

Effect of Different Harvesting Techniques on the Recovery and Quality of Bovine Cumulus Oocyte Complexes

Short Communication

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ABSTRACT

This study investigated the effect of different harvesting techniques on the recovery rate and quality of bovine cumulus oocyte complexes (COCs) and their subsequent developmental competence including *in vitro* maturation, fertilization and blastocyst formation. The COCs were retrieved from slaughter ovaries using aspiration, slicing, or aspiration followed by slicing. The COCs were examined microscopically then classed as A (at least three layers of cumulus), B (two layers of cumulus), C (one layer of cumulus, or degraded cytoplasm) or D (abnormal). The retrieved COCs yields per ovary differed ($P \leq 0.05$) between aspiration (2.31 ± 0.21), slicing (6.65 ± 1.65) and aspiration followed by slicing (3.72 ± 1.43). Differences in quality of collected COCs were observed for categories A, B and C but not for category D. Oocyte diameter and *in vitro* developmental competence did not differ between the retrieving methods. In conclusion, aspiration can recover moderate numbers of oocytes ovary and about half of them can not be retrieved by this method. Therefore when oocyte number is important, aspiration should be avoided and ovaries should be sliced.

KEY WORDS aspiration, cattle, cumulus oocyte complex, oocyte, slicing.

INTRODUCTION

A small proportion of primordial follicles, present in an ovary at birth, continually leave the resting pool and start to grow. However, only a tiny percentage of these oocytes grow to maturity and are released for fertilization. Most follicles undergo follicular atresia and die (Gardner *et al.* 2004). Slaughter ovaries, the cheapest and most abundant source of primary mammalian oocytes, are used in physiological and biotechnological research, reproductive technologies and *in vitro* embryo production (Wang *et al.* 2007).

Maximizing of recovered oocytes number is the goal of the oocyte recovery methods (Carolan *et al.* 1994; Nowshari, 2005). The quality of cumulus oocyte complexes (COCs) that can be retrieved from a given number of ova-

ries is also important (Alm *et al.* 1997). Oocytes have been collected from slaughtered animals by dissection of ovarian follicles, aspiration of oocytes from follicles, ovary slicing and puncture of visible surface follicles (Farahavar *et al.* 2010). Among these four methods, aspiration is widely used (Farahavar *et al.* 2010; Tetzner *et al.* 2011; Shabankarreh *et al.* 2011) because of COC quality (Hoque *et al.* 2011) and convenience. However ovary slicing is more successful than other techniques when number of COCs is taken into consideration (Das *et al.* 1996; Wang *et al.* 2007; Hoque *et al.* 2011).

Additionally, in some countries, female domestic animals are rarely slaughtered in order to increase the animal population. In such case the number of COCs which is initial and important step for further developmental process (*in vitro* maturation, fertilization and handling of blastocysts) is

particularly important. Therefore, this study was conducted to compare the performance of slicing with aspiration method.

MATERIALS AND METHODS

Ovaries of cows with unknown reproductive stage were collected from a slaughterhouse in the region of Ankara, Turkey. The ovaries were transported to a laboratory in 0.9% saline at 30-35 °C. In the laboratory, ovaries were washed once in 0.9% saline and once in COC collection medium (TCM 199 with Earle's salts supplemented by 1 mM L-glutamine, 5 mM sodium bicarbonate, 20 mM HEPES, 4 mg/mL BSA and 50 µg/mL gentamycin). Then COCs were harvested by aspiration, slicing, or aspiration followed by slicing. Ovaries were randomly assigned to one of the three oocyte collection methods in each replicate. For the aspiration procedure, an 18 gauge needle was connected to a 10 ml syringe. All visible follicles were aspirated by fine pressure, irrespective of size, position and health status, then they were gently tipped into a 15 mL test tube pre-warmed to 38 °C. For the slicing procedure, visible surface follicles were punctured with a sterile scalpel and fine cuts were made on the ovaries' surface. COCs were recovered immediately by rinsing and tapping the ovaries to release oocytes into a 400 mL beaker containing collection medium at 38 °C. The procedure of aspiration followed by slicing was performed to retrieve COCs which had not been recovered by aspiration alone.

The COCs were classed morphologically as describe other where (Tripp *et al.* 2000). Category A had normal cytoplasm surrounded by at least three layers of compact cumulus; Category B had normal cytoplasm surrounded by two layers of compact cumulus; Category C had normal cytoplasm surrounded by one layer of compact cumulus, or they had degraded cytoplasm; Category D had abnormal cytoplasm and / or they were surrounded by less than one complete layer of cumulus cells or an expanded cumulus. Categories A, B and C were designated as normal while category D was designated as abnormal. After imaging using a Leica DM IL (Leica, Wetzlar, Germany) microscope equipped with a camera, oocyte diameter was measured using LAS V4.1 software. Normal COCs were washed three times with maturation medium (TCM-199 supplemented with 0.4 mM sodium pyruvate, 1 mM L-glutamine, 100 µM cysteamine, 10% (v/v) fetal bovine serum (FBS), 40 IU PMSG (Chrono-GEST/PMSG), 2 µg/mL estradiol and 50 µg/mL gentamycin). They were cultured in groups of 20 in 100 µL maturation medium under mineral oil at 38.5 °C in a humidified atmosphere of 5% CO₂, 5% O₂ and 90% N₂. The prevalence of cumulus expansion (indicating oocyte maturation) was observed after culturing for 24 h.

20 COC/microdrop were co-cultured with 1×10^6 spermatozoa/mL (selected by the swim-up method) in 200 µl fertilization medium (Fert-SOF supplemented with 100 IU/mL penicillin, 100 µg/mL streptomycin, 2 mM caffeine and 10 µg/mL heparin) for 18 h in a humidified atmosphere as described for maturation. Then presumptive zygotes were removed from the fertilization medium, mechanically denoted, washed three times in culture medium (SOF-BE1) and incubated as before until eight days post insemination. Half of the culture medium was refreshed every 48 h and 10% FBS was added 72 h post insemination. The cleavage state was evaluated three days post insemination. The morula and blastocyst rate was recorded eight days post insemination.

Number of collected COCs, oocyte diameter, proportion of normal and abnormal COC, % prevalence of maturation, cleavage, morula and blastocyst formation from each treatment were used for statistical analysis. After testing of normality and arc-sine transformation of proportional data, analysis of variance (ANOVA) was performed in R to assess significant differences. The Duncan Range Test was performed by the Agricolae package program (Seefeld and Linder, 2007; de Mendiburu, 2014) to compare means ($P < 0.05$).

RESULTS AND DISCUSSION

Table 1 shows that number of recovered COCs per ovary differed ($P \leq 0.05$) between the retrieval methods. It was low after aspiration (2.31 ± 0.21), high after slicing (6.65 ± 1.65) and moderate after aspiration followed by slicing (3.72 ± 1.43). Table 1 also shows that oocyte diameter did not differ between the retrieval methods.

Table 1 Effect of COC retrieval methods on number of COC per ovary, number of retrieved COC and oocyte diameter

Treatment	Number of ovaries	COC per ovary	Oocyte diameter (µm)
Slicing	150	6.65 ± 1.65^a	98.91 ± 1.46^a
Aspiration	150	2.31 ± 0.21^b	98.91 ± 1.46^a
Aspiration followed by slicing	150	3.72 ± 1.43^b	100.64 ± 1.35^a

The means within the same column with at least one common letter, do not have significant difference ($P > 0.05$).

COC: cumulus oocyte complex.

However, COC quality differed ($P < 0.05$) between the retrieval methods (Figure 1). The number of category A COC was lower in aspiration (66.39 ± 0.6) than from slicing (72.6 ± 1.45) or aspiration followed by slicing. The number of category B COC was higher in aspiration (14.61 ± 0.9) than from slicing (7.47 ± 1.55) or aspiration followed by slicing (5.44 ± 1.64). The number of category C COC was lower in aspiration (3.36 ± 1.36) than slicing (5.51 ± 1.58) or

aspiration followed by slicing (9.31 ± 0.80). The number of category D COC did not differ ($P > 0.05$) between the retrieving methods.

In terms of developmental competence, the prevalence of maturation, cleavage, morula and blastocyst formation did not differ ($P > 0.05$) between collection methods (Table 2).

This study demonstrated that more COC can be collected by slicing than by aspiration. This finding agrees with those of Mantovani *et al.* (1999) and Das and Santra (2008) in cattle, Wani *et al.* (2000) in sheep, Alm *et al.* (1997) in equines, El-Harairy *et al.* (2007) and Nowshari *et al.* (2005) in camels. However, it does not agree with findings of Pawshe *et al.* (1994) who found aspiration to be more successful than puncturing and slicing ovaries from goats. In agreement with Carolan *et al.* (1994), the present study found no difference in oocyte diameter between the three retrieval methods.

It also found no difference in maturation, fertilization and embryo development between the three retrieval methods, agreeing with previous studies (Carolan *et al.* 1994; Alm *et al.* 2008; Pawshe *et al.* 1994).

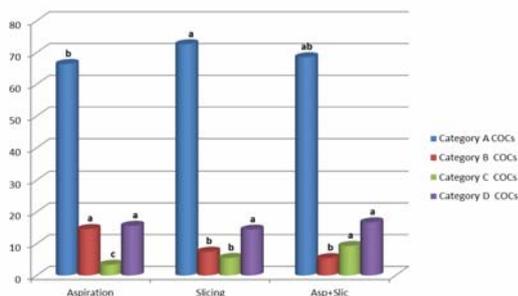


Figure 1 Effect of the collection method on the quality of recovered COC. The values above each column indicate significant differences ($P \leq 0.05$) between the columns with the same color

Table 2 Effect of COC retrieval methods on percentage of maturation, cleavage and produced embryos

Treatment	Matured oocyte	Cleavage	Embryo
Slicing	69.53 ± 3.74	31.87 ± 2.95	17.77 ± 0.61
Aspiration	71.26 ± 2.05	38.50 ± 3.45	21.63 ± 2.37
Aspiration followed by slicing	72.98 ± 5.37	31.87 ± 7.87	20.46 ± 1.98

The means within the same column with at least one common letter, do not have significant difference ($P > 0.05$).

On the other hand, the present study's findings differed from those of Takagi *et al.* (1992), who found better developmental competence of COC recovered by aspiration (%21) than in those from minced ovaries (%14). This difference might be due to the aspiration method used here, in which COC were collected from all visible follicles regardless of follicle size.

Harvesting fewer COC by aspiration alone, than when aspiration was followed by slicing, revealed that aspiration alone lost nearly 40% of COC. In an earlier study, the efficiency of aspiration was 37-43% and 67-56% of COC were lost (Alm *et al.* 1997).

Ovary slicing releases oocytes from follicles both on the ovary surface and in the ovarian cortex. This factor might have led to our high oocyte yield after slicing.

Additionally, some oocytes might be lost during follicle aspiration, which could be avoided by slicing (Wani *et al.* 2000). Our poor rate of oocyte retrieval after aspiration might have been affected not only by this method's restriction to superficial follicles but also by the rate of aspiration pressure. Additionally, the local and crossbreed cattle used in this study might have fewer superficial follicles relative to the number of follicles in the ovarian cortex. Low COC numbers per ovary were reported in previous studies, for unknown reasons (Mantovani *et al.* 1999; Carolan *et al.* 1994) or attributed to the age of slaughtered animals (Hasler, 1998), or to cattle breed (Pontes *et al.* 2010).

CONCLUSION

It can be concluded that ovary slicing was more efficient than aspiration, which led to excessive COC losses. Therefore slicing will be useful when the aim is to salvage oocytes from cows of high genetic potential, or to achieve high numbers of COCs. These aims might be important due to factors such as restrictions on the slaughter of female cattle or on the time of ovary harvesting.

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