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REVIEW ARTICLE

Leptin supplementation enhances *in vitro* embryo development

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ABSTRACT

Leptin is a 16kDa peptide hormone secreted mainly by the adipose tissue which plays an important role in the regulation of food intake, energy expenditure and reproductive success. The aim of the study is to find out the efficiency of leptin supplementation in oocyte maturation and embryo culture medium that enhances nuclear maturation rate of oocytes and blastocyst development. Leptin stimulate the growth of preantral follicles irrespective of the concentrations used which may be due to the presence of leptin receptors in the follicular cells (theca, granulosa and cumulus cells) and oocytes. Leptin stimulate the oocyte developmental competence in a dose dependent manner, enhances nuclear maturation via activation of the MAPK pathway, improves the ability of the oocyte to sustain embryonic development and had long-term effects on blastocyst apoptosis. In conclusion leptin has a beneficial impact on future stock breeding as it increases the production of healthy embryos.

Keywords: *Leptin, in vitro maturation, oocyte, blastocyst*

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INTRODUCTION

Leptin is a 16kDa peptide hormone secreted mainly by the adipose tissue which plays an important role in the regulation of food intake, energy expenditure and reproductive success [1, 2]. The type of medium used is also an important factor that can affect the IVM of mammalian oocytes. The culture media employed in IVM not only affect the proportion of mammalian oocytes undergoing fertilization but also influence the subsequent cleavage and developmental competency [3, 4]. Despite several efforts moderate success has been achieved for development of blastocysts, *in vitro* matured and fertilized oocytes. The availability of a sufficient number of oocytes is pre-requisite for IVP procedure, and ovaries from slaughtered animals are the cheapest and the most abundant source of them. For successful IVP of embryos *In vitro* maturation (IVM) is an essential step. Embryo development is also influenced by the events occurring during oocytes maturation *in vitro*. Various factors such as follicle size, follicular fluid or cells, hormones, serum, growth factors or vitamins in the IVM medium, age of the donor and the culture conditions are involved for successful IVM of oocytes [5]. In the past years leptin was used as an additive alone as well as in combination with other additives on *in vitro* maturation of sheep, camel, buffalo etc. The present study aims to evaluate the efficiency of leptin in *in vitro* maturation and blastocyst development in different species of animals.

Leptin in IVM of goat preantral follicles

On *in vitro* maturation of preantral follicles (PFs) of size 250–400 µm in goat for 10 days in 10 ng/ml, 50 ng/ml and 100 ng/ml concentrations of leptin and in combination with thyroxin (T4), FSH and epidermal growth factor (EGF), it was found that leptin with 10ng/ml showed highest proportion of PFs exhibiting growth (94.46 ± 2.07), increase in diameter (37.50 ± 3.65), antrum formation (81.41 ± 2.09) and oocytes exhibiting M-II (35.39 ±2.57). The proportion of PFs exhibiting growth, increase in diameter *in vitro* was decreasing as the concentration of leptin increased in culture medium but the proportion of PFs exhibiting antrum formation was significantly higher at 10ng/ml group than 50 and 100ng/ml leptin groups. The present results indicated that leptin stimulated the growth of PFs irrespective of the concentrations used which may be due to the presence of leptin receptors in the follicular cells (theca, granulosa and cumulus cells) and oocytes [6].

Leptin in IVM of sheep oocytes

In vitro culture of preantral follicles (PFs) isolated from sheep ovaries with human or ovine leptin revealed that among 12 different concentrations (0-1000ng/ml) of human leptin tested, the proportion of PFs exhibiting growth, mean increase in diameter, antrum formation, and maturation of the oocytes to MII stage were the best in 10 ng/mL. On culture of sheep ovarian PFs in TCM 199 supplemented with 10 ng/mL of human or ovine leptin, FSH (2.5 µg/mL), thyroxine (1 µg/mL), insulin like growth factor I (10 ng/mL), and GH (1 mIU/mL) resulted in significantly ($P \leq 0.05$) greater average increase in diameter (11 and 9 vs. 6 µm), better proportions of PFs exhibiting growth (66% and 58% vs. 48%), antrum formation (51% and 51% vs. 34%), and maturation of oocytes to MII stage (24% and 22% vs. 7%) than the control medium. It was concluded that the optimum dose of leptin for the growth of sheep PFs *in vitro* was 10 ng/mL, human or ovine leptin supported similar development *in vitro* of PFs in sheep, inclusion of leptin along with FSH, thyroxine, insulin like growth factor I, and GH resulted in only a marginal improvements in *in vitro* development of sheep PFs' [7].

The impact of leptin addition of 0, 10, 20, 50 and 100 ng/ml were studied to find out the efficiency of oocyte retrieval methods in relation to oocyte quality and quantity, effect of season on sheep oocytes quality and numbers, effect of presence of corpus Luteum (CL) on oocyte yield, and to study the effect of leptin addition on maturation media to compare the IVM (In vitro maturation) status like cumulus-oocyte complexes expansion and M-II stage of sheep oocytes in combination with traditional *in vitro* maturation medium. On retrieval of the oocyte by three different methods, it was found that highest number of oocytes were retrieved in aspiration method (65.8%) followed by slicing method (54.25%) and post aspiration slicing (36.1%). Ovaries collected in different seasons showed spring season (March-April) with highest number of oocytes recovery followed by winter (January-February) and summer (May-June). On presence of CL in ovaries showed reduced recovery of oocytes. The cumulus-oocyte-complexes (COC) expansion and polar body extrusion in IVM medium with 20ng/ml was found to be highest ($P<0.05$) with 81.90±1.87 and 56.87±2.92 per cent respectively. It can be concluded that leptin addition significantly improved the *in vitro* development of sheep oocytes [8].

Leptin in IVM of cattle oocytes

Maturation of cumulus-oocyte complexes (COCs) in serum-free medium containing 0 (control), 1, 10, or 100 ng/ml leptin or in medium supplemented with 10% (v/v) estrous cow serum (ECS) showed no effect on cleavage rate after fertilization. However, an increased proportion of oocytes that matured in presence of 1 or 10 ng/ml leptin developed to blastocysts, which exhibited increased cell numbers. The proportion of apoptotic cells was reduced in blastocysts originating from leptin- or ECS-treated oocytes. Transcript levels of the genes encoding leptin receptor (LEPR), signal transducer and activator of transcription (STAT3), BCL2 associated X-protein (BAX), and baculoviral inhibitor of apoptosis protein repeat-containing 4 (BIRC4, also known as XIAP), were determined by reverse transcriptase- quantitative polymerase chain reaction analysis of expanded and hatched blastocysts. Depending on the dose used, leptin treatment of oocytes resulted in increased LEPR, STAT3, and BIRC4 mRNA levels and reduced BAX mRNA levels in blastocysts. Leptin improved oocyte developmental competence in a dose-dependent manner and the oocyte developmental potential is a reflection of proper cytoplasmic maturation. In conclusion, leptin improved the ability of the oocyte to sustain embryonic development and had long-term effects on blastocyst apoptosis and transcript abundance of LEPR, STAT3, and apoptosis-associated genes [9].

Leptin in IVM of buffalo oocytes

Good quality buffalo oocytes aspirated from 2-8 mm visible follicles were subjected to maturation medium:TCM-199 supplemented with 10% FBS, 22µg/ml sodium pyruvate, 0.5 IU/ml ovine follicle-stimulating hormone (oFSH), 0.5 IU/ml ovine luteinizing hormone (oLH), 1 µg/ml oestradiol, 50 µg/ml gentamycin, and different concentrations of leptin [0,10, 50, and 100 ng/ml]. The percentage of

maturation in groups of leptin treated oocytes with 0, 10, 50 and 100 ng/ml were 74.65, 83.81, 77.85, and 75.40 per cent, while the percentage of oocytes apoptosis was 9.83, 9.54, 9.93, and 10.42 per cent, respectively. Results showed that addition of 10 ng/ml leptin to buffalo IVM medium increased oocytes maturation, significantly, whereas higher concentration of leptin did not show any extra beneficial effect [10].

The physiological function of leptin *in-vitro* maturation of oocytes and early embryonic development was studied to increase the efficiency of *in-vitro* embryo production in buffaloes. Oocytes were matured in TCM-199 medium supplemented with 10% FBS, 0.5µg/ml FSH, 10IU/ml LH, 1 µg/ml estradiol-17β, 20ng/ml EGF and 50 µM cysteamine in four groups i.e. without leptin, 10ng/ml leptin, 20ng/ml leptin and 30ng/ml leptin in CO2 incubator at 37ºC temperature, 5% CO2 and high humidity. The matured oocytes were fertilized *in-vitro* in modified synthetic oviductal fluid (mSOF) containing 10ng/ml heparin for 7 days to study the embryonic development. Results showed that oocytes supplemented with 30ng/ml leptin showed significantly higher (P<0.05) maturation percentage (92.16%) whereas, the percentage of cleaved zygote that developed into morula and blastocyst was found to be 58.82% and 44.12% respectively in medium supplemented with 20ng/ml leptin which was significantly higher (P<0.05) than that of 30ng/ml leptin. From the present study, it may be concluded that supplementation of Leptin at the rate of 20 ng/ml in *in vitro* maturation medium significantly increased the cleavage rate and embryonic development up to blastocyst [11].

Leptin in IVM of pig oocytes

Effects of leptin supplementation was studied on oocytes *in vitro* maturation (IVM), *in vitro* development of parthenogenetically activated (PA) and SCNT embryos and *in vivo* development of SCNT embryos after embryo transfer (ET). The results showed that supplementation of 0, 100 or 200 ng/ml leptin into the maturation medium resulted with 63.7± 3.8%, 71.3 ± 3.5%, 66.3 ± 2.3% did not greatly affect nuclear maturation of oocytes, or cleavage rates of PA and SCNT (P > 0.05). Blastocyst rates of PA and SCNT embryos were significantly improved when 100 or 200 ng/ml leptin was added to maturation medium, and the number of cells in PA blastocysts was also improved (P < 0.05). The number of cells in blastocyst of SCNT was improved, when 100 ng/ml leptin was added (P< 0.05). Furthermore, supplementation of 100 or 200 ng/ml leptin to the IVM medium may improve pregnancy rate and the delivery rate in pig cloning [12]. Expression of Ob-R was investigated in oocyte growth and maturation in porcine oocytes from small, medium, and large follicles and in oocytes in the germinal vesicle (GV), GV breakdown, and metaphase II (MII) stages at both the mRNA and protein levels. The leptin receptor (Ob-R) has six isoforms and can signal through either the MAPK or the Janus-activated kinase/signal transducer and activator of transcription signal-transduction pathway, depending on the isoform. The proportion of oocytes expressing Ob-R was maximal in oocytes from medium follicles and at the GV breakdown stage ($P \leq 0.05$), whereas the proportion of oocytes expressing the long isoform, Ob-Rb, was found to be consistently low throughout growth and maturation. When included in oocyte maturation medium, leptin significantly increased the proportion of oocytes reaching MII ($P<0.01$), elevated cyclin B1 protein content in MII-stage oocytes ($P < 0.05$), and enhanced embryo developmental potential ($P < 0.05$), suggesting that leptin plays a role in both nuclear and cytoplasmic maturation. During oocyte maturation, leptin increased phosphorylated MAPK content by 2.8-fold ($P < 0.05$), and leptin-stimulated oocyte maturation was blocked when leptin-induced MAPK phosphorylation was suppressed by a specific MAPK activation inhibitor, U0126 ($P < 0.01$), demonstrating that leptin enhances nuclear maturation via activation of the MAPK pathway. Leptin is expressed by the porcine oocyte and facilitates oocyte maturation *via* stimulation of the oocyte to progress to MII and up-regulation of cyclin B1 protein levels, as well as enhancing the developmental competency of parthenogenetic embryos [13].

Leptin in IVM of camel oocytes

Effect of leptin addition (0, 10, 20 and 30ng/ml) to maturation media was evaluated on *in vitro* maturation (IVM) and fertilization (IVF) of dromedary camel oocytes. After maturation of the oocytes in a CO2 incubator (5% CO2) at 38.5ºC and high humidity for 42h, the oocytes were categorized into Germinal Vesicle (GV), Germinal Vesicle Break Down (GVDB), metaphase-I (M-I), metaphase-II (M-II) and degenerated oocytes. In vitro fertilization was carried out for oocytes matured in TCM-199 with 10% FDCS plus 20ng/ml Leptin. Epididymal spermatozoa recovered from slaughtered camel testis and oocytes were co-incubated at 38.5ºC with 5% CO2 in air for 20-24h. On examination of the cleavage stages (2, 4 and 8-16 cell, morula and blastocyst stages) after five days of culture revealed that supplementation of leptin (20ng/ml) resulted highest (P<0.05) percentages of oocytes at M-II (58.8%) and lowest percentages of those at GV (7.8%), GVDB (9.8%), M-I (10.8%) and degenerated oocytes (12.7%) as compared to control medium and other levels of leptin. Supplementation of leptin matured with 20ng/ml Leptin increased fertilization rate (27 vs. 25.3%) and blastocyst rate (3.6 vs. 1.4%) as compared to

control medium. In conclusion supplementation of leptin to maturation medium (TCM-199) with 20ng/ml increases *in vitro* nuclear maturation rate of oocytes, fertilization and blastocyst production rate [14].

Leptin in IVM of horse oocytes

On maturation of compact and expanded-cumulus horse oocytes in medium containing different concentrations (1, 10, 100, 1000 ng/ml) of recombinant human leptin resulted in improved maturation 74% *vs* 44%, for 100 ng/ml leptin-treated and control groups, respectively; $P < 0.05$ and fertilization after ICSI 56% *vs* 23% for 10 ng/ml leptin-treated and control groups, respectively; $P < 0.05$. However, the developmental rate and quality of 8-cell stage embryos derived from leptin-treated oocytes (100 ng/ml) was significantly reduced. Ob and Ob-R proteins were detected up to the 8-cell stage with cortical and cytoplasmic granule-like distribution pattern in each blastomere [15].

Leptin in IVM of mouse oocytes

It was observed that on addition of 10ng/ml human recombinant leptin to the culture medium significantly increased the percentage of two-cell mouse embryos that developed into blastocysts and hatched blastocysts whereas the development rate was significantly inhibited with 100ng/ml leptin. The differential sensitivity to leptin was found to vary among embryos at different stages of development. The total cell numbers in the hatched blastocysts were significantly higher in the presence of 10 ng/ml leptin compared with controls and higher concentrations. Also Supplementation of leptin (10 ng/ml) to culture medium at two- to eight-cell stages resulted in a consistent stimulatory effect on embryo development which was found to be diminished on supplementation of 100ng/ml. On investigation of the expression pattern of leptin and its receptors, it was found that leptin mRNA was not detected in mouse two-, four-, eight-cell and blastocyst stage embryos, whereas three isoforms of leptin receptor (Ob-Ra, Ob-Rb and Ob-Re) were identified in these cells, indicating that leptin is likely to modulate embryo development via a paracrine signalling pathway [16]. Effects of different leptin concentrations on in vivo and in vitro development were investigated by embryo culture studies and quality of the embryo were assessed by embryo transfer to the recipient mice. It was seen that on addition of leptin into the embryo culture medium at 10 and 100 ng/mL doses resulted in 97.37±1.45% and 95.14±1.81% respectively. Moreover, leptin increases the total number of trophectoderm cells as well as the number of embryos implanted in 10 and 100ng/ml. Thus, in the present study, embryo culture media supplemented with 10 and 100 ng/mL leptin were found to support in vitro mouse embryo development. Trophectoderm cell lineage plays an important role in development for placenta and hence increases the rate of implantation [17].

Leptin in IVM of rabbit oocytes

It was seen that COC supplemented with 1, 10 and 100 ng/ml leptin had a significantly higher metaphase II (MII) percentage than those IVM without leptin ($P<0.05$) and a similar MII index compared to the group supplemented with 10% FCS. Leptin did not increase the percentage of cytoplasmically matured oocytes in terms of cortical granule migration rate, whereas a significantly higher index and higher oestradiol concentration was found in the FCS group ($P<0.001$). Leptin-stimulated nuclear oocyte maturation was significantly impaired when leptin-induced JAK2/STAT3 and MEK 1/2 activation was suppressed by the inhibitors ($P<0.001$). Steroid secretion of COC was not affected by leptin activation of JAK2/STAT3 or MEK 1/2 pathways. In conclusion, JAK2/STAT3 and MEK 1/2 pathways mediate the enhancement of nuclear oocyte maturation by leptin; however, neither cytoplasmic oocyte maturation nor steroidogenic response of COC were improved in the present rabbit model [18].

CONCLUSION

From the above observation and discussion it is clear that embryo production is of utmost importance for stock breeding (i.e. bovine, sheep, goat etc.). Increasing the production of healthy embryos through the use of leptin would contribute to the reclamation and development of national stockbreeding. Also leptin-supplemented culture media can be used in several reproductive biotechnology areas, such as somatic cell nuclear transfer, in *vitro* fertilization, or intracytoplasmic sperm injection. Therefore, we believe that further studies is required which would employ *in vitro* and *in vivo* developmental methods together to identify the effects of leptin on different animal species would contribute to the literature.

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