Journal of Zhejiang University SCIENCE B
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

# Qualitative and quantitative analysis of goat ovaries, follicles and oocytes in view of in vitro production of embryos

ISLAM M.R.[†1], KHANDOKER M.A.M.Y.[2], AFROZ S.[2], RAHMAN M.G.M.[2], KHAN R.I.[3]

([1]*Animal Division, National Institute of Biotechnology (NIB), Atomic Energy Research Establishment (AERE), EPZ, Savar, Dhaka-1349, Bangladesh*)

([2]*Reproductive Biotechnology Laboratory, Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh*)

([3]*Department of Animal Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh*)

[†]E-mail: mrislamnib_ad@yahoo.com

Received Dec. 21, 2006; revision accepted May 17, 2007

**Abstract:** Goat ovaries were collected from the slaughterhouse and categorized as right, left, corpus luteum (CL)-present and -absent group and evaluated on the basis of weight (g), length (cm), width (cm), number of follicles, follicles aspirated and number and state of cumulus-oocyte-complexes (COCs). Comparatively higher weight [(0.66±0.02) vs (0.64±0.02) g], length [(1.17±0.02) vs (1.11±0.02) cm] and width [(0.77±0.02) vs (0.74±0.02) cm] were found in right ovaries than those of left. On the other hand significantly ($P$<0.05) higher weight [(0.71±0.03) vs (0.64±0.01) g] and width [(0.76±0.03) vs (0.75±0.01) cm] were found in CL-present group than those of CL-absent group of ovaries. The left ovaries contained comparatively higher number of normal COCs [(1.06±0.09) per ovary] than right ovaries [(1.03±0.10) per ovary] and the similar trend was found in total number of follicles [(4.51±0.25) vs (4.30±0.23) per ovary] and follicles aspirated [(2.55±0.14) vs (2.52±0.12) per ovary]. But the total COCs per ovary was almost similar in both ovaries [right and left: (1.85±0.12) and (1.85±0.11) per ovary, respectively]. Higher number of total COCs [(1.87±0.09) vs (1.76±0.16) per ovary], total number of follicles [(4.45±0.19) vs (4.16±0.37) per ovary], follicles aspirated [(2.55±0.10) vs (2.48±0.21) per ovary] and normal COCs [(1.12±0.07) vs (0.76±0.14) per ovary] were found in CL-absent group than those of CL-present group of ovaries.

**Key words:** Goat ovary, Follicles, Cumulus-oocyte-complexes (COCs), In vitro production (IVP)
**doi:**10.1631/jzus.2007.B0465 **Document code:** A **CLC number:** Q954.4

## INTRODUCTION

For successful in vitro production (IVP) of embryos, the evaluation of ovaries, the efficient collection and grading of oocytes is very important. Extensive research on in vitro maturation (IVM), in vitro fertilization (IVF) and in vitro culture (IVC) of the resulting zygotes has so far been reported (Cognie *et al*., 2003) but limited information so far has been found on the evaluation of goat ovaries, the efficient collection and grading of oocytes. Rahman *et al*.(1977) reported that right ovary is heavier (0.90 g) than the left ovary (0.85 g), and the length of right ovary (1.30 cm) is lower than the left ovary (1.35 cm). Although, several methods of oocytes recovery have been described, such as slicing of the ovaries, flushing the follicles with phosphate buffered saline (PBS) or rupturing the isolated follicles and the later one may increase the number of recovered oocytes as compared with that of aspiration of follicular materials (Alm and Torner, 1994). The average number of quality oocytes recovered from ovaries without corpora lutea was more as compared to the ovaries with corpora lutea, which can be effectively used for IVF (Kumar *et al*., 2004). Average number of normal follicles was reportedly significantly higher in normal breeder than acyclic, cyclic but not conceived and post partum anestrous Black Bengal does (Salim, 2004). Oocytes

recovered from large antral follicles have the ability to develop to the blastocysts stage compared to small and medium follicles (Crozet *et al*., 1995). Fulka and Okolski (1981) reported that compact COCs matured in vitro and subsequent studies further confirmed the observation (Zhang *et al*., 1989). Eckert and Niemann (1995) divided the collected COCs into two morphological categories: COCs with a homogenous evenly granulated cytoplasm possessing at least three layers of compact cumulus cells designated as category I and COCs with less than three layers of cumulus cells and partially denuded possessing a homogenous evenly granulated cytoplasm as category II and better results were found with the former category of COCs. In addition it has been shown that a comparable higher maturation rate could be reached within 24 h of culture if the oocytes had a compact cumulus investment. Denuded oocytes or oocytes with few cumulus cells are usually rejected because of their low capacity of fertilization and/or in vitro development (Leibfried-Rutledge *et al*., 1986). From that stand point this present research work has been undertaken for collection and evaluation of slaughterhouse goat ovaries, follicles and COCs with the view of IVP of embryos.

## MATERIALS AND METHODS

The experiment was conducted at the Reproductive Biotechnology Laboratory, from Jan. 2004 to May 2005 under the Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh.

### Collection and processing of ovaries

Ovaries were collected from local slaughterhouse with their reproductive history being unknown. The ovaries were then recorded as right, left and the presence or absence of corpus luteum (CL) was also recorded. They were then kept in collection vial containing 0.9% physiological saline in a thermo flask at 25 °C to 30 °C and transported to the laboratory within 4 to 5 h of slaughter. The ovaries were then transferred to sterilized petridishes and rinsed thoroughly by physiological saline at 25 °C before further processing.

### Measurement of weight, length and width

After trimming individually right, left, CL-present and -absent ovaries were weighed and the weight in gram was recorded in tabular form. The length and width in cm of the right, left, CL-present and -absent group ovaries were measured with the help of a measuring scale.

### COCs aspiration and grading

The numbers of visible follicles on the surface of different category of ovaries were counted and recorded. The ovaries were washed 2 to 3 times in saline solution at 30 °C. Ten milliliters syringe was loaded with PBS (1~1.5 ml), and the needle (19 G) was put in the ovary parenchyma near the vesicular follicles of more than 2 mm diameter and all follicles were aspirated near the point. After aspirating the follicles from one ovary, the aspirated follicular materials were transferred slowly into a 90-mm petridish, avoiding damage of the cumulus cells and the COCs were searched and graded under microscope at low magnification. The COCs were classified according to the slight modification of the method of Khandoker *et al*.(2001) into 2 grades, normal: oocyte completely surrounded by cumulus cells; abnormal: oocyte partially surrounded by cumulus cells or completely denuded. The numbers of different grades of COCs in each category were recorded. In the meantime another petridish of Dulbeccos phosphate buffered saline (D-PBS) was prepared for pooling COCs and the COCs were picked up with an appropriate glass micropipette.

The tip diameter of the pipette was checked under the microscope to ensure COCs, which could be easily aspirated without damaging the cumulus cells. Basically the glass micropipettes were prepared slowly stretching the tip of Pasteur pipette above burners flame. Then the picked up COCs were washed 2 to 3 times into D-PBS.

All values were expressed as mean±*SE*. Statistical significance of differences between different parameters was evaluated by using Students unpaired *t*-test. The statistical analysis was done by SPSS (Statistical Package for Social Sciences, SPSS Inc., 1999; Microsoft Corporation, 1998) windows package and MINITAB.

## RESULTS AND DISCUSSION

From local slaughterhouses, goat ovaries were collected and recorded as right and left. On the presence or absence of corpus luteum (CL) they were also categorized as CL-present or -absent group. Among 220 ovaries CL was found in 65 ovaries with the remaining 155 ovaries having no CL. The result of the different parameters is summarized in Tables 1 and 2.

In different categories of ovaries the mean weight, length and width were distinctly higher (Table 1) in right ovaries [(0.66±0.02) g, (1.17±0.02) cm and (0.77±0.02) cm respectively] compared to that of left ovaries [(0.64±0.02) g, (1.11±0.02) cm and (0.74±0.02) cm respectively]. The mean weight, length and width in the present study were found higher in right ovaries than those of left ovaries. Normal physiological explanation of ovarian activity is that right ovaries are more active than left ones, according to previous reports (Singh *et al*., 1974; Rahman *et al*., 1977; Sarkar, 1993). In the present study, values that have not reached statistical significance may be ascribable to the species.

On the other hand, the mean weight was significantly higher ($P<0.05$) and width was comparatively higher in the ovaries (Table 2) with CL [(0.71±0.03) g and (0.76±0.03) cm respectively] than those of ovaries without CL [(0.64±0.01) g and (0.75±0.01) cm respectively], while the mean lengths were found higher in the ovaries without CL [(1.14±0.02) cm]. The result is very usual as the hypertrophy of luteinized granulosa cells, hyperplasty of fibroblasts of the connective tissues and vascularity contribute to an increase in size of the CL (Jablonka-Shariff *et al*., 1993). The maximum diameter of CL is reached 6~9 d after ovulation and then regression starts between days 13 and 16 in ewes if maternal recognition does not occur (Jablonka-Shariff *et al*., 1993).

A total of 968 follicles were recorded on the surface of the ovaries and out of this 557 aspirable (>2 mm diameter) follicles were aspirated from right and left ovaries [277 were obtained with a mean of 2.52±0.12 per ovary (Table 1) from right and 280 from left ovaries with a mean of (2.55±0.14) per ovary]. The collected COCs number was found to be almost the same in right and left ovaries (Table 1). When the COCs were classified into normal and abnormal groups, the higher numbers of normal COCs were recorded in left [(1.06±0.09) per ovary] than that of right ovary [(1.03±0.10) per ovary]. The total numbers of COCs were found almost similar in both ovaries with a mean of 1.85 COCs per ovary which is lower than the 4.00 oocytes per ovary reported by Wahid *et al*.(1992) in sheep, 2.17 oocytes per ovary by Datta *et al*.(1993) in sheep and 2.75 oocytes per ovary by Das *et al*.(1996) in bovine and these types of variation or lower number of COCs per ovary obtained in this study might be due to the non-cyclicity of the animals.

In another case, 557 follicles were aspirated out of 968 follicles on the surface of ovaries from CL group and from without CL group. Comparatively higher number of follicles were aspirated from

**Table 1 Qualitative and quantitative parameters in right and left ovaries**

| Ovary (*n*) | Weight (g) | Length (cm) | Width (cm) | *TNFS* | *NFA* | Collected COCs per ovary | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Total | Normal | Abnormal |
| Total (220) | 0.65±0.01 | 1.14±0.02 | 0.75±0.01 | 4.40±0.17 | 2.53±0.09 | 1.85±0.08 | 1.06±0.06 | 0.79±0.06 |
| Right (110) | 0.66±0.02 | 1.17±0.02 | 0.77±0.02 | 4.30±0.23 | 2.52±0.12 | 1.85±0.12 | 1.03±0.10 | 0.82±0.09 |
| Left (110) | 0.64±0.02 | 1.11±0.02 | 0.74±0.02 | 4.51±0.25 | 2.55±0.14 | 1.85±0.11 | 1.06±0.09 | 0.75±0.09 |

Data are express as mean±*SE*; TNFS: Total number of follicles in the surface per ovary; NFA: Number of follicle aspirated per ovary; Figures in the parenthesis indicate the total number of ovary by ovaries

**Table 2 Qualitative and quantitative parameters in corpus luteum-present and -absent groups of ovaries**

| Ovary (*n*) | Weight (g) | Length (cm) | Width (cm) | *TNFS* | *NFA* | Collected COCs per ovary | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Total | Normal | Abnormal |
| Total (220) | 0.65±0.01 | 1.14±0.02 | 0.75±0.01 | 4.40±0.17 | 2.53±0.09 | 1.85±0.08 | 1.06±0.06 | 0.79±0.07 |
| CL-present (65) | 0.71±0.03$^{*}$ | 1.13±0.04 | 0.76±0.03 | 4.16±0.37 | 2.48±0.21 | 1.76±0.16 | 0.76±0.14$^{*}$ | 1.00±0.17 |
| CL-absent (155) | 0.64±0.01$^{**}$ | 1.14±0.02 | 0.75±0.01 | 4.45±0.19 | 2.55±0.10 | 1.87±0.09 | 1.12±0.07$^{**}$ | 0.74±0.07 |

Data are express as mean±*SE*; TNFS: Total number of follicles in the surface per ovary; NFA: Number of follicle aspirated per ovary; $^{*}$ and $^{**}$: Means with different superscripts differ significantly from each other within the same column ($P<0.05$); Figures in the parenthesis indicate the total number of ovary by ovaries

CL-absent ovaries than from CL-present group with a mean of (2.55±0.10) and (2.48±0.21) follicles per ovary respectively (Table 2). The causes of higher number of follicles found in CL-absent group ovaries than those of CL group were understood well as it fits the endocrinological explanation. It is well established that all female mammals are born with a large number of follicles which rapidly decline as puberty approaches; but whether this early losses represent a mechanism of physiological wastage or not, is not definitely known. Follicle growth initiation is one of the most important and least understood aspects of ovarian biology and represents a major challenge for experimental study. When compared with the total number of COCs the higher numbers of COCs were obtained in CL-absent group ovaries compared to CL group, with a mean of (1.87±0.09) and (1.76±0.16) oocytes per ovary respectively. It was also found that higher numbers of normal COCs were obtained from CL-absent group ovaries than from CL group, with a mean of (1.12±0.07) and (0.76±0.14) COCs per ovary respectively. An interesting result was obtained in average number of collected COCs per ovary. The significantly ($P$<0.05) higher number of normal COCs was obtained in CL-absent group than in CL-present group (Table 2). The negative effect of progesterone might not be effectively functional in this group. So the higher number of COCs in this category than that of CL functional group explains the role of hormonal balance on goat folliculogenesis. Within the category, the higher number of normal COCs than that of abnormal COCs further supports the above statement. The findings of CL-absent group ovaries explain the role of progesterone on goat follicular degeneration and further strengthen the previous statement. Total number of COCs was found significantly ($P$<0.05) higher in CL-absent group ovaries with a mean of 2.55 COCs per ovary which is lower than those of previous reports in ovine and bovine. The results further confirmed the species variation.

The less reproductively performing goats are usually slaughtered and most of them might be non-cyclic. So there is the possibility to get more non-cyclic ovaries from the slaughterhouse. The less number of CL group ovaries obtained in this experiment further supports the above statement.

Finally, it can be concluded that the CL-absent group ovaries comprise higher number as well as superior quality of COCs than CL-present group ovaries and the CL-absent group ovaries can be used to collect the quality COCs for IVP of goat embryos.

## References

Alm, H., Torner, H., 1994. In vitro maturation of horse oocytes. *Theriogenology*, **42**(2):345-349. [doi:10.1016/0093-691X(94)90279-8]

Cognie, Y., Baril, G., Poulin, N., Mermillod, P., 2003. Current status of embryo technologies in sheep and goat. *Theriogenology*, **59**(1):171-188. [doi:10.1016/S0093-691X(02)01270-0]

Crozet, N., Ahmed, A.M., Dubos, M.P., 1995. Developmental competence of goat oocytes from follicles of different size categories following maturation, fertilization and culture in vitro. *J. Reprod. Fert.*, **103**(2):293-298.

Das, S.K., Chauhan, M.S., Palta, P., Katiyar, P.K., Madan, M.L., 1996. Replacement of fetel bovine serum and FSH with buffalo follicular fluid in in vitro maturation of buffalo oocytes. *Theriogenology*, **45**(1):245. [doi:10.1016/0093-691X(96)84718-2]

Datta, T.K., Goswami, S.L., Das, S.K., 1993. Comparative efficiency of three oocyte recovery methods from sheep ovaries. *Indian J. Anim. Sci.*, **63**(11):1178.

Eckert, J., Niemann, H., 1995. In vitro maturation, fertilization and culture to blastocyst of bovine oocytes in protein-free media. *Theriogenology*, **43**(7):1211-1225. [doi:10.1016/0093-691X(95)00093-N]

Fulka, J.Jr, Okolski, A., 1981. Culture of horse oocytes in vitro. *J. Reprod. Fert.*, **61**(1):213-215.

Jablonka-Shariff, A., Grazul-Bilska, A.T., Redmer, D.A., Reynold, L.P., 1993. Growth and cellular proliferation of ovine corpora lutea throughout the estrous cycle. *Endocrinology*, **133**(4):1871-1879. [doi:10.1210/en.133.4.1871]

Khandoker, M.A.M.Y., Imai, K., Takahashi, T., Hashizume, K., 2001. Role of gelatinase on follicular atresia in the bovine ovary. *Biol. Reprod.*, **65**(3):726-732. [doi:10.1095/biolreprod65.3.726]

Kumar, N., Paramasivan, S., Sood, P., Singh, M., 2004. Micrometry of different category oocytes recovered from goat ovaries. *Indian J. Anim. Sci.*, **74**(3):259-260.

Leibfried-Rutledge, M.L., Critser, E.S., First, N.L., 1986. Effects of fetal calf serum and bovine serum albumin on in vitro maturation and fertilization of bovine and hamster cumulus-oocyte complexes. *Biol. Reprod.*, **35**(4):850-857. [doi:10.1095/biolreprod35.4.850]

Rahman, A., Hossain, A., Ahmed, M.V., Sen, M.M., 1977. Studies on some reproductive performances and biometry of the female genital tract of Black Bengal goat. *J. Anim.*

*Sci.*, **47**:714-725.

Salim, M.S., 2004. Causes of Infertility in Black Bengal Does Investigated by Anatomical and Histological Tools. Master of Science Thesis Submitted to the Dept. of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Sarkar, M.K., 1993. Studies on the Incidence of Reproductive Abnormalities in Female Goat (*Capra hircus*). Master of Science Thesis Submitted to the Dept. of Anatomy, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Singh, S.K., Bhattacharay, A.R., Luktura, S.N., 1974. Studies on biometry of genital organs of female goat. *Indian Vet. J.*, **51**(1):81-85.

Wahid, H., Gordan, I., Sharif, H., Lonergan, P., Monaghan, P., Gallagher, M., 1992. Effect and efficiency of recovery methods for obtaining ovine follicular oocytes for in vitro procedures. *Theriogenology*, **37**(1):318. [doi:10.1016/0093-691X(92)90387-7]

Zhang, J.J., Boyle, M.S., Allen, W.R., Galli, C., 1989. Recent studies on the in vitro fertilization of in vitro matured horse oocytes. *Equine. Vet. J.*, **8**(Suppl.):101-104.