EFFECT OF CORPUS LUTEUM AND STATUS OF ANIMAL ON RECOVERY RATE OF OOCYTES IN OVINE

K. A. Sofi[1], M. Z. Khan[2], R. Islam[3] and F. A. Lone[1]

Division of Animal Reproduction, Gynaecology & Obstetrics
Faculty of Veterinary Sciences and Animal Husbandry
S.K. University of Agricultural Sciences & Technology of Kashmir
Shuhama, Srinagar - 190 006, J&K.

ABSTRACT

Genital organs of slaughtered ewes were collected from abattoirs in Srinagar city and transported to the laboratory in warm normal saline solution containing 50µg gentamicin sulphate per ml. Upon arrival to the laboratory; the status of the reproductive tract (gravid or non-gravid) and ovaries for corpus luteum (with or without) were observed and recorded. The oocytes were collected separately from ovaries of gravid and non-gravid uteri as well as from ovaries with or without corpus luteum (CL) using slicing method and graded as good, fair and poor for each ovary under stereo-zoom microscope. The good quality, usable and total oocytes harvested from ovaries without CL were significantly ($p < 0.05$) higher (3.13, 4.91 and 6.57) than the ovaries containing CL (2.55, 4.14 and 5.62). However, the number of good quality, usable and total oocytes recovered was non-significantly ($p > 0.05$) higher for ovaries of non-gravid uteri (3.14, 4.92 and 6.45) than for ovaries of gravid uterus (1.96, 3.38 and 5.04). In conclusion, ovaries without CL were better for oocyte collection from abattoir ovaries in ovine.

Keywords: Recovery rate; Corpus luteum; Pregnancy status; Ovine

The number of high quality oocytes harvested from an ovary is an important aspect in the *in vitro* maturation and production of embryos. Oocytes for various assisted reproductive technologies are collected from different sources like oviducts soon after ovulation, mature follicles shortly before ovulation; ovaries of pre-pubertal animals by ovum pick up (OPU) or immature and atretic follicles usually from abattoir material. Ovaries of abattoir origin are generally used for the large scale production of domestic animal embryos as they are less expensive and most abundant source of primary oocytes. In the past, follicular dissection was employed for the recovery of ovine follicular oocytes and currently slicing (Wahid *et al.*, 1992; Wang *et al.*, 2007) and aspiration (Watson *et al.*, 1994; Wang *et al.*, 2007) are routinely employed for oocyte recovery in sheep. The oocyte collection techniques generally used are slicing, puncture and aspiration but the recovery rate of total oocytes as well as usable oocytes was higher for slicing method of oocyte recovery (Wani et al., 1999; Wang et al., 2007). Previous works have reported on the effect of season of collection (Arangasamy *et al.*, 2008; Nandi and Kumar, 2008), collection methods (Wahid *et al.*, 1992; Wani *et al.*, 1999; Wang *et al.*, 2007) and age of the animal (Wani et al., 1999) on the recovery rate of oocytes. However, the studies on the effect of presence of corpus luteum on the ovary and/or status of the donor animal (Pregnant/ non pregnant) on the oocyte recovery rate particularly in sheep is scanty. Thus, the present study was conducted to evaluate the effect of corpus luteum and pregnant status on the recovery rate of oocytes from abattoir ovaries.

Sheep ovaries were collected from local abattoirs and transported immediately to the laboratory in a warm normal saline solution supplemented with 50µg Gentamicin sulphate (Sigma: Steinheim, Germany) per ml in a thermos flask. Upon arrival at the laboratory, extraneous tissues were trimmed off from the ovaries and washed with 70% ethanol to control contamination. Out of 88 ovaries collected and processed, 42 ovaries were with corpus luteum (CL) and 46 were without CL. Out of total 90 ovaries collected, 66 were from non-gravid and 24 were from gravid uterus. The biometry of each ovary was measured by digital caliper. The oocytes were collected from the ovaries by slicing method. Ovaries were placed in a Petri dish (35mm) containing 2-3ml of saline solution supplemented with 50 µg Gentamicin sulphate per ml and all visible follicles were sliced with a surgical blade. The Petri dishes were

1M.V.Sc. Student, 2Professor & Head, 3Senior Assistant Professor

kept undisturbed for 1-2 minutes; excess saline solution was taken out with a pipette gently and observed under stereo zoom microscope.

Grading of oocytes was done according to the character of cumulus cells (Pawshe *et al.*, 1994); Good: Oocytes with many complete layers of cumulus cells and uniform cytoplasm, Fair: Oocytes with thin or incomplete layers of cumulus cells and uniform cytoplasm and Poor: Oocytes with few or no cumulus cells and total number of oocytes along with Good, Fair and Poor oocytes was recorded for each ovary.

The data was analyzed by paired T-test and significance was determined by Wilcoxon Signed Ranks test using statistical software SPSS version-17 (SPSS Corporation, USA). All the data are expressed as Mean ±S.E.M. The level of significance was set at $p<0.05$.

The mean number of oocytes collected per ovary from ovaries containing CL (n=42) and without CL (46) were compared and the good quality, usable and total oocytes harvested from ovaries without CL were significantly higher (3.13 ± 0.19, 4.91 ±.0.26 and 6.57 ± 0.31; pd"0.05) than the ovaries containing CL (2.55 ± 0.24, 4.14 ± 0.28 and 5.62 ± 0.31). However, the recovery rate of fair oocytes was non-significantly higher for ovaries without CL (1.76± 0.11 v/s 1.60± 0.11), respectively. Similarly, the effect of status of donor animal *viz.* non-gravid (n=66) and gravid (n=24) on the collection rate of oocytes were compared. The number of good quality, usable and total oocytes recovered was higher for ovaries of non-gravid uteri (3.14±0.17, 4.92 ±0.21 and 6.45 ±0.25) than for ovaries of gravid uteri (1.96±0.26, 3.38±0.33 and 5.04 ±0.38), but the difference was non-significant.

An important aim of oocyte collection method from the ovaries collected from abattoirs is to maximize the total number of oocytes and the yield of good quality oocytes at low cost, which can be used for *in vitro* embryo production (IVEP). Based on the present results, the effect of presence of CL on oocyte recovery rate revealed that the usable and total oocytes collected per ovary from ovaries without CL were significantly higher (4.91 ± 0.26 and 6.57 ± 0.31; pd"0.05) than the ovaries containing CL (4.14 ± 0.28 and 5.62 ± 0.31). This is in accordance with the results of Wani *et al.* (1999) and Jamil *et al.* (2008). A significantly higher recovery rate of oocytes recorded from the ovaries without CL than the ovaries with CL might be attributed to the fact that the animal with CL were either pregnant, infertile or in diestrus phase in which the corpus luteum covers the major portion of ovary. It also maintains the non- conducive environment for follicle development. Similarly, the number of good quality, usable and total oocytes recovered was non-significantly higher for ovaries of non-gravid uteri (3.14±0.17, 4.92 ±0.21 and 6.45 ±0.25) than for ovaries of gravid uteri (1.96±0.26, 3.38±0.33 and 5.04 ±0.38). The results obtained in the present study pertaining to the status of donor animal (pregnant vs. non pregnant) could not be compared and discussed due to paucity of literature on this aspect. However, it might be attributed to the fact that CL of pregnancy covers the 3/4th portion of the ovary (Roberts, 2004) thereby less chances for the development of antral follicles during pregnancy. In conclusion, ovaries without CL were better for oocyte collection in sheep.

ACKNOWLEDGMENT

The authors would like to thank all the staff of Division of Animal Reproduction, Gynecology and Obstetrics, Faculty of Veterinary Sciences, Shuhama, SKAUST-K for their cooperation in completing this study.

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