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Effect of multiple vs. single preovulatory follicle on oocyte quantity and quality and *in vitro* maturation of goat oocytes

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Abstract

The present study was conducted to investigate the effects of multiple vs. single preovulatory follicle on oocyte quantity and quality and *in vitro* maturation of goat oocytes. Slaughtered goat ovaries were collected and ranked into 2 types according to the number of preovulatory follicles on their surface: Type I (with multiple preovulatroy follicles, ≥ 2 follicles), Type II (with single preovulatory follicle). The number of goat oocytes retrieved from follicles (2-6 mm Ø) was recorded for each ovarian type. The released immature goat oocytes were scored for cumulus - oocyte cell adhesion into one of 3 grades (C^{+}; cumulus - enclosed oocytes, $C^{+/-;}$ cumulus partially enclosed oocytes, C^{-} ; cumulus - free oocytes). Grade C^{+} and grade $C^{+/}$ were matured in tissue culture media (TCM -199) supplemented with 10% Fetal Calf Serum for 30 h in CO_2 incubator at 38.5ºC and 95% humidity. The results indicated that a greater number of aspirated oocytes were found in type I than type II. The number of Grade C+ and Grade C+/- oocytes in Type I was significantly higher ($P < 0.05$) than type II. The in vitro maturation rate of oocytes recovered from goat ovaries was non significantly different in both types but the number of cumulus - full expanded oocytes (CE^{+}) appeared to be higher in ovaries having multiple than single preovulatory follicle. In conclusion, higher quantity, quality and maturation rate of oocytes may produce in multiple preovulatory follicles of goat ovaries.

Keywords: follicle number, oocyte, in vitro maturation, goat

Introduction

In mammals, the ovary contains primordial follicles and growing follicles of which small number are visible on the ovarian surface. The differentiation of one mature preovulatory follicle, the so-called dominant follicle, is the result of a complex interaction between the cohort of ovarian follicles on the growth trajectory and the hypothalamo-pituitary system with intraovarian endo-, para- and autocrine regulatory factors and mechanisms. Differentiation of the dominant tertiary follicle is a two-stage process in which, as a result of gonadotrophic stimulation, antral follicles are advantaged by more intensive growth. The development of normal healthy tertiary follicles with a diameter surpassing 2 mm is termed recruitment and the developing, growing antral follicles with a diameter of 2 - 5 mm are termed recruited. Differentiation and maturation advance to the process of selection of those tertiary follicles that reach a diameter of more than 5 mm. This process of selection usually results in one, seldom in two and only exceptionally in multiple, 3 or 4 dominant ovulatory follicles. All other follicles undergo atresia (DiZerega, 1981; Presl, 1989; Dolezel 1995).

In mammalian reproduction, the oocyte depends on the ovarian follicle for most of its growth, they form a bipolar partnership and the status of one will impact the functioning of the other (Sirard, 2011). There are several factors affecting the number of different categories of oocytes relative to the number of the ovarian follicles per ovary and its frequency distribution to the totally recovered oocytes. Mogas *et al.* (1992), Thakre (1993), Islam *et al.* (2007) and Khillare (2008) reported that age, pregnancy status, stage of estrous cycle, presence of CL and season variations had an important role on the yield and quality of goat oocytes. However, effect of presence multiple or single preovulatory follicle in goat ovaries has not been recognized during previous studies. Moreover, there is no information available on the effect of follicular number on maturation of goat oocytes.

Yadav *et al.* (1997) reported that the higher maturation rate of goat oocytes is 30 h cultured period and also Previous studies on goat oocytes have shown maturation rates of 68-97% (Martino, *et al.* 1995), 50-52% (Nagar and Purohit, 2005), 30-40% (Majeed *et al.* 2011) and 60-80% (Wang, *et al.* 2007).

Recent advances in *in vitro* maturation, fertilization and culture technology allowed progress in an increasing the number of off springs produced from genetically superior females but this progress still has important factors affect the yielding and quality of the oocytes. The influence of number of preovulatory follicles on oocyte quality has been largely considered, as a factor of high impact.

Our aim was to study the effect of multiple vs. single preovulatory follicle of adult goat ovaries on the quantity and quality of oocytes and *in vitro* maturation of goat oocytes.

Materials and Methods

Chemicals

All materials were purchased from Sigma Chemical Company (St. Louis, MO, USA) unless otherwise indicated.

Ovary collection

A total of 90 ovaries from slaughtered goats of unknown reproductive history were collected from the butchers in Beni-Suef governorate. The ovaries were separated shortly after slaughtered native goat and maintained in a thermos flask containing 0.9% sterile normal saline and gentamycine 0.5 mg/ml. Ovaries were transported to laboratory within 2 h (Abd-Allah, 2010).

Under aseptic condition the ovaries were washed with Ethanol 70% (to remove adhering blood), and avoid contamination. Other tissues were dissected away from the collected ovaries, then the ovaries were rinsed by sterile wormed saline at 37ºC three times to further remove any contaminants on the ovary surface and the traces of ethanol (Abd-Allah, 2010).

2.3. Processing of the ovaries

The collected ovaries were classified according to the number of follicles on their surface into two types: Type I (with multiple preovulatroy follicles, ≥ 2 follicles) and Type II (with single preovulatory follicle). The presence of a corpus luteum was not considered.

Collecting and qualifying the oocytes

The follicles of 2-6 diameters were aspirated with an 18-gauge needle connected to disposable syringe containing 0.5 ml warm TCM-199 at 38.5ºC.

The number of goat oocytes retrieved from follicle (2-6 mm) was recorded for each ovarian type. The released immature goat oocytes were scored for cumulus - oocyte cell adhesion as previously described for bovine by Combelles and Albertini (2003) with minor modification by Abd-Allah (2009) as fellow:

C^+ for cumulus - enclosed oocytes

$C^{+/-}$ for cumulus partially - enclosed oocytes (whenever there were granulosa cell - free regions on the oocyte surface)

C^- for cumulus - free oocytes.

The number of oocytes with different grades (C^+, $C^{+/-}$ and C^-) for each ovarian type (I and II) was recorded.

Maturation of oocytes in vitro

The grades (C^+ and $C^{+/-}$) COCS oocytes were cultured in culture media (TCM-199 supplemented with 10% Fetal Calf Serum) for 30 h at 38.5ºC in 5% CO_2 incubator and 95% humidity and the degree of cumulus cell expansion was the criteria for maturation of the oocyte.

The matured oocytes were scored for cumulus - oocyte cell expansion as previously described for bovine by Lorenzo *et al.* (1994) with minor modification: CE^+ for cumulus - full expanded oocytes, $CE^{+/-}$ for partially expanded oocytes, (whenever there were few expansion of cumulus layers or cumulus cells were non-homogeneously spread and clustered cells were still observed or moderate expansion of cumulus layers) and CE^- for cumulus - non expanded oocytes. The degree of cumulus cell expansion was the criteria for maturation of the oocyte.

Statistical analysis

Data were analyzed using Chi-square analysis (Snedecor and Cochran, 1980).

Results and Discussion

From a total of 90 ovaries collected, 40 were categorized as Type I and 50 as Type II (Table 1). The highest number of oocytes was observed in Type I followed by Type II. Depending upon the microscopic morphology of collected oocytes, the highest ($P < 0.05$) number of Grade C^+ and Grade $C^{+/-}$ oocytes was observed in Type I followed by Type II ovaries.

A significantly greater ($P < 0.05$) number of Grade C^+ was observed in Type I. Also, a significant difference was observed in the number of Grade $C^{+/-}$ in Type I ovaries compared to other type.

The number of cumulus - enclosed oocytes (C^+) appeared to be the highest followed by cumulus partially enclosed oocytes ($C^{+/-}$) with partial and then the granulosa - free oocytes (C^-) in both types.

The in vitro maturation rate of oocytes recovered from goat ovaries was non significantly different in both types but the number of cumulus - full expanded oocytes (CE^+) appeared to be higher in ovaries having multiple than single preovulatory follicle (Fig. 1).

Table1. Population of Oocytes quality and yield and maturation rate obtained from goat ovaries for different ovarian types.

Criteria		Ovaries Type I	Ovaries Type II
Number of Ovaries		40	50
Number of Recovered Oocytes		60	55
Recover Rate		1.5	1.1
Morphological observation of immature oocytes (%)	C^+	22(36.7%)^a	14(25.5%)^b
	$C^{+/-}$	24(40.0%)^a	17(30.9%)^b
	C^-	14 (23.3%)^a	24(43.6)^b
Number of cultured oocytes (%)		46(76.7%)^a	31(56.4%)^b
Number of Matured Oocytes (%)		30(65.2%)^a	20(64.5%)^a
Cumulus Expansion of Matured Oocytes (%)	CE^+	22(47.8%)^a	8(25.8%)^b
	$CE^{+/-}$	8(17.4%)^a	12(38.7%)^b
	CE^-	16(34.8%)^a	11(35.5%)^a

Within the same raw, Values with the different superscripts are significantly different from each other ($P < 0.05$).

Ovaries type I: Ovaries bearing multiple Preovualtory follicles

Ovaries type II: Ovaries bearing single preovulatory follicle

C+ : Cumulus - enclosed oocytes

C+/– : Cumulus partially - enclosed oocytes (whenever there were granulosa cell-free regions on the oocyte surface)

C– : Cumulus -free oocytes.

CE+ : Cumulus-full expanded oocytes.

CE+/–: Cumulus-partially expanded oocytes (whenever there were few expansion of cumulus layers or cumulus cells were non-homogeneously spread and clustered cells were still observed or moderate expansion of cumulus layers)

CE– : Cumulus-non expanded oocytes

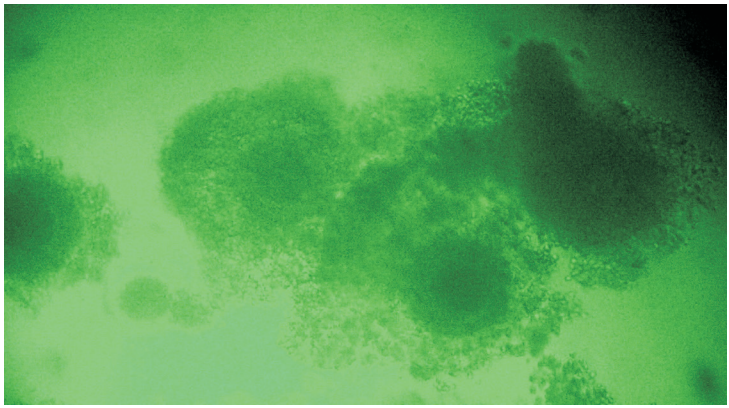

Fig. 1. Morphological normal mature goot oocytes (40 X).

In ovaries, there are three wave patterns of follicular growth although two waves or sometimes four waves can occur during the estrous cycle. Each wave of follicular development is characterized by simultaneous emergence of medium-sized (> 4 mm in diameter) growing follicles from a pool of smaller follicles. One of these groups of follicles rapidly emerges as the dominant follicle (7 - 9 mm in diameter) and continues to develop while the others undergo atresia and regress. It usually takes 5 to 7 days for the dominant follicle to develop to ovulatory size (Savio *et al.*, 1993). The greatest number of oocytes that were found in Type I ovaries in the present study might reflect the optimum level of gonadotropins and steroids.

In the current study, Grades C^{+} and $C^{+/-}$ oocytes were found in significantly greater numbers in Type I ovaries. Therefore, 2 - 6 mm follicles from Type I ovaries can be used as a source of oocytes for *in vitro* studies.

In the present study, ovaries bearing multiple provulatory follicles had significantly higher number of good quality oocytes than those bearing single ones. This may attributed to the negative effect of progesterone might not be effectively functional in this group. So the higher number of good quality of COCs in this category than that of single preovualtory group explains the role of hormonal balance on goat folliculogenesis.

The present results revealed that maturation rate of goat oocytes was 64.5-65.2%. These results are contradictory to those reported by Martino, *et al.* (1995) 68-97%, Nagar and Purohit (2005) 50-52%, Wang, *et al.* (2007) 60-80% and Majeed *et al.* (2011) 30-40%.

The difference in maturation rates might be due to the factors like method of oocytes collection, system of classification, duration of incubation period, maturation media used and media additives. Also, might be due to the differences in age, pregnancy status, presence of CL, season of ovarian collection and site of ovary, nutritional and genetic status of the oocyte donors obtained from a slaughterhouse source.

Conclusion

In conclusion, goat ovaries with multiple preovulatory follicles resulted in a greater number of recovered oocytes and good quality oocytes compared to those with single follicle. Moreover, oocytes recovered from ovaries bearing multiple preovulatory follicles had better *cumulus expansion* than those bearing single ones, Therefore, 2 - 6 mm follicles from ovaries bearing multiple preovulatory follicles can be used as a source of oocytes for *in vitro* maturation and subsequent culture study.

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