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EFFICACY OF DIFFERENT COLLECTION TECHNIQUES ON YIELD AND QUALITY OF EGYPTIAN BUFFALO OOCYTES.

Hammad, M.E.*; Sh.A. Gabr.*; I.T. El-Ratel and Maha A. Gad***
*** Anim. Prod. Dept., Fac. Agric., Tanta Univ., Egypt.**
****Biotech. Dept., Anim. Prod. Res. Inst., Agric. Res. Center, Egypt.**

ABSTRACT

Buffaloes ovaries obtained from slaughterhouses were used to study the influence of the oocyte collection techniques (dissection, aspiration, slicing, and aspiration plus slicing) on the availability of oocytes quantity and quality of buffalo oocytes. The oocytes were collected aseptically from the ovaries by the four methods. In all methods, oocytes were classified into 5 classes on the basis of the morphology of compact, denuded, degenerated, expanded and partial denuded oocytes. Results showed that the oocyte recovery rate from ovaries was higher ($P<0.05$) by aspiration plus slicing (84.67%) and slicing (83.30%) than aspiration (72.68%), while dissection technique showed the lowest ($P<0.05$) oocyte recovery rate (52.04%). Percentage of cumulus oocyte complexes collected by slicing (63.17%) was higher ($P<0.05$) than aspiration (51.34 %), aspiration plus slicing (51.24%) and dissection (42.03%). The corresponding percentages of expanded cumulus oocytes were 29.93, 30.31, 27.55 and 32.68%, respectively ($P<0.05$). Such results may indicate efficacy of slicing technique as a collection method on quantity and quality of buffalo oocytes.

Keywords: Buffalo, oocytes, collection method, recovery rate, categories.

INTRODUCTION

Assisted reproductive technologies such as ovarian hyper-stimulation, collection of immature oocytes, *in vitro* maturation (IVM), artificial insemination (AI), *in vitro* fertilization (IVF), and embryo transfer (ET) have been introduced to increase the number of offspring from selected females, and to reduce the generation intervals in buffaloes (Suresh *et al*., 2009). Ovaries obtained from the slaughterhouse constitute an economical source of oocytes. This allows for large scale and economical production of embryos (Sianturi *et al*., 2002).

The quantity and quality of oocytes which can be retrieved from a given number of ovaries play a key role in the acquisition of oocyte developmental competence *in vitro* (Amer *et al*., 2008). The ability to identify good quality oocytes is of considerable importance. The appearance of an oocyte and its cumulus cells investment have been used to estimate or assess the developmental potential of the oocyte, i.e. the ability of an oocyte to undergo normal maturation, fertilization and development to the blastocyst stage (Sianturi *et al*., 2002). The efficacies of different methods of oocyte collection were compared on the basis of cumulus oocyte complexes (COCs) per ovary (Abid *et al*., 2011).

In cattle, COCs were collected from the visible follicles from immediate slaughter house ovaries (Hoque *et al*., 2011; Dolakasaria *et al*.,

2013). However, a serious problem associated with the production of buffalo embryos through IVM is the very poor recovery of good quality immature oocytes (Palta and Chauhan, 1998). Oocyte quality can be expressed both morphologically and intrinsically, in which latter is described as developmental competence of an oocyte to develop into an embryo after fertilization (Merton *et al*., 2009).

Three methods for collection of oocytes have been described in domestic animals: aspiration of the oocyte from follicles (Datta *et al*., 1993; Boediono *et al*., 1995), slicing the ovaries (Carolan *et al*., 1992; Mogas *et al*., 1992; Pawshe *et al*., 1994) and puncture of visible surface follicles (Wani *et al*., 1999; Shirazi *et al*., 2005), and these methods were used with varying degrees of success. The number of oocytes recovered from ovaries will vary with different collection procedures, like dissection (Lonergan *et al*., 1991), aspiration (Totey *et al*., 1991; Jain *et al*., 1995), slicing (Mermillod *et al*., 2006; Wang *et al*., 2007) and post aspiration slicing (Suresh and Maurya, 2000) of ovarian follicles.

Therefore, the aim of this study was to compare the efficiency of dissection, aspiration, slicing, and aspiration plus slicing as harvesting techniques methods on the availability of quantity and quality of slaughterhouses buffalo oocytes.

MATERIALS AND METHODS

This study was carried out at the International Livestock Management Training Center (ILMTC), belonging to the Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, in cooperation with Department of Animal Production, Faculty of Agriculture, Tanta University.

Ovarian Collection:

A total of (668) buffalo ovaries were collected from local slaughter houses (El-Batanoun–Menofeya governorate) immediately after slaughter. The collected ovaries were placed into thermos at 27–30 °C in normal saline (0.9%) containing gentamicin (50 µg/ml) and transported to the laboratory within three hours post slaughter. In the laboratory, extraneous tissues were removed and the ovaries were washed three times in phosphate buffer saline (PBS, pH 7.3).

Methods of oocyte collection.

In the laboratory, the oocytes were collected aseptically from the ovaries by four methods, including dissection, aspiration, slicing and aspiration plus slicing. In all collection techniques, the visible ovarian follicles (2-8 mm in diameter) were counted before oocyte collection.

Dissection technique: the ovaries were placed in a sterile glass Petri dish containing 2 ml of PBS. All the visible follicles were carefully subjected to blunt dissection with the help of forceps and the remaining ovarian tissues were removed after a brief rinsing. The follicles were ruptured and the follicular fluid was allowed to flow into the PBS in Petri dish (Singh *et al*., 2013).

Aspiration technique: the oocytes were aspirated from individual ovaries after carefully removing the extraneous tissues and placed in Petri dish containing 1 ml of PBS (Rao and Mahesh, 2012). Oocytes were aspirated from the visible follicles presented on the ovarian surface. Oocytes were aspirated with 22 gauge needle fixed to 5 ml disposable syringe containing 1~2 ml of PBS.

Slicing technique*:* the ovaries were held firmly with the help of forceps in a sterile glass Petri dish containing 2 ml of PBS. The ovaries were sliced into possible thin sections with a blade fixed to the artery forceps. The oocytes containing PBS medium were placed in Petri dish and examined under Stereomicroscope (Abid *et al*., 2011).

Aspiration plus slicing technique*:* the aspirated ovaries were subjected to further slicing to obtain count of the residual oocytes (post aspiration slicing) according to Suresh and Maurya (2000). Then number of oocytes collected from both techniques was counted.

In all the four techniques, the Petri dishes were kept undisturbed for 5 min, allowing the oocytes to settle down. The Petri dishes were examined under stereomicroscopy and the oocytes were transferred to a searching dish containing PBS for grading. Phosphate buffer saline (PBS) medium was prepared according to Gordon (1994) as shown in Table (1).

Table (1): Composition of phosphate buffer saline (PBS) medium.

Ingredient	g/l	Ingredient	g/l
CaCl2. 2H2O	0.133	Kh2PO4	1.0
MgSO4. 7H2O	0.120	Glucose	1.0
NaCl	8.0	Sodium pyruvate	0.036
KCl	0.2	Streptomycin	100 mg
NaHPO4	2.17	Sodium Penicillin G	100,000 I.U

About 2 mg from bovine serum albumin was added to each one ml PBS. The pH value of the medium was adjusted to 7.2-7.4 using pH-meter and to osmolarity of 280-300 mOsmol/kg using osmometer. The medium was filtered by 0.22 µm millipore filter.

Oocyte evaluation:

Oocytes were examined under stereomicroscopy and classified according to their compaction, number of cumulus cell layers and homogeneity of ooplasm according to Ravindranatha *et al*. (2003) into 5 categories: 1) *Cumulus oocytes–complexes* (COCs) with compact cumulus cells (≥3 layers) and homogenous ooplasm, 2) *Expanded cumulus cells oocytes*, 3) *Denuded oocytes* with completely devoid cumulus cells and heterogeneity ooplasm, 4) *Partial denuded oocytes* with cumulus cells present either incompletely surrounding the oocyte and 5) *Degenerated oocytes* with ooplasm shrunken away from the zona pellucida or not evenly filling the zona pellucida.

Recovery rate:

Oocyte yield from each method was counted and calculated per ovary. Oocyte recovery rate (ORR) was determined as the following:

ORR (%) = (Number of recovered oocytes/ total number of follicles) x 100.

Statistical analysis:

Data in term of 9 runs (20-22 ovaries in each method) were statistically analyzed using general linear model procedures of SAS (2001) to determined the effect of collection technique. The significant differences among means were performed using Duncan's Multiple Range Test (Duncan's, 1955). The percentages values were adjusted to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from transformed values to percentages.

RESULTS AND DISCUSSION

Effect of collection technique on oocyte recovery rate:

Data in Table (2) showed significant (P<0.05) effect of collection techniques on oocyte recovery rate from buffalo ovaries, being the higher for aspiration plus slicing and slicing alone (84.7 and 83.3%) than for aspiration alone (72.7%), while dissection technique significantly (P<0.05) showed the lowest oocyte recovery rate (52.0%). It is of interest to note that there was insignificant and slight increase in recovery rate when oocytes were collected by additional post-aspiration slicing, although number of follicles on the ovarian surface and recovered oocytes were different for each collection technique.

Table (2): Effect of collection technique on number and recovery rate (%) of buffalo oocytes.

Item	Dissection	Aspiration	Slicing	Aspiration plus slicing
Total ovary (n)	262	206	200	206
Total follicles (n)	1087	921	1062	921
Total oocytes (n)	567	665	831	665
Visible follicles/ovary	4.16±0.05^{b}	4.33±0.21^{b}	5.24±0.43^{a}	4.33±0.21^{b}
Recovered oocytes/ovary	2.16±0.39^{c}	3.14±0.16^{b}	4.10±0.36^{a}	3.67±0.20^{ab}
Oocyte recovery rate (%)	52.0±0.76^{c}	72.7±1.59^{b}	83.3±0.95^{a}	84.7±1.72^{a}

a, b and c: **Means denoted within the same row with different superscripts are significantly different at P<.0.05.**

In accordance with the present results, Rao and Mahesh (2012) showed that among the three harvesting techniques, the slicing method appeared to be superior in terms of both total recovery and number of culture grade oocytes. Slicing yielded significantly (P<0.01) higher number of oocytes (7.98±0.70) per ovary compared to the puncture (3.46±0.31) and aspiration methods (2.38±0.19). Also, Gasparrini (2001) reported that the oocytes yield increased by using slicing method for collection of buffalo oocytes. Moreover, Khan *et al*. (1997) reported that oocytes were recovered via aspiration from 55% of follicles, as compared to the slicing method, which recovered oocytes from 78% of follicles from buffalo ovaries. In cow, Zheng *et al*., (2007) concluded that the recovery of oocytes using then slicing and

puncture techniques yielded more oocytes per ovary than other aspiration method.

In the present study, post aspiration slicing of buffalo ovaries provided additional 0.53 oocyte/ovary versus 0.46 oocytes/ovary as reported by Suresh and Maurya (2000). In buffaloes, Das *et al*. (1996) found that number of oocytes per ovary (follicles with 2-6 mm in diameter) recovered by slicing was significantly (P<0.01) higher (5.7/ovary) than that achieved by follicles puncture (2.6 / ovary) and aspiration (1.7/ovary).

Higher oocyte recovery in ovarian slicing may be due to their release from both surface follicles as well as from deeper cortex (Das *et al.,* 1996). Generally, the lower number of oocytes recovered by the aspiration method may be attributed to the presence of some follicles embedded deeply within the cortex, which are released by slicing of the ovary. Some of the oocytes may even be lost during aspiration of follicles, which is not possible when using the slicing method.

On the other hand, Shirazi *et al*. (2005) documented that the number of oocytes per ovary for slicing and aspiration didn't differ significantly in ewes. Also, Gupta and Sharma (2001) stated that there was no effect of slicing, aspiration and combined methods on the recovery of buffalo oocytes.

The conflicted results in this respect may be attributed to the effect of interaction between collection method and reproductive status. In this line, Das *et al*. (1996) found that buffalo ovaries with and without corpus luteum differ in the total oocyte yield/ovary, only when the aspiration method was used.

Effect of collection technique on oocyte quality:

Frequency distribution of recovered availability of oocyte categories from buffalo ovaries with different collection methods are presented in Table (3). Using different collection technique showed that buffalo oocytes were classified into COCs, expanded, denuded, partial denuded and degenerated oocytes according to the number of layers of COCs.

Table: (3): Effect of collection technique on categories (%) of buffalo oocytes.

Oocyte category	Dissection	Aspiration	Slicing	Aspiration plus slicing
COCs	42.03±0.90^{c}	51.34±0.47^{b}	63.17±1.19^{a}	51.24±0.83^{b}
Expanded	32.68±0.83^{a}	30.31±0.83^{b}	29.93±0.44^{b}	27.55±0.86^{c}
Denuded	10.59±0.56 ^{a}	3.34±0.54^{bc}	1.89±0.37^{c}	4.93±0.98^{b}
Partial denuded	3.25±0.73^{b}	11.01±0.84^{a}	3.05±0.92^{b}	10.89±0.77^{a}
Degenerated	11.45±0.32^{a}	4.00±0.39^{b}	1.96±0.37^{c}	5.39±0.39^{b}

a, b and c: **Means denoted within the same row with different superscripts are significantly different at P<.0.05.**

Results showed significant (P<0.05) effect of collection technique on frequency distribution of oocyte categories. Percentage of COCs was significantly (P<0.05) the highest (63.17%) using slicing technique, followed by aspiration or aspiration plus slicing techniques (51.34 and 51.24%,

respectively. Meanwhile dissection technique showed significantly (P<0.05) the lowest percentage of COCs (42.03%). On the other hand, dissection technique showed significantly (P<0.05) the highest percentage of expanded oocytes (32.68%) versus the lowest percentage (27.55%) in aspiration plus slicing techniques. Also, slicing technique showed significantly (P<0.05) the lowest percentages of denuded, partial denuded and degenerated oocytes (1.89, 3.05 and 1.96%) compared with other techniques, respectively (Table 3).

The present results showed that the COCs recovery rate or even acceptable oocytes (COCs and expanded oocytes) from slaughterhouse ovaries was better with slicing (93.1%) than with aspiration (81.6%) and aspiration plus slicing techniques (78.7%). Although the present results were much higher than that obtained by Rao and Mahesh (2012), they also reported that the slicing method appeared to be superior in term of number of culture grade oocytes. The slicing method was reflected in a greater number of mean good, fair, poor quality and culture grade oocytes, being 38.46, 27.75, 33.79 and 66.21%, respectively. Also, the present results were much higher than earlier findings of some authors (Madan *et al*., 1994; Das *et al*., 1996; Jamil *et al*., 2008) in buffaloes and cattle (Carolan *et al.,* 1994).

In accordance with the present results, Abid *et al.* (2011) found that the COC recovery rate of buffaloes was better with slicing than with the aspiration method (P<0.05). Also, Mistry and Dhami (2009) demonstrated that slicing was a simple effective method for collecting a high quality oocyte yield for *in vitro* culture. Also, Sianturi *et al.* (2002) found that the percentage of acceptable oocytes (cumulus oocyte complexes and expanded oocytes) of total oocytes was lower using aspiration (27.71and 40.66%) than slicing (33.8 and 34.02%), but the difference was not significant.

On the other hand, Gasparrini (2001) found a higher proportion of poor oocytes using slicing technique in buffaloes due to heterogeneous population of oocytes retrieved from all follicles which distributed through the stromal of ovaries.

The recorded variation due to the different methods used for COC recovery may be associated with seasonal effects, and variation in the reproductive status of the slaughtered buffaloes (Greve and Madison.,1991). Another explanation is due to the age of animal, state of ovary and the ovarian environment at time of collection (Hammam *et al*., 1997) or age, season, nutritional status (body condition) and cyclicity of animals at the time of slaughter, size and functional status of follicles, method of oocyte retrieval (Das *et al*., 1996; Nandi *et al*., 2001; Zoheir *et al.,* 2007; Amer *et al*., 2008).

CONCLUSION

The number of high quality oocytes recovered per ovary is an important consideration in the *in vitro* production of embryos. Therefore, to obtain a larger number of good quality oocytes (culture grade oocytes), it is necessary to choose the appropriate recovery method under certain

conditions. In buffaloes, Mistry and Dhami (2009) demonstrated that slicing was a simple effective method for oocyte collection for *in vitro* culture.

In conclusion, the recovery of oocytes using slicing technique increased oocyte recovery rate and produced higher percentage of good quality oocytes (COCs and expanded oocytes as compared to the dissection, aspiration and aspiration plus slicing techniques. Such results may indicate efficacy of slicing technique as a collection method on quantity and quality of buffalo oocytes.

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كفاءة طرق الجمع المختلفة على محصول وجودة بويضات الجاموس المصري

محمـد الفـاتح حمـاد*، شـريف عبـد الـو نـيس جبـر*، إبـراهيم طلعـت الرطـل و مها عبد المنصف جاد***

*** قسم الإنتاج الحيواني– كلية الزراعة – جامعة طنطا.**

**** قسم التكنولوجيا الحيوية - معهد بحوث الإنتاج الحيواني – مركز البحوث الزراعية.**

تهدف هذه الدراسة الى تقييم كفاءة طرق جمع مختلفة للبويضات من مبايض الجاموس في المجازر (التقطيع – الشفط - التشريح والشفط مع التشريح) على عدد وجودة بويضات الجاموس المصري. تم جمع البويضات من مبايض الحيوانات المذبوحة بالمجازر بأربع طرق مختلفة وتم تصنيف البويضات في كل طريقة من هذه الطرق إلى خمسة أنواع وذلك على حسب عدد طبقات الخلايا الركامية ومدى تجانس السيتوبلازم (بويضات جيدة- بويضات مقبولة – بويضات معراة جزئيا – بويضات معراة كليا- بويضات مضمحلة). وقد أظهرت النتائج أن معدل الاسترداد الكلى للبويضات المستردة من المبايض كانت أعلى معنويا بطريقة الشفط مع التشريح (٨٤%) وطريقة التشريح (٨٣,٣٠%) عن طريقة الشفط (٧٢,٦٨%) بينما أظهرت طريقة التقطيع اقل معدل لاسترداد الكلى للبويضات معنويا عن الطرق الأخرى (٥٢,٠٤%). كانت النسبة المئوية للبويضات الجيدة المستردة من المبيض بطريقة التشريح (٦٣,١٧%) أعلى معنويا عن تلك المستردة بطريقة الشفط (٥١,٣٤%) وكذلك طريقة الشفط مع التشريح (٥١,٢٤%) وعن طريقة التقطيع أيضا (٤٢,٠٣%) بينما كانت النسبة المئوية للبويضات المقبولة أعلى في طريقة التشريح عن طريقة الشفط و طريقة الشفط مع التشريح وطريقة التقطيع وكانت القيم المناظرة هي ٢٩,٩٣- ٣٠,٣١- ٢٧,٥٥ و ٣٢,٦٨%، على التوالي. وتشير هذه النتائج إلى كفاءة تقنية التشريح كطريقة لجمع البويضات على عدد وجودة البويضات في الجاموس المصري.