Journal of Applied Biological Sciences 5 (3): 27-31, 2011
ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

Morphological Evaluation of Ovary in Relation to Recovery, Quality of Oocytes and Steroid Production in Sheep

Mohamed A.M. ALSAFY [1] K.H. EL-SHAHAT[2]

[1]Anatomy & Embryology department, Fac.Vet. Med., Alex. Univ., EGYPT

[2]Theriogenology department, Fac.Vet. Med., Cairo Univ., EGYPT

*Corresponding Author
e-mail:Safy73@yahoo.com

Received: May 27, 2011
Accepted: May 31, 2011

Abstract

The purpose of current study was to evaluate the effect of follicles, corpus luteum (CL) and steroid production (progesterone and estradiol) on the morphology of sheep ovaries, with the view of in vitro recovery, quality, and maturation of oocytes. Fifty ovarian pairs grouped into right, and left, CL bearing and non-CL -bearing ovaries were used in the current study. The weight, length, width and thickness of ovaries were recorded. The follicles were classified into 3 groups small (>2mm), medium (2-4mm) and large (>4mm) follicles. Oocytes were classified according to their morphology into 3 grades COCS (Compact cumulus oocyte complexes), POCS (Partially invested with less than three layers of cumulus cells), DO (denuded oocyte). The concentration of progesterone and estradiol 17β in the follicular fluid were estimated. The dimensions of ovaries between right and left ovaries were not significantly different. However, the ovarian dimensions as well as their weight was significantly ($P < 0.05$) affected by presence of CL, being higher in CL bearing ovary than that of non-CL bearing ovary. The average number of large follicles were significantly ($P < 0.05$) increased in the right ovary (1.0±0.14) when compared to the left ovary (0.6±0.10). The recovered COCs number was found to be significantly higher ($P < 0.05$) in the right than left ovaries (2.0±0.13; 1.2 ±0.09 respectively). A greater number of vesicular follicles and aspirated COCS were found in non-CL bearing ovary (5.0 ±0.97;1.25±0.09 respectively) than in CL bearing ovary (3.0±0.67;0.80±0.13 respectively). The CL-absent ovaries provide large numbers as well as high quality of COCs when compared to CL-present ovaries and that CL-absent ovaries can be used to collect quality COCs for in vitro production of sheep embryos.

Keywords: Morphology; Corpus Luteum; Follicles; Oocytes; Ovary

INTRODUCTION

In vitro production (IVP) of embryos is currently the central focus in livestock industry. For any successful IVP program in sheep and other livestock, artificial removal of cumulus-oocyte complexes (COCs) from mature follicles, culturing, and maturing them is a primary requirement where the optimal rates of embryo production in vitro can be attained by selecting ovaries and follicles, which provide oocytes to undergo maturation, successful fertilization, and in vitro development [19, 23, 24]. Extensive research on in vitro maturation (IVM), in vitro fertilization (IVF) and in vitro culture (IVC) of the resulting zygotes in sheep and goat has been reported by [7] but limited information has been reported on the evaluation of sheep ovaries or methods for the efficient collection and grading of oocyte. The average number of high quality oocytes recovered from ovaries that can be effectively used for IVF recorded by [18, 26]. Oocytes recovered from large antral follicles have the ability to develop to the blastocysts stage compared to small and medium size follicles [25]. The morphology of the cumulus investment is commonly used as selection criteria prior to IVM, which greatly influence to the maturity of oocytes, COCs matured in vitro and subsequent studies, further confirmed the observation, Denuded oocyte with few cumulus cells are usually rejected because of their low capacity of fertilization and or in vitro development [24, 25]. Various factors such as follicle size; follicular fluid or cells; hormones, serum, growth factors or vitamins in the IVM medium, age of the donor goat and the culture conditions are involved for successful IVM of goat oocytes reported by [24].

The morphology of sheep ovaries in relation to follicular growth, corpus luteum, with the view of in vitro recovery, quality, and maturation of oocytes still need more investigation where the Quantitative aspects of follicular growth have been studied in sheep by [20] and in bovine [30], whatever the in vitro embryo-production procedures developed for sheep have been improved significantly, but many factors influencing their efficiency still need to be investigated.

The present research work has been undertaken for collection and evaluation of slaughterhouse ovaries, follicles and follicular fluid to evaluate the effect of follicles, corpus luteum (CL) and steroid production (progesterone and estradiol) on the morphology of sheep ovaries.

MATERIAL AND METHODS

Collection of Ovaries

One hundred ovaries from sheep aged 2-3 years with unknown reproductive histories were obtained from abattoir during January and March 2011. The ovaries were then kept in a thermos containing 0.9% physiological saline containing 100 IU/ml penicillin-G sodium and 100mg/ml streptomycin sulfate in a thermo flask at 37 °C and were transported to the laboratory within 2 to 3 hrs of slaughter.

Processing of Ovaries

The ovaries were then recorded as right and left. The presence or absence of corpora lutea (CL) was recorded. Upon arrival at the laboratory the ovaries were washed three times in phosphate buffer saline prepared according to [14] supplemented with the above-mentioned antibiotic. After trimming, individually right, left, CL bearing and non-bearing ovaries were weighed (gm). The length, width and thickness in cm of the right, left, CL bearing and non-bearing ovaries were measured with a measuring scale, the ovaries were collected and weighed and the values recorded (Fig.1) . The morphometric dimensions of the ovary were recorded by using a measuring tape and Vernier calliper. The length of each ovary was determined as the maximal distance from pole to pole along an axis parallel to the ovarian mesenterial attachment. The width was determined as the furthest distance along an axis vertical to the longitudinal axis. While the thickness was taken as the greatest distance along, an axis at right angles to the two axes [3, 6]. All antral follicles for each ovary were counted and classified according to their diameter into small (>2mm), medium (2-4mm) and large follicles (>4mm).

Oocyte Recovery and Classification

Follicular oocytes 2-5mm in diameter were aspirated from the surface of ovary with an 18-gauge needle fixed on a disposable syringe filled with 1ml of the aspiration solution used modified phosphate buffer (M-PBS) enriched with sodium pyruvate (0.036g/ml). The media used for aspiration of follicular oocytes enriched with 3% heat inactivated fetal calf serum (FCS, Sigma, USA) and contained the same previous mentioned antibiotic. Oocytes was collected using a stereomicroscope, and were washed twice in the previous media. The average number of oocyte per ovary was recorded and evaluated according to their morphological shape [12, 23, 24] into:

1. COCS (Compact cumulus oocyte complexes) with homogenous evenly granulated cytoplasm possessing at least three layers of cumulus cells (Fig.2/A).
2. POCS (Partially invested with less than three layers of cumulus cells) (Fig.2/B).
3. DO (Denuded oocyte) without cumulus cells (Fig.2/C).

Hormonal Assay

Follicular fluid of small, medium and large follicles of right and left ovaries with or without CL were aspirated using 1ml insulin syringe. The follicular fluid was pooled and stored frozen at -20°C until subsequent assay for progesterone and estradiol 17β. The concentration of progesterone and estradiol 17β in follicular fluid were assayed in duplicate using radioimmunoassay. Prior to analysis, the samples of follicular fluid were thawed and centrifuged at 1200 g. for 30 minutes at 4°C to remove the debris and follicular cells. Progesterone concentrations were measured in follicular fluid according to [11]. The intra and inter assay coefficients of variation, calculated by method described by [10] were found to be 7.5% and 8% respectively. The sensitivity of assay was 0.11ng/ml for the quantitation of estradiol 17β a commercial radioimmunoassay kit (Baxter Merz plus Dade AG, CH 3186 Duedingen). The intra and inter assay coefficients of variation were found to be 10.2% and 11.5% respectively. The sensitivity of the assay was 0.50ng/ml.

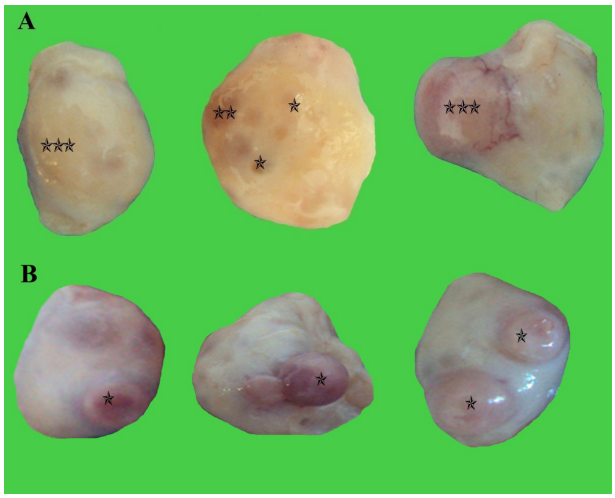

Fig.1. The morphology of the ovary, A. Non CL ovaries (Stars (*) indicate follicles, (*) small follicle, (**) medium follicle, (***) large follicle). B. CL ovaries (Stars (*) indicate corpus luteum

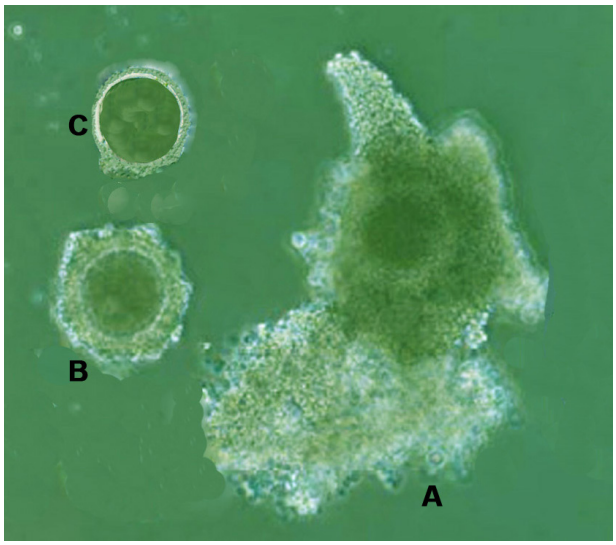

Fig.2. Different categories of a freshly collected immature sheep oocyte (X-100), A. COCS (Compact cumulus oocyte complexes), B. POCS (Partially invested with less than three layers of cumulus cells), C. DO (denuded oocyte).

Statistical Analysis

All value were expressed as mean ± SE. the data were analyzed using general linear model of [27], while the difference between means was detected by ANOVA and Duncan's Multiple Range Test.

RESULTS

Morphometry of Ovaries in Relation to Corpus luteum & Follicles

Data presented in Table 1 showed that the mean weight, length, width and thickness were not significant differed in right ovaries (1.34±0.17 gm, 1.02±0.12 cm, 0.73±0.07 cm, and 0.54±0.05 cm, respectively) compared to that of left ovaries (1.27±0.13 gm, 0.97±0.13 cm, 0.71±0.06 cm, and 0.52±0.06 cm, respectively). CL presence was exhibited in 30 ewes (60.0%) from 100 ovaries of 50 slaughtered ewes were examined, 16 ewes had an active right bearing CL and inactive left ovary (53.3%), 14 ewes had an inactive and active left ovary (46.6%), and 20 ewes (40.0%) had inactive ovary (no CL). The ovarian weight recorded was significantly ($P < 0.05$) affected by presence of CL, where the CL bearing ovaries (1.40±0.19 gm) were heavier than that of non-CL bearing ovaries (0.77±0.06 gm) (Table 2). Similar trends were observed in length, width and thickness being higher in CL bearing ovary (1.14±0.09 cm, 0.82±0.07 cm, 0.84±0.05 cm, respectively) than that of non-CL bearing ovary (0.75±0.05 cm, 0.66±0.06 cm and 0.60±0.06 cm, respectively) as shown in Table 2. The number of follicles visible on the surface in relation to the CL bearing right and left ovaries was counted as shown in Table 3. There were 490 follicles recorded on the surface of the ovaries (250 follicles of mean 5.0±0.87 were found on the right ovary and 240 follicles

Table.1. Comparative dimensions of right and left ovary (Mean± SEM).

Parameters	Right ovary (50)	Left ovary (50)
Weight(gm)	1.34±0.17[a]	1.27±0.13[a]
Length(cm)	1.02±0.12[a]	0.97±0.13[a]
Width(cm)	0.73±0.07[a]	0.71±0.06[a]
Thickness (cm)	0.54±0.05[a]	0.52±0.06[a]

Within the same row with different superscripts (a, b) are different ($p<0.05$).
Figures in parenthesis indicate the total number of ovaries.

Table.2. Comparative dimensions of CL or non-CL bearing right and left ovary (Mean± SEM).

Parameters	CL bearing ovary (60)	Non CL bearing ovary (40)
Weight(gm)	1.40±0.19[a]	0.77±0.06[b]
Length(cm)	1.14 ±0.05[a]	0.75±0.09[b]
Width(cm)	0.82±0.07[a]	0.66±0.06[b]
Thickness (cm)	0.84±0.05[a]	0.60±0.06[b]

Within the same row with different superscripts (a, b) are different ($p<0.05$).
Figures in parenthesis indicate the total number of ovaries.

Table.3. Follicular development in right and left ovary (Mean± SEM).

Parameters	Right ovary	Left ovary
Total number of follicles	5.0±0.87[a] (250)	4.8±0.92[a] (240)
Small follicle	3.0±0.80[a] (150)	3.1±0.88[a] (155)
Medium follicle	1.0±0.24[a] (50)	1.1±0.22[a] (55)
Large follicle	1.0±0.14[a] (50)	0.6±0.10[b] (30)

Within the same row with different superscripts (a, b) are different ($p<0.05$).
Figures in parenthesis indicate the total number of ovaries.

Table.4. Follicular development in CL and non-CL bearing ovary (Mean±SEM).

Parameters	CL bearing ovary	Non-CL bearing ovary
Total number of follicles	3.0±0.67[a] (180)	5.0±0.97[b] (200)
Small follicle	1.60 ±0.97[a] (100)	1.75±0.68[a] (70)
Medium follicle	0.83±0.18[a] (50)	1.75±0.22[b] (70)
Large follicle	0.83±0.27[a] (50)	1.50±0.36[b] (60)

Within the same row with different superscripts (a, b) are different ($p<0.05$).
Figures in parenthesis indicate the total number of ovaries.

Table.5. Effect of site of sheep ovary on the mean number of recovered oocyte and their quality per follicles (Mean± SEM).

Parameters	Right ovary	Left ovary
Number of aspirated follicles /ovary	4.0±0.87[a] (100)	4.8±0.92[a] (120)
Total number of oocytes	350	300
COCS	2.0±0.13[a] (200)	1.2±0.09[b] (150)
POCS	1.0±0.27[a] (100)	1.0±0.26[a] (120)
DO	0.50±0.01[a] (50)	0.25±0.00[a] (30)

Within the same row with different superscripts (a, b) are different ($p<0.05$).
Figures in parenthesis indicate the total number of oocytes.

of mean 4.8±0.92 were found on the left ovaries). The mean numbers of small and medium sized follicles were equivalent without a significant difference in right and left ovary. However, the average number of large follicles were significantly ($P < 0.05$) increased in the right ovary (1.0±0.14) than that in the left ovary (0.6±0.10). Non-CL bearing ovaries had a significantly higher number of total follicles (5.0 ±0.97, $P < 0.05$) than those of CL bearing ovaries (3.0±0.67). A significantly ($P < 0.05$) higher number of follicles were medium and large sized follicles in the non-CL bearing ovaries (1.75±0.22; 1.5±0.36, respectively) as compared to CL bearing ovaries (0.83±0.18; 0.83±0.27 respectively) as shown in Table 4. The mean numbers of aspirated follicles were similar in right ovary (4.0±0.87) and left ovary (4.8±0.92). The collected COCs number was found to be significantly higher ($P < 0.05$) in right ovaries (2.0±0.13) than that in left ovaries (1.2 ±0.09). However, the mean numbers of POCS and DO were equivalent between right (1.0±0.27, 0.50±0.01, respectively) and left ovaries (1.0±0.26, 0.25±0.00, respectively) as shown in Table 5. A significantly higher number of aspirated follicles were found in non-CL bearing ovaries (4.0±0.44 follicles per ovary) than those observed in CL bearing ovary (2.5±0.51 follicles per ovary). On average the number of COCs recovered the non-CL bearing ovary was 1.25±0.09 where that of CL bearing ovary was 0.80±0.13. Oocytes with few dispersed layers of cumulus (POC) were recovered with mean of 0.80±0.27 of CL bearing ovaries and 0.62±0.26 in non-CL bearing ovaries as shown in Table 6.

Table.6. Effect of presence or absence of CL on the mean number of recovered oocyte and their quality per follicles (Mean± SEM).

Parameters	CL bearing ovary	Non CL bearing ovary
Number of aspirated follicles / ovary	2.50±0.51[a] (150)	4.0±0.44[b] (160)
Total number of oocytes	250	350
COCS	0.80±0.13[a] (100)	1.25 ±0.09[b] (200)
POCS	0.80±0.27[a] (100)	0.62±0.26[b] (100)
DO	0.33±0.01[a] (50)	0.31±0.00[a] (50)

Within the same row with different superscripts (a, b) are different ($p<0.05$).

Figures in parenthesis indicate the total number of oocytes.

Table.7. Effect of presence or absence of CL on recovered follicular oocyte and steroid production (Mean± SEM).

Ovary	Progesterone	Estradiol
CL bearing ovary	27.75±2.3[a]	8.43±1.4[a]
Non CL bearing ovary	12.33±1.5[b]	22.10±3.3[b]

Within the same column with different superscripts (a, b) are different ($p<0.05$).

Hormonal Assay

Table 7 represents the concentrations of progesterone and estradiol 17β of follicular fluid of CL bearing ovaries and in non-CL bearing ovaries. The progesterone concentration of follicular fluid was significantly different ($P < 0.01$) in CL and non-CL bearing ovaries (27.75±2.3ng/ml; 12.33±1.5ng/ml respectively). Non-CL bearing ovaries had significantly ($P < 0.01$) higher concentration of estradiol 17β than those found in CL bearing ovaries (22.10±3. 3 pg/ml; 12.33±1.5pg/ml, respectively).

DISCUSSION

The mean weight, length and width of ovaries in the present study were found similar in both right and left ovaries similar finding was obtained by [20] in sheep and goat. In the present study, CL presence was more pronounced in the right ovary (53.3%) than that in the left ovary (40%) that indicates the right ovaries are more active than the left ones these findings were in the same line with [4, 28] Statistical analysis of the present results revealed that the length, width and thickness of CL bearing ovaries were significantly ($P<0.05$) higher than those of non-CL bearing ovaries this is in agreement with that recorded by [16]. The mean number of follicles was the same in the right and left ovaries (5.0±0.87 and 4.4±0.92, respectively). However, the average number of large follicles were significantly ($P<0.05$) increased in right ovary (1.0±0.14) than that in left ovary (0.6±0.10) this is attributed to that the right ovary is more active than left one similar finding reported by [4, 28]. The number of COCs per ovary obtained in this study was 2.0±0.13 to right ovary and 1.2±0.09 to left ovary these findings were similar to that recorded by [9] in sheep but different than that recorded by [21, 32] whose stated that the average number of COCs were found to be lower than the 4.00 oocytes per ovary. The current work indicated that the ovary without corpus luteum had higher follicular activity than that bearing corpus luteum (5.0±0.97vs. 3.0±0.67 follicles per ovary respectively) as reported in cattle [22], camel [13] and buffalo [5]. The presence of CL on ovaries in the present study explain the role of progesterone on sheep follicular growth and recovery of oocytes as indicated by higher level of progesterone in CL bearing ovaries (27.75±2.3 ng/ml) than those in no CL ovary (12.33±1.5ng/ml) in follicular fluid similar to reported that [15]. The presence of CL on the ovaries decreased the number of oocytes per ovary in goats and cows [2], which might be the result of a major portion of the ovary being occupied by the lutein cells [8,17]. Furthermore, [1] showed that total number of oocytes recovered from ovaries without CL (9.1/ovary) was higher ($P<0.01$) than those with CL (6.8/ovary). However in bovine, oocyte yield per ovary was higher ($P<0.01$) for ovaries with than without CL [31]. In addition, [29] observed similar number of buffalo oocytes on ovaries with or without CL.

CONCLUSIONS

It can be concluded that the ovaries without CL comprise higher number as well as superior quality of COCs than those obtained from ovaries bearing CL. So the ovaries without CL can be used to collect good quality of COCs in view of in vitro production of sheep embryos (IVP).

REFERENCES

[1] Abdoon, A.S. and O.M. Kandil, 2001. Factors affecting number of surface ovarian and oocytes yield and quality in Egyptian buffaloes. Reprod. Nutr. Dev., 41,71-77.

[2] Agrawal, K.P. 1992. Factors affecting oocyte recovery from caprine ovaries of abattoir origin. In : Recent advances in Goat production, Lokeshwar RR(ed),Proc 5th Int. Conf. Goat 1207-1210.

[3] Al-Baggal, H.A.R., S.Y.A. Al-Dahash and A.F. Alwan, 1993. Microscopic study of the female genital system of Iraqi goats. Small Rumin. Res. 9,341-346.

[4] Alosta, R.A., L. Vaughan and J.D. Collins, 1998. An abattoir survey of ovine reproductive tracts in Irlaland. Theriogenology 50,457-464.

[5] Amer, H.A., A.O. Hegab and S.M. Zaabal, 2008. Effects of ovarian morphology on oocytes quantity and quality, granulose cells, in vitro. Anim. Reprod. 5,55-62.

[6] Bukar, M.M., J.D. Amin, M.N. Sivachelvan and A.Y. Ribadu, 2006. Postnatal histological development of the ovaries and uterus and the attainment of puberty in female kid goats. Small Rumin. Res., 65,200-208.

[7] Cognie, Y., G. Baril, N. Poulin and P. Mermillod, 2003. Current status of embryo technologies in sheep and goat. Theriogenology 59(1)171-188.

[8] Das, G.K., G.C. Jain, V.S. Solanki and V.N. Tripathi, 1996. Efficacy various collection methods for oocyte retrieval in buffalo. Theriogenology, 46: 1403-1411.

[9] Datta, T.K., S.L. Goswami and S.K. Das, 1993. Comparative efficiency of three oocyte recovery methods from sheep ovaries. Ind. J. Anim. Sci., 63(11)1178-1179.

[10] Dighe, K.K. and W.M. Hunter, 1976. A solid phase radioimmunoassay for plasma progestrone. Biochem. J., 143, 219-223.

[11] Dobeli, M., 1980. Comparative studies in radioimmunoassay of progesterone in plasma and milk of cows using double antibody technique and dextran-coated charcoal separation. Intl. Symp. Vet. Lab. Diagn., 2: 207-215

[12] Eckert, J. and H. Niemann, 1995. In vitro maturation, fertilization and culture to blastocyst of bovine oocytes in protein-free media. Theriogenology, 43(7)1211-1225.

[13] Ghoneim, I.M., 2001. Ovarian follicular dynamic and some steroid hormones of follicular fluid of pregnant and non pregnant camels (Camelus Dromedarius). J. Egy. Vet. Med. Asssoc., 61(5)195-199.

[14] Gordon, I., 1994. Laboratory Production of Cattle Embryos. Wtory allingford, UK, CAB International.

[15] Hafez, E.S.E., 1993. Folliculogenesis, Egg Maturation and Ovulation: Reproduction in farm animals. 5th Ed., Lea and Febiger, Phaildelphia, 114-143.

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

[17] Kumar, A., V.S. Solanki, S.K. Jindal, V.N. Tripathi and G.C. Jain, 1997. Oocyte retrieval and histological studies of follicular population in buffalo ovaries. Anim. Reprod. Sci. 47,189-195.

[18] Kumar, N., S. Paramasivan, P. Sood and M. Singh, 2004. Micrometry of different category oocytes recovered from goat ovaries. Ind. J. Anim. Sci., 74(3)259-260.

[19] Madison, V.J., 1988. Factors influencing in vitro maturation and fertilization of ovine oocytes. Ph.D. Thesis, Swiss Federal Institute of Technology, Zurich.

[20] Mohammadpour, A.A., 2007. Comparative histomorphological study of ovary and ovarian follicles in Iranian Lori-Bakhtiari sheep and native goat. Pakistan Journal of Biological sciences 10(4)673-675.

[21] Mostafizur Rahman, M.G., P.C. Goswami, M.A.M. Yahia Khndoker, K.M.A. Tareq and S.Z. Ali, 2003. Collection of bovine Cumulus-oocyte-complexes (COCs) from slaughterhouse ovaries in Bangladesh. Pak. J. Biol. Sci., 6: 2054-2057.

[22] Pierson, R.A. and O.J. Ginther, 1987. Reliability of diagnostic ultrasonography for identification and measurement of follicles and detecting the corpus luteum in heifers. Theriogenology, 28,929-936.

[23] Rahman, A.N.M.A., R.B. Abdullah and W.E. Wan Khadijah, 2007. Goat embryo development from in vitro matured oocytes of heterogeneous quality through intracytoplasmic sperm injection technique. Biotechnology, 6: 373-382.

[24] Rahman, A.N.M.A., R.B. Abdullah and W.E. Wan-Khadijah, 2008a. Recovery and grading of goat oocytes with special reference to laparoscopic ovum pick-up technique: A review. Biotechnology, 7: 612-622.

[25] Rahman, A.N.M.A., R.B. Abdullah and W.E. Wan-Khadijah, 2008b. In vitro maturation of oocytes with special reference to goat: A review. Biotechnology, 7: 599-611.

[26] Salim, M.S., 2004. Causes of infertility in black bengal does investigated by anatomical and histological tools. Master Thesis, Bangladesh Agricultural University, Bangladesh.

[27] SAS, 1992. SAS User's Guide: Statistics. 5th Edn., SAS Institute: Cary, NC, USA.

[28] Scaramuzzi, R.J. and J.A. Downing, 1997. The distribution of ovulation from the ovulation from the ovaries of Merino and Border Leicester X Merino ewes and the effect on the survival of their embryos. Anim. Reprod. Sci. 47,327-336.

[29] Shamiah, S.M., 2004. Studies on in vitro fertilization in Egyptian buffaloes. Ph.D. Thesis, Faculty of Agriculture, Mansoura University, Egypt.

[30] Singh, J. and G.P. Adams, 2000. Histomorphometry of dominant and subordinate bovine ovarian follicles. Anat. Rec. 258,58-70.

[31] Varisanga, M.D., C. Sumantri, M. Murakami, M. Fahrudin and T. Suzuki, 1998. Morphological classification of the ovaries in relation to the subsequent oocyte quality for IVF-produced bovine embryos. Theriogenology 50,1015-1023.

[32] Wahid, H., I. Gordan, H. Sharif, P. Lonergan, P. Monaghan and M. Gallagher, 1992. Effect and efficiency of recovery methods for obtaining ovine follicular oocytes for in vitro procedures. Theriogenology 37:318-318.