57% in ovine. Bhajoni *et al*. (2018) recorded the cytoplasmic maturation rate and nuclear maturation rate of 82.24 % and 56.82 % in bovines, respectively, with cysteamine supplemention. Ranjbar *et al*. (2019) and Alsalim *et al.* (2020) recorded the maturation percentage about 74 and 80.89±2.17, respectively, in cattle. In contrast to the present findings, Borah *et al*. (2018) documented the mean percent of maturation based on polar body extrusion as 43.03 ± 1.48. Studies have shown that the presence of reactive oxygen species during *in vitro* maturation (IVM) can negatively influence oocyte development (de Matos *et al.,* 2000). Also, it was observed that the intracellular synthesis of GSH was crucial for the cytoplasmic maturation of oocytes (Gordon, 2003). Cysteamine (CYST) act as an antioxidant, improving oocyte maturation outcomes by reducing the oxidative stress to the oocytes (de Matos *et al.,* 1995; Gasparrini *et al.,* 2003), also it increases the GSH levels in matured oocytes (de Matos *et al.,* 1995), which will have positive effect on oocyte development.

In the present study, the oocytes matured in EGF group (Group II) were also significantly greater than in the control group. The result was in parallel with Bhajoni *et al.* (2018), who reported the rate of cytoplasmic maturation about 86.92% in cattle using TCM 199 treated with EGF. Yousef *et al*. (2018) recorded the rate of cumulus cell expansion in buffaloes as 76.96 ± 9.04 %. Baishya *et al.* (2024) documented the rate of cumulus cell expansion and polar body extrusion

Table 2: Rate of maturation of goat oocytes abattoir ovaries using various maturation media (Mean ± SE, %)

Maturation media	No of oocytes set for maturation	Degree of COC expansion					No of oocytes matured		Polar body extruded	
		Degree 0	Percent Mean	Degree 1	Percent Mean	Degree 2	Percent Mean	Degree 1 + 2	Percent Mean	No	Percent Mean
Control	1591 (n=50)	743	47.13 ±1.91^{a}	486	30.24 ±1.19^{a}	362	22.38 ±1.55^{a}	848	52.63 ±1.84^{a}	464	31.64 ±2.68^{a}
Group-I (CYST)	1252 (n=33)	282	22.48 ±1.57^{b}	510	40.11 ±2.00^{b}	460	37.44 ±2.10^{b}	970	77.56 ±1.57^{b}	770	64.11 ±1.93^{b}
Group-II (EGF)	1242 (n=31)	275	22.03 ±1.32^{b}	529	42.25 ±2.10^{b}	438	36.61 ±1.95^{b}	967	77.51 ±1.34^{b}	751	61.66 ±3.07^{b}
Group-III (CYST+EGF)	1214 (n=30)	279	21.58 ±2.24^{b}	487	40.31 ±1.87^{b}	448	38.10 ±1.76^{b}	935	77.02 ±2.24^{b}	636	55.01 ±3.00^{b}

n=Number of trials or replicates, Means ±SEs bearing different superscripts in a column differ significantly ($p<0.05$)

as 77.59±5.48 and 70.00±14.49, respectively. The current findings on the mean percent nuclear maturation of oocytes in Group II was less than the 91.5% maturation rate reported by Bastan *et al*. (2010). EGF, a potent mitogenic protein, activates the mitogen-activated protein kinase (MAPK) pathway during IVM of goat cumulus-oocyte complexes (Gall *et al*., 2005). This interaction begins with EGF binding to its receptor, triggering its intrinsic tyrosine kinase activity, which in turn leads to receptor autophosphorylation and phosphorylation of downstream targets (Carpenter and Cohen, 1990). This sequence of events is crucial for the oocyte to advance to metaphase II (M II) and acquire the capability for fertilization and subsequent development into a mature embryo.

In the present study a combination of cysteamine and EGF in maturation media (Group III) also had a significantly higher oocytes maturation rate compared to control medium. This finding concurred well with Borah *et al.* (2018), who found the maturation rate on cumulus cell expansion and first polar body extrusion as 88.74 ± 1.85 and 61.71± 1.61 % in goat oocytes. Nguyen *et al*. (2024) documented the maturation rate in Co-goat oocytes with 74.33 ± 1.08 %, whereas Kumar *et al.* (2020) recorded the total maturation rate of 91.30±1.27 % in bovine, which was superior from the present work. However, there is scarcity of literature on combined use of EGF and cysteamine. Oyamada and Fukui (2004) investigated the impact of co-supplementing EGF and cysteamine during IVM and reported no significant improvement in nuclear maturation. However, they observed enhanced cleavage rates and embryonic development, which may be attributed to increased GSH production during IVM. Similarly, El-Ratel and Fouda (2016) found significantly improved maturation rates, cleavage outcomes, and blastocyst cell numbers, while reducing the incidence of apoptosis in blastocysts with the addition of 10 ng/mL EGF and 100 µM cysteamine to TCM-199.

Conclusion

In conclusion, this study indicates that the goat oocytes maturation rate was significantly promoted by addition of both Cysteamine and EGF alone or in combination in the maturation media, *i.e.,* it had a beneficial effect on *in vitro* maturation of goat oocytes.

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