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Nuclear maturation of abattoir derived cumulus oocyte complexes in goats

Ashitha Suresh, MK Shukla, Dharmendra Kumar, OP Shrivastava and BC Sarkhel

Abstract
The present experiment was undertaken to study oocyte retrieval from abattoir derived goat ovaries and to study their nuclear maturation for *in vitro* embryo production. A total of 348 abattoir derived goat ovaries were subjected to aspiration technique for oocyte retrieval resulting in recovery of 1035 cumulus oocyte complexes (COCs) from 1422 follicles with an average of 3.20±0.32 COCs per ovary and recovery rate of 73.93±1.49. The nuclear maturation rate of 89.78±0.50 was also observed in *in vitro* matured goat oocytes after staining with Hoechst 33342 dye.

Keywords: Cumulus oocyte complex, nuclear maturation, *In vitro* maturation, goat

Introduction
Since the first report of *in vitro* fertilization in goats by Hanada (1985) [1], the technique of *in vitro* embryo production has been the subject of intense study. It is an excellent source of low-cost embryos for all kinds of research which require large numbers of embryos (Cognié *et al*., 2003) [2]. The availability of substantial number of acceptable quality oocytes is a major issue in goat IVP research laboratories (Rahman *et al.* 2007) [11]. The cumulus oocyte complexes (COCs) obtained from slaughtered animals has been found highly variable in their developmental competence after *in vitro* maturation (IVM) (Bilodeau-Goeseelsand Panich 2002) [12]. Although the mammalian ovary contains thousands of oocytes, only a small proportion of these oocytes can be used for *in vitro* embryo production with the currently available methods (Pawshe *et al*., 1994) [3]. The use of slaughterhouse ovaries as a source of oocytes has led to the development of *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) techniques. Oocytes can be recovered from slaughterhouse ovaries by follicle dissection, aspiration or slicing. Aspiration of follicles from the ovaries is the most economic and convenient technique for oocyte retrieval (Martino *et al*., 1994) [4].
Oocytes gradually and sequentially acquire capacity to undergo *in vitro* maturation, *in vitro* fertilization and competence for further embryonic development during the course of folliculogenesis as they grow and their companion somatic cells differentiate (Eppig *et al*., 1994) [5]. The present study was undertaken to study the oocyte retrieval from abattoir- derived goat ovaries and the nuclear maturation rate of the acquired oocytes.

Materials and Methods
The study was carried out with oocytes retrieved from ovaries collected from local small animal slaughterhouse at Jabalpur, Madhya Pradesh as per the procedure described below. All the chemicals, media and reagents used in the experiment were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) unless otherwise indicated.

Collection and grading of oocytes
Goat cumulus oocyte complexes (COCs) were obtained from ovaries of goats of unknown genetics and reproductive status slaughtered at a small animal abattoir in Jabalpur, Madhya Pradesh, India. The ovaries were transported to the laboratory within 2-3 hours of slaughter in sterile warm (37 ºC) normal saline (0.9% NaCl) solution supplemented with 0.06 mg/ml penicillin and 0.1% streptomycin sulphate.
Aspiration of COCs was done from 3-8 mm follicles using a 20 gauge needle attached to a 10 ml syringe according to previously described technique in our laboratory (Kumar *et al*., 2016) [6]. The aspirate was transferred to a gritted petridish containing Dulbecco's phosphate buffer

saline (DPBS). COCs were searched and graded under a stereo zoom microscope and the isolated COCs were placed in 50 µl droplet of TCM-199 medium in 35 mm petridish.

***In vitro* maturation of oocytes**

In vitro maturation of the oocytes was done as per the technique previously described for the caprine species in our laboratory (Kumar *et al*., 2016; Suresh *et al*., 2018) [6, 17]. Briefly, the COCs were washed three times in *in vitro* maturation medium TCM-99 enriched with 0.8 mM sodium pyruvate, 10 µg/ml FSH, 10 µg/ml LH, 1 µg/ml oetsradiol, 7.5% v/v FBS (Hyclone) and 50 µg/ml gentamicin. The 50 µl droplets of maturation medium were prepared in a 35 mm petri dish and oocytes were transferred in groups of 20-25 oocytes per droplet. The droplets were then covered with sterile pre-equilibrated (38.5°C) mineral oil and incubated in CO_2 incubator with >95% humidity, 5% CO_2 at 38.5°C for 27 hours. For the assessment of nuclear maturation, the matured COC's were denuded bytreating with 0.1% hyaluronidase. To stain the denuded oocytes, they were washed with PBS and treated with Hoechst 33342 stain (10 µg/ml) for 10 min in a dark chamber. The oocytes were kept on a glass slide with a coverslip and examined under inverted fluorescence microscope for the presence of polar body. The percent of oocytes showing extrusion of first polar body on fluorescent microscopy were considered to have undergone nuclear maturation.

Statistical Analysis

The data pertaining to number of follicles per ovary, cumulus oocyte complexes (COCs) per ovary, recovery rate and per cent nuclear maturation were subjected to standard statistical procedure for calculation of mean and standard error using statistical software Systat version 11.

Results

A total of 13 replicates of the experiment were taken for the study of oocyte recovery and maturation rate. Oocytes were retrieved from abattoir ovaries by aspiration. A total of 1422 follicles were aspirated from 348 ovaries. The average number of follicles per ovary was 4.35±0.43. A total 1035 cumulus oocyte complexes (COCs) aspirated with an average of 3.20±0.32 COCs per ovary. The mean recovery rate was found to be 73.93±1.49% (Table 1). Out of 793 COCs set for *in vitro* maturation, 89.78±0.50 percent COCs showed nuclear maturation as assessed by extrusion of first polar body and visualized under fluorescent microscopy after staining with Hoechst 33342.

Table 1: Study of oocyte recovery and maturation rate in goats

Sr. No.	Parameters	Number/ Mean ±SE
1	Number of replicates	13
2	Number of ovaries	348
3	Number of follicles	1422
4	Number of follicles per ovary	4.35±0.43
5	Number of Cumulus Oocyte Complexes (COCs)	1035
6	Cumulus Oocyte Complexes (COCs) per ovary	3.20±0.32
7	Recovery rate (%)	73.93±1.49
8	Nuclear maturation	89.78±0.50

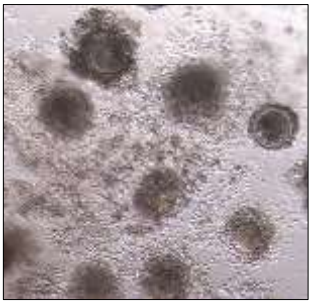

Fig 1: *In vitro* matured goat oocytes

Fig 2: Visualization of first polar body extrusion by Hoechst 33342 staining

Discussion

The oocytes in the present study were obtained by the follicular aspiration technique. This method has been reported to result in higher recovery of quality oocytes (Shirazi *et al*., 2005 and Wang *et al*., 2007) [7, 8]. A total of 1035 cumulus oocyte complexes (COCs) were retrieved with an average of 3.20±0.32 COCs per ovary and oocyte recovery rate of 73.93±1.49%. These results corroborate the findings of oocyte retrieval reported by various workers in the past (Yadav *et al*., 1998; Shirazi *et al*., 2005; Hoque *et al*., 2011) [9, 7, 10]. Similarly, lower oocyte yield from abattoir derived goat ovaries has also been reported in the past (Singh *et al*., 2013) [13]. The variations may be due to differences in pubertal status of the animals (Martino *et al*., 1994) [4], presence of corpus luteum (Islam *et al*., 2007) [14] and technique of oocyte retrieval. A lower number of COCs retrieved by the aspiration technique may also be due to the presence of some follicles embedded deeply within the cortex, which cannot be harvested by aspiration (Hoque *et al*., 2011) [10]. Overall, the aspiration technique was found to be satisfactory for retrieval of oocytes for *in vitro* embryo production.

Developmental competence of caprine oocytes has been previously studied by various workers including Kumar *et al*., 2016 [6] who reported a significantly higher ($P \leq 0.01$) number of grade A oocytes (95.25±0.82%) showed extrusion of polar body as compared to grade-B oocytes (90.68±0.5%). The *in vitro* maturation rate of oocytes depends on the ability to identify good quality oocytes prior to *in vitro* culture (Kharche *et al*., 2011) [15]. The integrity of the cumulus oocyte complex (COC), defined by cumulus cell density and homogeneity of oocyte cytoplasm are the main criteria for selection of oocytes (De Souza-Fabjan *et al*., 2014) [16]. In the

present study, oocytes were evaluated and graded morphologically based on the number of cumulus cell layers and ooplasm homogeneity. The nuclear maturation, as indicated by extrusion of first polar body was assessed by Hoechst staining method.

In conclusion, oocytes for *in vitro* embryo production can be easily and conveniently recovered by the follicular aspiration technique. Evaluation and grading of oocytes using parameters of number of cumulus cell layers and ooplasm homogeneity leads to satisfactory *in vitro* maturation rates.

References

1. Hanada A. *In vitro* fertilization in goats. Japanese Journal of Animal Reproduction 1985;31:21-26.
2. Cognié Y, Baril G, Poulin N, Mermillod P. Current status of embryo technologies in sheep and goat. Theriogenology 2003;59:171-188.
3. Pawshe CH, Totey SM, Jain SK. A comparison of three methods of recovery of goat oocytes for *in vitro* maturation and fertilization. Theriogenology 1994;42(1):117-125.
4. Martino A, Palomo MJ, Mogas T, Paramio MT. Influence of the collection technique of prepubertal goat oocytes on *in vitro* maturation and fertilization. Theriogenology 1994;42(5):859-873.
5. Eppig JJ, Schultz RM, O'Brien M, Chesnel F. Relationship between the developmental programs controlling nuclear and cytoplasmic maturation of mouse oocytes. Developmental Biology 1994;164(1):1-9.
6. Kumar D, Jain SK, Sarkhel BC. A comparison of *in vitro* fertilization potential of sperm obtained from refrigerated (4°C) epididymis and fresh ejaculation of buck under different embryo culture media. Indian Journal of Animal Sciences 2016;86(8):883-886.
7. Shirazi A, Shams-Esfandabadi N, Hosseini SM. A comparison of two recovery methods of ovine oocytes for *in vitro* maturation. Small Ruminant Research 2005;58(3):283-286.
8. Wang ZG, Xu ZR, Yu SD. Effects of oocyte collection techniques and maturation media on *in vitro* maturation and subsequent embryo development in Boer goat. Czech Journal of Animal Science 2007;52(1):21-25.
9. Yadav PS, Saini A, Kumar A, Jain GC. Effect of oviductal cell co-culture on cleavage and development of goat IVF embryos. Animal Reproduction Science 1998;51(4):301-306.
10. Hoque SM, Kabiraj SK, Khandoker MY, Mondai A, Tareq KMA. Effect of collection techniques on cumulus oocyte complexes (COCs) recovery, *in vitro* maturation and fertilization of goat oocytes. African Journal of Biotechnology 2011;10(45):9177-9181.
11. Rahman ANMA, Abdullah RB, Embong WKW. Goat embryo development from *in vitro* matured oocytes of heterogeneous quality through intracytoplasmic sperm injection technique. Biotechnology 2007;6:373-382.
12. Bilodeau-Goeseels S, Panich P. Effects of oocyte quality on development and transcriptional activity in early bovine embryos. Animal Reproduction Science 2002;71:143-155.
13. Singh LR, Rao KS, Mohan KM. Oocyte retrieval methods, grade and percentage of oocytes in goats. International Journal of Molecular Veterinary Research 2013;3(2):4-6.
14. Islam MR, Khandoker MAMY, Afroz S, Rahman MGM, Khan RI. Qualitative and quantitative analysis of goat ovaries, follicles and oocytes in view of *in vitro* production of embryos. Journal of Zhejiang University Science B 2007;8(7):465-469.
15. Kharche SD, Goel P, Jha BK, Goel AK, Jindal SK. Factors influencing *in vitro* embryo production efficiency of caprine oocytes: A review. Indian Journal of Animal Sciences 2011;81(4):344-61.
16. De Souza-Fabjan JMG, Panneau B, Duffard N, Locatelli Y, De Figueiredo JR, Freitas VJF *et al*. *In vitro* production of small ruminant embryos: Late improvements and further research. Theriogenology 2014;81(9):1149-1162.
17. Suresh A, Verma N, Kumar D, Shukla MK, Shrivastava OP. Simulated physiological oocyte maturation augments the developmental competence of *in vitro* produced goat embryos. In proceedings of XXXIV Annual Convention and International Symposium of ISSAR on Productivity enhancement through augmenting reproductive efficiency of livestock for sustainable rural economy, 28-30th December, Anand 2018, 63.