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Full Length Research Paper

Effect of the presence of corpus luteum on the ovary and the new oocyte recovery method on the oocyte recovery rate and meiotic competence of ovine oocytes

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This study was designed to identify the effects of the presence of corpus luteum (CL) on the meiotic competence of the ewe oocytes. In addition, due to the pivotal roles of oocyte recovery methods on efficiency of *in vitro* maturation and *in vitro* fertilization, this study considered the new oocyte recovery method "Oocyte Recovery with Centrifuge" (ORC); Ovine ovaries were obtained from local abattoir at Karaj, Iran. The ovaries were placed in 0.9% saline which was supplemented with penicillin/streptomycin in thermo flask at 37°C and transported to the laboratory within 1 to 2 h of slaughter. The ovaries were assigned to two groups; group-1 with functional CL and group-2 without CL. The oocytes were recovered by means of aspiration pump or ORC. After oocyte recovery, they were cultured in TCM-199 for 24 h. The mean number of oocyte recovery per ovary in group 1 ovaries (1.8 ± 0.01 via aspiration and 3.84 ± 0.05 via ORC) was lower (P<0.05) than group 2 ovaries (2.2 ± 0.00 via aspiration and 5.43 ± 0.01 via ORC). There were no significant differences (P>0.05) between percentage of nuclear maturation in oocytes which were recovered from group 2 ovaries via aspiration and ORC (75.20 ± 0.00 vs. 74.95 ± 0.00, metaphase II; M-II) method. The nuclear maturation in oocytes which were obtained via ORC from group 2 ovaries was higher (P<0.05) than group 1 ovaries (74.95 ± 0.00 vs. 60.07 ± 0.00b). Nuclear maturation for oocytes obtained via ORC from group 1 ovaries was (P<0.05) lower than oocytes obtained via aspiration (60.07 ± 0.00 vs. 74.70 ± 0.00). Result of the present study showed that the presence of CL on ovaries lead to decrease in the quality and the quantity of oocytes. ORC method increased the quantity and quality of recovered oocytes.

Key words: Aspiration, corpus luteum, *in vitro* maturation (IVM), oocyte recovery.

INTRODUCTION

Today, an *in vitro* embryo production (IVP) is considered as one of the most important aspects of modern science. IVP has two main subdivision; *in vitro* maturation (IVM) and in vitro fertilization (IVF). To improve the efficiency of IVP, many laboratories and expertise are involved in developing the subdivision of IVP. Choosing the best and the most efficient oocyte recovery method is the most important and the first step for successful embryo production (Buccione et al., 1990).

Ivm and Ivf have enormous potential for the generation of a large number of domestic animal embryos for research and for the application of other technologies. A demand exists in small ruminants, especially in sheep and goat, for basic research on zygote development. Ovaries of the slaughtered animals are the cheapest and the most abundant source of primary oocytes for large scale production of embryos through IVM-IVF. IVF not only provides more embryos from valuable animals than would be possible with conventional multiple ovulation and embryo technology (MOET) programs, but IVM-IVF embryos are estimated to be five times cheaper than the embryos from superovulated donors (Wooliam and wilmut, 1989).

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Abbreviations: CL, IVM, etc

The meiotic competence, the quantity and quality of oocytes recovered per ovary has been an important consideration in the production of IVM-IVF embryos (Wooliam and Wilmut, 1989). This experiment was aimed to record the effect of new oocyte recovery method "Oocyte Recovery with Centrifuge" (ORC) and presence or absence of corpus luteum on the meiotic competence and the quantity and quality of oocytes recovered per ovary.

MATERIALS AND METHODS

Collection and processing of ovaries

The ovaries from ewes were collected from local slaughterhouse at Karaj, Iran. The ovaries were placed in 0.9% saline which was supplemented with penicillin/streptomycin in thermo flask at 37°C and transported to the laboratory within 1 to 2 h of slaughter. The ovaries in the laboratory were handled aseptically. They were then categorized into two groups. Group 1 ovaries had at least one corpus luteum (CL) on their surface and group 2 ovaries had no CL on their surface. All visible antral follicles were harvested by two different methods.

Oocyte collections methods

Aspiration

Aspiration of follicular oocytes was carried out by using a 20 gauge needle connected with an aspiration pump by 10 ml/min water flow rate (Morton et al., 2008). Aspiration flow rate was measured and expressed as ml water/min because aspiration flow rate at the tip of the needle varies with the other components of the aspiration device (tubing inner diameter and length + needle gauge), and this measure more accurately reflects the physical forces experienced by oocytes than mmHg vacuum pressure (Bols et al., 1996). The oocytes were collected in 50 ml falcon tube which contained pre-incubated (at 38.6°C, 5% CO_2 and 98% humidity) oocyte washing medium (OWM), which was supplemented with 3 µl/ml heparin. After that, the oocytes were picked up from the bottom of a falcon tube and they were washed two times with pre-incubated (at 38.6°C, 5% CO_2 and 98% humidity) OWM and two times with pre-incubated (at 38.6°C, 5% CO_2 and 98% humidity) oocyte maturation medium (OMM) (Morton et al., 2008).

New oocytes recovery method

In this method, ovaries were initially washed with 0.9% saline which was supplemented with penicillin/streptomycin and then all the superfluous tissues were separated; so ovaries were cleaned and placed in modified Falcon tubes (MFTs) which were designed and prepared for this method. The cleaned ovaries were placed in MFTs. The MFTs were filled with 3 ml of OWM which was supplemented with heparin (30 µl; Sigma). The surface of ovaries was scratched several times with scalpel blade. After these procedures, the MFTs were placed in a centrifuge. The centrifuge was set at 750 rpm for 5 min. Then, the oocytes were collected from the bottom of the MFTs, these oocytes were subjected to washing, grading and finally they were cultured for IVM in TCM-199 for 24 h.

IVM

Ovine oocyte were recovered by means of aspiration or ORC and collected in a pre-incubated Hepes-modified TCM 199 medium. The medium used for IVM was tissue culture medium 199 (TCM199; Invitrogene Co.) supplemented with 10% heat-inactivated fetal calf serum (FCS; Sigma), 0.2 mM sodium pyruvate, 5 µg/L gentamycin, 10 µg /ml FSH and 1 µg/ml estradiol (Sigam; Arav et al., 1996). Oocytes were divided into five groups by considering the number of cumulus layers. The oocytes were cultured in drops of IVM medium (100 µg) covered with mineral oil (Sigma) for 24 h at 38.5°C in 5% CO_2 in air and ≥ 95% humidity.

Morphological evaluation and nuclear maturation of oocytes

All oocytes were examined under a light microscope to evaluate the cumulus-oocytes complex (COCs) quality and classification. The COCs were graded from level I to V which is illustrated in Figure 1. The *in vitro*-matured oocytes were fixed in acetic acid : ethanol (1:3) in D-PBS (50%) followed by no diluted acetic acid : ethanol for at least 48 h. They were subsequently stained with aceto-orcein (1% in 45% acetic acid solution) and assessed for the nuclear stage of meiosis under a phase-contrast microscope. Oocytes were considered to have matured when they reached the M-II stage.

Statistical analysis

The data were analyzed by proc gen-mod and proc means. *In vitro* maturation and oocyte grade were considered significant at $P<0.05$.

RESULTS

The mean number of oocyte recovery per ovary in group 1 ovaries (1.8 ± 0.01 via aspiration and 3.84 ± 0.05 via ORC) was lower ($P<0.05$) than group 2 ovaries (2.2 ± 0.00 via aspiration and 5.43 ± 0.01 via ORC). The new recovery method yielded a higher ($P<0.05$) number of oocytes per ovary (3.84 ± 0.05 from group 1 and 15.43 ± 0.01 from group 2) as compared to the aspiration method (1.80 ± 0.01 from group 1 and 2.2 ± 0.00 from group 2). In general, group 1 ovaries yielded significantly ($P<0.05$) lower numbers of oocytes than group 2 ovaries via either the aspiration method or the new method (ORC).

In the case of oocyte recovery from group 2 ovaries, the percentage of good oocytes however, was similar for both techniques (Table 1). But in the case of oocyte recovery from group 1 ovaries via ORC method, the percentage of good oocytes significantly lower ($P<0.05$) than oocytes were recovered from the same type of ovaries via aspiration method. The results of percentage of oocytes which were recovered from the two groups of ovaries via ORC and aspiration technique are shown in Table 1.

The results of this study in Table 1 showed that the percentage of meiotic competence in oocytes which were recovered from group 1 ovaries via ORC method (60.07 ± 0.00) was significantly ($P<0.05$) lower than oocytes which were recovered from group 1 ovaries by aspiration technique (74.70 ± 0.00). The percentages of meiotic competence in oocytes recovered by aspiration method from group 2 ovaries (75.20 ± 0.00) and had no significant differences with oocytes were recovered by

Table 1. The recovery rate and percentages (±SD*) of oocytes which were recovered from the two groups of ovaries via aspiration and ORC technique.

Recovery	Ovary	Oocyte quality*					Recovery rate	Metaphase II*
		I	II	III	IV	V		
Aspiration	Group 1 (n = 100)	24.44[a]	30.55[a]	34.43[a]	5.00[a]	5.54[a]	1.80±0.01[a]	74.70[a]
	Group 2 (n = 100)	24.52[a]	29.51[a]	35.88[a]	6.78[a]	3.17[a]	2.20±0.00[b]	75.20[a]
ORC	Group 1 (n = 106)	17.60[b]	18.10[b]	17.33[b]	29.12[b]	29.05[b]	3.84±0.05[c]	60.07[b]
	Group 2 (n = 103)	25.38[a]	30.10[a]	33.90[a]	5.55[a]	5.56[a]	5.43±0.01[d]	74.95[a]

*±SD = ± 0.00; different superscripts ([a,b]) in the same column indicate a significant difference (P < 0.05).

ORC method from group 2 ovaries (74.95 ± 0.00). All results are presented in Table 1.

DISCUSSION

Several techniques have been used for the collection of oocytes from ovaries in sheep (Wahid, 1992), cattle and goats (Iwasaki and Hanada, 1987). The most common technique used is the aspiration of visible ovarian follicles (Watson et al., 1994). In this study, the new method significantly yielded more oocytes than aspiration. The aspiration techniques resulted in the production of more debris than new method, interfering with the recovery of the oocytes. The new method of oocytes recovery can be used as an alternative to the aspiration technique from ovine ovaries. The lower number of oocytes recovered by the aspiration method may be attributed to the presence of some follicles embedded deeply within the cortex (Carolon et al., 1992; Katska, 1984). In the case of oocyte recovery rate, similar study was conducted by Wani (1999). In our study, it was observed that the presence of CL adversely affected the number of oocytes recovered via aspiration technique. The cause of a low number of visible follicles per ovary which were subjected to oocyte recovery by means of aspiration technique may be attributed to the fact that a large body of CL may inhibit the growth of follicles on the ovary and increase their atresia (Hafez, 1993). Hafez (1993) reported that progesterone secreted by the luteal cells of the CL inhibited estrus and gave the negative feedback on the anterior pituitary to secret follicle stimulating hormone (FSH). As a result, the growing follicles regressed and became atretic. The low number of oocytes per ovary with corpus luteum can be explained in the light of studies described by Wani and Sahni (1988). The highest number of follicles that were found in group 2 ovaries in the study might reflect the optimum level of gonadotropins and steroids. Group 2 ovaries did not contain the CL and the negative effect of progesterone on anterior pituitary was not functional in this type of ovaries. Least number of follicles in type-I ovaries further confirmed the earlier statement as functional CL was found in type-I ovaries. Huma Jamil et al. (2006) reported that significantly higher (P<0.05) oocyte recovery rate was obtained from ovaries in which the corpus luteum was absent.

The new method of oocytes collection provides access to some follicles embedded deeply within the cortex. It is well established that all female mammals are born with a large store of follicles (Webb et al., 1999); after pubertal age, only a few number of these follicles undergo the growth and development. Populations of oocytes were collected from surface visible (peripheral) and cortical follicles from the same ovaries (Arlotto et al., 1996). When the number of oocytes from both peripheral and cortical follicles was combined, the yield of oocytes was approximately double that collected from 1 ovarian site alone (Arlotto et al., 1996). So, the pool of invisible follicles was used for oocyte recovery by means of the new recovery method and increased the number of oocytes which was recovered.

When the stage of the estrous cycle was observed, it was found to have no effect on developmental potential (Arlotto et al., 1996). The maturational competence of oocytes for IVM was not influenced by the reproductive status (Leibfried-Rutledge et al., 1985). Our results demonstrate that the oocytes recovered by aspiration technique reached more MII oocytes than those recovered by the new technique oocytes from cortical follicles which were smaller than those from the surface population, and the smaller cortical oocytes had a lower potential for both meiotic maturation and embryo development. Only cortical oocytes with the largest diameters underwent IVM and subsequently developed to blastocysts at rates comparable to oocytes from peripheral follicles (Arlotto et al., 1996).

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