LEPTIN INCREASES *IN VITRO* DEVELOPMENTAL POTENTIAL OF PREPUBERTAL AND PUBERTAL DECCANI EWE OOCYTES

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ABSTRACT

The impact of leptin addition (0, 10, 20, 50 and 100 ng/ml) in maturation media on Deccani ewe oocytes (prepubertal, 383; pubertal, 376) was investigated during non-breeding season. The cumulus oocyte complex expansion and oocytes at M-II stage with extrusion of first polar body in prepubertal (63.8±1.5% and 61.1±2.5%, respectively) and pubertal (68.2±1.3% and 66.2±2.%, respectively) ewes was higher ($p<0.05$) following the addition of leptin at 20 ng/ml compared to other leptin concentrations tested. In brief, leptin addition (20 ng/ml) in maturation media increases the developmental potential of prepubertal as well as pubertal Deccani ewe oocytes.

Keywords: COCs, Ewe, Leptin, M-II stage, Oocytes

INTRODUCTION

Assisted reproductive technologies like *in vitro* embryo production can be used to improve the breeding performance of small ruminants (Mondal *et al.*, 2008). The oocytes derived from antral follicles of prepubertal cattle (Armstrong *et al.*, 1992), sheep (O'Brien *et al.*, 1997) and goat (Martino *et al.*, 1995) can be matured and fertilized *in vitro*. Oocytes of prepubertal females may shorten generation interval resulting in faster genetic change. On the other hand developmental ability of oocytes from prepubertal animals is compromised in farm animals (Lv *et al.*, 2010). Leptin hormone is mainly secreted by white adipocytes. An efficient role of leptin hormone-augmented medium during *in vitro* maturation was reported in cattle (Cordova *et al.,* 2011). Therefore, the impact of leptin addition on *in vitro* maturation of prepubertal and pubertal Deccani ewe oocytes was studied during non-breeding season.

MATERIALS AND METHODS

Between February to April, the ovaries were collected immediately after the slaughter of Deccani breed ewe (98 prepubertal ovaries and 90 pubertal ovaries). Oocytes harvested by slicing techniques were ategorized into four quality grades (Aruna Kumari *et al.,* 2010). The oocytes retrieval and overall efficacy was determined.

Before culturing, the oocytes were washed twice in handling media then pre-incubated in *in vitro* maturation (IVM) media (bicarbonate-buffered TCM 199 with 10 µg/mL each of FSH, LH, and bovine serum albumin, 5 µg/mL estradiol-17 β, 50 µg/mL gentamicin sulphate, and 10% v/v estrus sheep serum). Various concentrations of leptin (0, 10, 20, 50 and 100 ng/ml) were used in IVM media. In culture plates, grade 1 and 2 oocytes were used and were placed in six droplets of 50 µl IVM media along with different concentrations of leptin. Each droplet was covered by autoclaved mineral oil and was incubated under an atmosphere of 5% CO_2, 95% humidity at 38.5°C for 24 h.

The degree of oocyte maturation was assessed under stereo zoom microscope (SZX-12, DF PLAPO 1X, Olympus, Japan) based upon cumulus cell expansion. The maturation was classified as grade-1 or full cumulus cell expansion (cumulus cells spread homogenously and amplification of cumulus cell was

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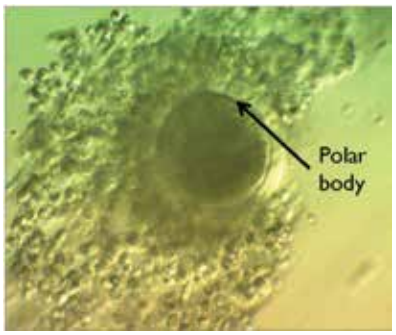
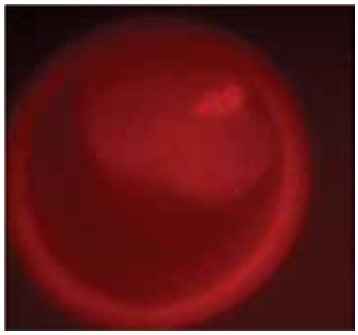
Culture grade oocyte



Matured oocyte with polar body



M-II stage

Figure 1: *in vitro* maturation of oocyte in Deccani ewe

at least three times diameter away from zona pellucid), grade-2 or moderate cumulus cell expansion (cells were spread non-homogenously and enlargement of cumulus cell to at least two times diameter away from zona pellucid) and grade-3 or slight expansion (cumulus cells were extremely adhering to zona pellucid). Only grade-1 and -2 cumulus cell expansion oocytes were considered as matured. Moreover, oocytes maturation at 24 h post-IVM was evaluated based upon staining with propidium iodide. The oocytes containing M-II nuclei with the 1st polar body were considered for complete nuclear maturation (Figure 1). The data obtained was analyzed by SPSS software (version 17) using one-way ANOVA followed by Duncan's multiple range test.

RESULTS AND DISCUSSION

Oocyte retrieval results of the present study (Table 1) were in accordance with a previous study in which the oocytes retrieval efficiency in prepubertal and pubertal goats was 77% and 80%, respectively (Khatun *et al.,* 2011). The quality of oocyte recorded in prepubertal and pubertal ewes of present study revealed higher ($p<0.05$) numbers of grade 3 and 4 oocytes from prepubertal ewe ovaries (Table 1). The majority of ovaries were collected during non-breeding season that might be the reason for low-grade oocyte recovery rate.

Table 1: Oocyte recovery and efficacy from prepubertal and pubertal Deccani ewe ovaries

Status	**Prepubertal**	**Pubertal**
Ovaries, n	98	90
Oocytes recovered, n	383	376
Oocytes per ovary, n	3.9±0.5[a]	4.2±0.6[b]
Efficacy, %	66.3±0.6[a]	71.3±0.1[b]
Oocyte quality		
Grade 1	2.3±0.2[a]	3.6±0.4[b]
Grade 2	3.4±0.4	3.1±0.3
Grade 3, 4	3.9±0.3[a]	1.9±0.2[b]

[a vs b]$p< 0.05$, within a row for a parameter

The indicators of maturation of ovine oocyte are expanded cumulus cells, change in dimension of perivitelline space, expulsion of first polar body and formation of second metaphase spindle tangential to the surface of vitelline membrane (Gordon, 2003). The oocytes incubated with 20 ng/ml leptin in maturation media exhibited higher ($p<0.05$) percentage of maturation rate in comparison to other concentrations used (Table 1). In agreement with present study, it was reported that 20 ng/ml leptin enhances buffalo oocytes maturation rate, cleavage and *in vitro* embryo production (Singh *et al.,* 2012). This could be due to leptin enhanced autocrine and paracrine factors that are helpful in oocyte maturation. It is widely

Table 2: Impact of different leptin concentrations on *in vitro* maturation of pre-pubertal and pubertal Deccani ewe

Status	**Leptin**, ng/ml				
	0	**10**	**20**	**50**	**100**
Prepubertal					
Replicates / No. of oocyte	7/98	7/105	7/105	7/105	7/105
% COC expansion	48.2±1.1^{a}	58.5±1.3^{b}	63.8±1.5^{c}	46.5±1.5^{a}	54.1±1.3^{b}
% Metaphase-II	46.3±2.1^{a}	57.1±2.6^{b}	61.1±2.5^{c}	49.9±2.1^{a}	48.0±2.4^{a}
% Unclassified	5.83± 2.1^{a}	5.11±2.1^{a}	3.11±1.4^{b}	8.28±1.1^{c}	11.2±2.2^{c}
Pubertal					
Replicates / No. of oocyte	7/98	7/105	7/105	7/105	7/105
% COC expansion	52.1±1.2^{a}	66.1±0.2^{b}	68.2±1.3^{c}	62.2±1.7^{b}	65.2±1.7^{b}
% Metaphase-II	47.8±2.3^{a}	59.1±2.5^{b}	66.2±2.2^{c}	51.7±2.4^{b}	54.2±2.3^{b}
% Unclassified	6.33± 2.1^{a}	5.71±2.1^{a}	2.18±1.4^{b}	7.18±1.1^{c}	18.1±2.1^{c}

$^{a\ vs\ b\ vs\ c}p< 0.05$, within a row for a parameter; COC, Cumulus oocyte complex

accepted that leptin activates the mitogen activated protein kinase pathway leading to induction of cellular maturation.

In brief, the ovaries of prepubertal Deccani ewe could be a good source for *in vitro* maturation. The addition of 20 ng/ml leptin in IVM media can upsurge the developmental potential of prepubertal oocytes.

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