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Ovarian Morphology and Oocyte Quality in Relation to in Vitro Embryo Production in Ruminant Animals

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Abstract: The aim of this study was to evaluate the reproductive status of small (sheep and goat) and large ruminants (cow and buffalo) slaughtered out of slaughterhouse through the characteristics of ovaries and oocytes. Ages of slaughtered animals were determined through the teeth. They were classified into young with milk teeth and adult with 2-8 permanent incisors. Genitalia were taken immediately after slaughtering the animals. The collected genitalia were normal and from non-pregnant animals. Ovaries were examined to determine the reproductive status according to the estrus cycle. Ovaries were classified into follicular or luteal phase. Weight of reproductive systems and ovaries as well as the number of follicles per animals were recorded. Oocytes were aspirated from the large follicles using an 18-gauge needle and examined under a stereomicroscope to identify and evaluate the quality of oocytes morphologically. Weights of ovaries were increased with increasing weights of reproductive systems. Also, weights of ovaries were increased significantly ($P<0.05$) during the luteal phase compared to the follicular phase. Most of the examined ovaries were showed large number of follicles. The results indicated that the majority of the collected oocytes were evaluated and classified morphologically as grade I. The collected oocytes can be used for in vitro production of embryos and other purposes.

Introduction

Ovaries are the primary reproductive organs in the female because they produce the female gamete. Numerous of follicles are produced during the reproductive cycles in ruminants (Fitzpatrick and Entwistle, 1997; Cerri *et al.*, 2009; Lauderdale 2009). The ovary of the ruminants contains several hundred growing follicles (McNatty *et al.*, 1982). Kaulfuss *et al.*, (1994) found an average of 44 visible vesicular follicles at different stages during the estrus cycle and the number of follicles did not differ significantly between the left and right

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ovaries. Depletion of the ovarian reserve is associated with reproductive senescence in mammalian females and there is a positive relationship between the size of the ovarian reserve and the number of antral follicles on the surface of the ovary (Cushman et al., 2009). Normally cow and buffalo produce one ovum per estrus cycle whereas sheep and goat produce one or more ova each estrus cycle. Age at puberty is affected by both genetic and environmental factors. Genetic factors can be seen by comparing breeds within a species. Average age at puberty is 5 to 7 months for does, 6 to 9 months for ewes, 8 to 11 months for European-type dairy cows, 10 to 15 months for European-type beef cows, 17 to 27 months for Zebu-type cows (Moran et al., 1989; Hassan et al., 1992; Waldron 1999).

There is a relationship between peak number of antral follicles and follicular waves, hormone concentrations, superovulatory response and embryo quality in beef heifers (Ward et al., 2006). Oocytes could be collected from follicles and used for in vitro production of embryos. Production of embryo in vitro is generally referred to as three-step procedures, namely maturation, fertilization and culture of the in vitro-derived oocytes (Mohammed et al., 2005). Although there are different methods for in vitro maturation (IVM); in vitro fertilization (IVF) and in vitro culture of oocytes in ruminants, however, more studies are still required for further improvement.

In vitro maturation (IVM) of oocyte is an important reproductive technology for generating mature oocytes which are capable of supporting preimplantation and post implantation development of embryos to term. Although, there is great clinical and commercial incentive to improve the efficiency of this technique; however, progress has been slow over the past decade (Gilchrist and Thompson. 2007).

The oocyte is a unique and highly specialized cell responsible for creating, activating, and controlling the embryonic genome, as well as supporting basic processes such as cellular homeostasis, metabolism, and cell cycle progression in early embryo development. During oogenesis, oocyte accumulates myriad of factors to execute these processes, consequently, oogenesis is critically dependent upon correct oocyte–follicle cell interactions. Disruptions of oogenesis via environmental factors and changes in maternal health and physiology can compromise oocyte quality, leading to arrested development, reduced fertility, and epigenetic defects that affect long-term health of the offspring (Mtango et al., 2008). In this context,

preimplantation and postimplantation development of embryos was affected by the oocyte quality in addition to the culture media (Mohammed et al., 2005; Mtango et al., 2008).

The ovaries, oocytes and embryos (fresh & frozen) can be used for different purposes; preservation of species and producing offspring. Therefore, the aim of this study is to identify and evaluate the animals slaughtered out of slaughterhouse and their reproductive status through the ovarian follicles and oocytes.

Materials And Methods

1) Sample collection: Slaughtered animals were classified according to their teeth into young (milk teeth) and adult (2-8 permanent incisors). Thereafter, reproductive system of the slaughtered animals was collected and transported within 4-6 hours to the laboratory at 30-33°C in thermos for further evaluation.

Sample evaluation: The young and adult reproductive systems were classified according to the stage of estrus cycle into follicular and luteal phase. Weights of reproductive systems and ovaries were recorded. Also, numbers of visible antral follicles were counted per animal according to animals' age (young and adult) and the stage of estrus cycle (follicular and luteal phase).

Oocyte recovery, selection and classification: Oocytes were aspirated from the large follicles by using 18-gauge needle and syringe. Then, oocytes were counted and classified into three classes based on the cumulus cells and homogeneity of the cytoplasm as recommended by Ganguli et al. (1998), as follow:-

Grade 1: Oocytes were completely invested with cumulus cell layers (good oocytes).

Grade 2: Oocytes were surrounded with scantly cumulus cell layers (fair oocytes)

Grade 3: Naked (denuded) oocytes.

Statistical analysis: Data are presented as means ± SD. Differences between mean values were determined by ANOVA procedures of SAS (1 998) followed by Duncan's multiple range test for mean separations.

Results And Discussion

I. Evaluation of reproductive systems of the slaughtered animals

Out of 54 and 24 slaughtered sheep's and buffalos, forty and eighteen of the former slaughtered animals respectively were young whereas the remaining slaughtered animals were adult (Tables 1 & 3). Ovaries weight were significantly ($P<0.05$) higher during the luteal phase than the follicular phase. Variations in the number of visible antral follicles were

observed among animals. Numbers of aspirated large follicles and recovered oocytes were not significantly differed between ages or stages of estrus cycle (Tables 1-4). Numbers of visible antral follicles, aspirated large follicles and recovered oocytes were significantly ($P<0.05$) higher in goats than sheep (Table 1 &2).

Table (1). Reproductive system characteristics during follicular and luteal phases of young and adult sheep

Trait	Young	Adult	
Stage of estrus cycle	**Follicular phase**	**Follicular phase**	**Luteal phase**
No. reproductive systems	40	8	6
Reproductive system weight (g)	$11.05^{b} \pm 3.23$	$23.17^{a} \pm 2.65$	$22.85^{a} \pm 1.24$
Ovaries weight (g)	$00.44^{b} \pm 00.10$	$0.56^{b} \pm 0.046$	$1.28^{a} \pm 0.37$
No. follicles	$10.70^{a} \pm 6.87$	$11.5^{a} \pm 3.81$	$8.00^{a} \pm 0.89$
No. aspirated follicles	$03.10^{a} \pm 01.32$	$2.75^{a} \pm 0.88$	$3.33^{a} \pm 0.51$
No. recovered oocytes	$01.55^{a} \pm 0.78$	$1.5^{a} \pm 0.53$	$1.83^{a} \pm 0.41$

Values are presented as means ± standard deviation a,b: Values with different superscripts on the same row are significantly different ($P<0.05$)

Table (2). Reproductive system characteristics during follicular and luteal phases of adult goat

Trait	Adult	
Stage of estrus cycle	**Follicular phase**	**Luteal phase**
No. reproductive systems	8	6
Reproductive system weight (g)	$46.51^{a} \pm 10.34$	$28.64^{b} \pm 3.34$
Ovaries weight (g)	$1.83^{a} \pm 0.57$	$2.05^{a} \pm 0.13$
No. follicles	$21.25^{a} \pm 11.14$	$22.0^{a} \pm 10.95$
No. aspirated follicles	$6.25^{a} \pm 1.38$	$6.66^{a} \pm 0.51$
No. recovered oocytes	$3.75^{a} \pm 0.83$	$3.16^{a} \pm 0.75$

Values are presented as means ± standard deviation a,b: Values with different superscripts on the same row are significantly different ($P<0.05$)

Table (3). Reproductive system characteristics during follicular and luteal phases of young and adult buffalo

Trait	Young buffalo	Adult buffalo
Stage of estrus cycle	**Follicular phase**	**Luteal phase**
No. reproductive systems	18	6
Reproductive system weight (g)	$63.14^{b} \pm 3.64$	$215.16^{a} \pm 13.9$
Ovaries weight (g)	$01.80^{b} \pm 0.18$	$3.00^{a} \pm 0.37$
No. follicles	$10.44^{a} \pm 0.88$	$10.16^{a} \pm 1.83$
No. aspirated follicles	$02.11^{a} \pm 0.78$	$2.16^{a} \pm 0.75$
No. recovered oocytes	$01.10^{a} \pm 0.60$	$1.50^{a} \pm 0.51$

Values are presented as means ± standard deviation a,b: Values with different superscripts on the same row are significantly different ($P<0.05$)

Table (4). Reproductive system characteristics during follicular and luteal stages of adult cattle

Trait	Adult cattle	
Stage of estrus cycle	**Follicular phase**	**Luteal phase**
No Reproductive systems	5	5
Reproductive system weight (g)	$460^{a} \pm 42.19$	$475.8^{a} \pm 29.55$
Ovaries weight (g)	$4.92^{b} \pm 00.86$	$5.46^{a} \pm 0.73$
No. follicles	$19.0^{a} \pm 4.36$	$19.2^{a} \pm 4.60$
No. aspirated follicles	$3.2^{a} \pm 0.84$	$3.6^{a} \pm 1.34$
No. recovered oocytes	$2.2^{a} \pm 0.44$	$2.0^{a} \pm 0.70$

Values are presented as means ± standard deviation a,b: Values with different superscripts on the same row are significantly different ($P<0.05$)

In general, it was observed that weights of reproductive systems are increased simultaneously with increasing the body weights (Bukar et al., 2006) and with parturition (Morgan and Davis 1936). The weights of ovaries were increased significantly ($P<0.05$) during the luteal phase compared to the follicular phase. This increase might be due to the presence of corpus luteum. Corpora lutea weights on days 3 and 14 of the natural estrus cycle were 0.47 and 4.7g respectively (Fields and Fields, 1996). Osman and Shehata (2005) found that the corpus luteum represents

30.1% of the ovarian weight in buffalo.

The collected ovaries were almost contained large numbers of visible antral follicles. The numbers of visible antral follicles were not differed significantly with advancing age (from young till adult) or with the stage of estrus cycle of animals. This may be due to variations in animals' age. Recent study by Murasawa *et al.*, (2005) in cattle demonstrated that the number of antral follicles is highly variable among animals. Cushman et al., (2009) concluded that antral follicle count in beef cows and heifers is influenced by birth weight and age but not by stage of the estrus cycle.

The results indicated that oocytes recovery rate of punctured follicles was 50-60%. Recovery of oocytes by aspiration of antral follicles, using syringe and needle, has been the method employed with ovaries retrieved from the slaughtered animal. One of the difficulties initially associated with the aspiration approach lay in the fact that oocytes might only be recovered from some 30–60% of the punctured follicles. The advantage of follicle aspiration is related to the speed of operation. Scott et al., (1989) found that recovery rates were significantly ($P<0.01$) higher in 18- to 20-mm follicles and lower ($P<0.001$) in those ≤ 11 mm. Zoheir et al., (2007) found that number of recovered oocytes per ovary was 1.7-2.20 in buffalo.

II. Oocytes quality

Results showed that the quality of the oocytes collected during the luteal phase were better than the follicular phase (Table 5). Although the quality of collected oocytes was comparable in sheep and goat, it was higher in cattle than those in buffaloes (Table 5).

Table (5). Quality of recovered oocytes from young and adult animals during follicular and luteal phases

Animal	Age	Stage of estrus cycle	No. of oocyte	Oocyte quality		
				Good	Fair	Denuded
Sheep	Young	Follicular phase	62	64.5 (40/62)	32.3 (20/62)	3.2 (2/62)
	Adult	Follicular phase	12	58.3 (7/12)	41.7 (5/12)	00.0 (0/12)
		Luteal phase	11	72.2 (8/11)	27.8 (3/11)	00.0 (0/11)
Goat	Adult	Follicular phase	30	53.3 (16/30)	43.3 (13/30)	3.4 (1/30)
		Luteal phase	19	63.1 (12/19)	31.6 (6/19)	5.3 (1/19)
Buffalo	Young	Follicular phase	20	50.0 (10/20)	50.0 (10/20)	00.0 (0/20)
	Adult	Luteal phase	9	55.5 (5/9)	44.5 (4/9)	00.0 (0/9)
Cattle	Adult	Follicular phase	11	63.6 (7/11)	27.3 (3/11)	9.1 (1/11)
		Luteal phase	10	70.0 (7/10)	30.0 (3/10)	00.0 (0/10)

The morphological quality of recovered oocytes from young and adult slaughtered animals during the follicular and luteal phases was evaluated. Although it seems that the age of animals had no effect on the quality of oocytes morphologically, however, the stage of estrus was affected. The percentages of good oocytes were increased during the luteal phase compared with the follicular phase. Taken over the full range of mammalian species, concern is often expressed regarding the quality of oocytes recovered either from the very young or the very old. Oocytes from calves are developmentally less competent than those from adult animals (Adulyanubap et al., 1998; Gandolfi et al., 2000). It is known that calf oocytes may be smaller in diameter than those of adult cattle; other features that may mark them out after exposure to sperm include delayed sperm aster formation and asynchronous pronuclear formation (Duby et al., 1995). By comparing the developmental competence of IVM oocytes derived from lambs and ewes; Kochhar *et al.* (2002) found that the cleavage rate was similar and the blastocyst yield was significantly lower in lamb-derived oocytes.

Oocyte quality was recorded during different stages of the estrus cycle and according to whether follicles are located ipsilateral or contralateral to the corpus luteum. Boediono *et al.* (1995) tested the hypothesis that higher-quality oocytes can be obtained from ovaries in the luteal phase and from ovaries bearing the corpus luteum; their results were consistent with that hypothesis. Varisanga *et al.* (1998) attempted to classify bovine ovaries into five categories according to their morphological features and to determine whether such features affected the recovery and developmental competence of oocytes. Oocyte morphology was to some extent determined by stage of cycle; results supported the concept that the intraovarian environment to which oocytes are exposed can play a major role in determining their developmental competence.

The results indicate that the number of follicles and oocyte quality of cattle were higher than buffalo. Tan *et al.* (1998) recorded that buffalo ovaries contain five oocytes per ovary compared with 14.3 oocytes per ovary for cattle; maturation and cleavage rates were both lower in buffaloes than the equivalent values in cattle. Moreover, the numbers of antral visible follicles and recovered oocytes of goat were higher than sheep. Taken together into consideration, this may be related to genetic variability between species.

It could be concluded that the results are important for; I) in vitro production of embryos (IVP) which elevates with

increasing the oocyte quality, II) ovum synchronization, an efficient method adopted to regulate dominant follicle growth and ovulation during the follicular waves occurring in estrus cycles. Animal with relatively high number of follicles per wave respond best to standard superovulation protocols, III) Biological resource banks (BRBs) which are important tools for the conservation of species and valuable breeds, and have been strongly developed during the last decade (Felipe et al., 2005). The term BRB comprises many techniques and protocols, the purpose of which is to collect, preserve and utilize tissues and germplasm of selected individuals in order to ensure the continuity and the genetic variability of breeds, populations and species.

Further studies are required for adopting and improving *in vitro* embryo production systems (maturation, fertilization and culture) of collected oocytes from local mammalian animals (cattle, buffalo, sheep and goat). Technological manipulation of the mammalian oocytes may increase the production of meat, milk and conserve species.

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الملخص العربي

مورفولوجيا المبيض وجودة البويضات للحيوانات المجترة وعلاقتها بإنتاج الأجنة خارجيا

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الهدف من البحث هو تقييم الحالة التناسلية للمجترات الصغيرة (الاغنام والماعز) والكبيرة (الابقار والجاموس) المذبوحة من خلال صفات المبيض والبويضات. تم جمع المتاح من الاجهزة التناسلية من الحيوانات المذبوحة. تم تقييم الاجهزة التناسلية حيث كانت طبيعية ومن حيوانات غير حاملة. تم تقدير العمر للحيوانات المذبوحة عن طريق الاسنان حيث قسمت الحيوانات الي صغيرة ذات اسنان لبنية وبالغة ذات ٢-٨ اسنان مستديمة. فحصت المبايض لتقدير الحالة التناسلية للحيوانات المذبوحة علي حسب دورة الشبق وقسمت الي الطور الحويصلي والطور الليوتيني. تم تقدير وزن الاجهزة التناسلية وكذلك المبيض كما تم تقدير عدد الحويصلات الموجودة علي سطح المبيض. تم سحب البويضات من الحويصلات الكبيرة وفحصت تحت الميكروسكوب لتقدير جودتها المورفولوجية. أظهرت النتائج زيادة وزن المبيض بزيادة وزن الجهاز التناسلي. كما زاد وزن المبيض في الطور الليوتيني عن الطور الحويصلي. احتوت معظم المبايض في الاجهزة التناسلية علي عدد كبير من الحويصلات. كما ان اغلب البويضات كان من طراز الدرجة الأولي مورفولوجيا. تستخدم البويضات المجموعة في إنتاج الأجنة لاستخدامها في الأغراض المختلفة.