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Effect of leptin supplementation during *in vitro* oocyte maturation and embryo culture on bovine embryo development and gene expression patterns

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Abstract

Leptin is a metabolic hormone related to body condition and nutritional status that influences fertility in assisted reproductive technologies modulating oocyte and embryo quality. The aim of the present study was to establish the effect of various leptin concentrations (0, 10, 100 ng/mL) during *in vitro* oocyte maturation (IVM) and *in vitro* embryo culture (IVC) on bovine embryo development and quality in terms of gene expression. The relative mRNA abundance of the genes encoding solute carrier family 2 (facilitated glucose transporter) member 1 *(SLC2A1)*, Bcl-2-associated X protein (*BAX*), placenta-specific 8 (*PLAC8*), aldo-keto reductase family 1 member B1 (*AKR1B1*) and leptin receptor (*LEPR*) were determined on Day 7 blastocysts by qRT-PCR. Cleavage rate ($P < 0.005$) and blastocyst yield ($P = 0.05$) was significantly lower when cumulus-oocyte complexes (COCs) were matured with 100 ng/mL leptin compared to 0 or 10 ng/mL leptin. No significant effect of different concentrations of leptin added during IVC on blastocyst yield was observed. The presence of 100 ng/mL leptin in both IVM and IVC further decreased cleavage rate ($P < 0.005$) and blastocyst yield compared to the control group without leptin ($P = 0.05$) and those supplemented with 10 ng/mL leptin or FCS ($P < 0.005$). There was no evidence of any leptin-induced difference in the relative transcript abundance of *SLC2A1, BAX* and *PLAC8* genes in Day 7 blastocysts. Expression of *AKR1B1* was significantly lower in blastocysts from COCs matured with 100 ng/mL leptin compared to those matured with 0 or 10 ng/mL leptin ($P < 0.005$). *LEPR* expression was up regulated when leptin concentration was increased from 0 ng/mL during IVM to 10 ng/mL during IVC, but it was down-regulated in the opposite situation ($P < 0.005$). In conclusion, high leptin concentrations possibly related to obesity seem to be more detrimental rather than the absence of this hormone for preimplantation embryo survival; this effect is independent of *LEPR* gene expression and it does not influence expression of *SLC2A1, BAX* and *PLAC8* genes in Day 7 blastocysts.
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Keywords: Leptin; Oocyte maturation; Embryo quality; Embryo development; Bovine

1. Introduction

Nutritional status and an extreme body mass index (BMI) (lean or obese) can impair reproductive function through different endocrine and metabolic alterations.

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Among known metabolic markers, leptin, a 16-kDa-peptide hormone that is encoded by the *obese* gene and secreted mainly by the adipose tissue appears to play an important role. In addition to its action on food intake and energy expenditure, it plays an important function in female reproductive biology [1,2]; mice lacking leptin *(ob/ob)* are obese and infertile [3], but administration of exogenous leptin restores fertility in these animals [4]. Leptin is present in follicular fluid of different species [5,6], and in oviductal and uterine fluids during early pregnancy [7–9], and thus it is available to the oocyte and embryo. Furthermore, leptin receptor *(LEPR)* mRNA has been detected in granulosa and cumulus cells [5,10,11], oocytes [12,13] and blastocysts [14], suggesting that both oocytes and preimplantation embryos could be sensitive to leptin.

In women, serum and follicular fluid leptin concentrations are highly correlated to each other and with BMI [15–17], and a minimum level of leptin is required for maintenance of reproductive function [18]. In periparturient [19,20] and cyclic cows [21], physiological concentrations of serum leptin are around 10 ng/mL. These concentrations appear to be beneficial at the end of follicular growth and early embryogenesis also in other species [22,23]. During the postpartum period in high yielding dairy cows, lactation is the metabolic priority at the expense of the cow's own body reserves, resulting in a negative energy balance (NEB) and a decrease in body condition, which results in a decrease in leptin concentration [24]. This decrease may have direct or indirect consequences on oocyte or embryo quality resulting in early embryonic mortality, a major cause of reproductive failure in cattle [25]. In contrast, high leptin concentrations may be also detrimental, as they have been associated with obesity and polycystic ovarian syndrome (PCOS) in women, providing a link between these metabolic disorders and low fertility [26]. These findings indicate that the association of BMI and reproductive success is of an 'inverted U shape', implying that both under- and overweight have a deleterious effect on assisted reproduction outcome, reducing the probability of achieving pregnancy [27].

In vitro studies adding leptin during oocyte maturation and embryo culture allow the testing of a possible direct effect of this metabolic marker on early embryo survival through its receptor. Previous studies have indicated that leptin plays a role on early embryo development, regulating the expression of genes related to metabolism, apoptosis and implantation [14]. In cattle, most studies to date have focused on the effect of leptin on oocyte competence [11,12,14], but as previously mentioned, embryos are also exposed to leptin in the oviductal fluid. In addition, it is known that while the quality of oocyte is crucial in determining blastocyst yield, blastocyst quality is to a large extent determined by the environmental conditions occurring during post-fertilization development [28].

With this background, the aim of the present study was to assess the direct effect of leptin on bovine oocytes and embryos by analyzing the impact of different concentrations of leptin during oocyte maturation and *in vitro* culture on embryo development and mRNA relative abundance of leptin receptor and genes related to metabolism, apoptosis and implantation at the blastocyst stage.

2. Material and methods

Unless otherwise stated, all chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA).

2.1. Experimental design

To determine the effect of leptin supplementation during oocyte maturation and embryo culture on embryo development and quality, we performed a dose-response study over seven replicates [n = 1343 cumulus–oocyte complexes (COCs)]. Human recombinant leptin, (cat # L4146), which exhibits high homology to bovine leptin (84%), was used. Embryo development (cleavage rate and blastocyst yield), embryo quality (gene expression) and mRNA expression of leptin receptor in blastocysts were assessed.

2.1.1. Experiment 1—Effect of leptin supplementation during oocyte maturation (IVM)

COCs were pooled and randomly allocated to one of the following treatments: IVM with 0, 10 or 100 ng/mL leptin, without leptin supplementation during embryo culture (0/0 lep, 10/0 lep and 100/0 lep groups, respectively).

2.1.2. Experiment 2—Effect of leptin supplementation during in vitro culture (IVC)

Presumptive zygotes obtained after IVM and *in vitro* fertilization (IVF) in the absence of leptin (see below), were pooled and randomly allocated to one of the following treatment groups: IVC with 0, 10 or 100 ng/mL leptin (0/0 lep, 0/10 lep and 0/100 lep groups, respectively).

2.1.3. Experiment 3—Effect of leptin supplementation during both in vitro maturation and embryo culture

Four additional groups were performed. For three groups, both maturation and culture medium were supplemented with the same concentration of leptin (i.e. 0, 10 or 100 ng/mL leptin, named as 0/0 lep, 10/10 lep or 100/100 lep groups, respectively). A fourth control group was supplemented with 10% foetal calf serum (FCS) during both oocyte maturation and preimplantation embryo development (FCS/FCS group).

2.2. In vitro embryo production

The techniques for producing embryos *in vitro* have been previously described in detail [28]. COCs were obtained by aspirating 2 to 8 mm follicles from the ovaries of cows collected at slaughter. COCs with compact cumulus cells were washed four times with PBS supplemented with 36 g/mL pyruvate, 50 g/mL gentamycin and 0.5 mg/mL BSA (Bovine Serum Albumin) and once in maturation media without leptin. Groups of 50 COCs were placed in 500 μL maturation medium in four-well dishes (Nunc, Roskilde, Denmark) and cultured for 24h at 39 °C under an atmosphere of 5% CO_2 in air with maximum humidity. Maturation medium consisted of TCM-199 supplemented with 3 mg/mL BSA, 10 ng/mL epidermal growth factor, and 0, 10 or 100 ng/mL human recombinant leptin. For the FCS/FCS group, BSA was substituted by 10% FCS. IVF was performed with frozen-thawed percoll-separated sperm (GE Healthcare Bio-Sciences, Uppsala, Sweden) from one bull at a concentration of 1×10^6 spermatozoa/mL. Gametes were co-incubated for 22h at 39 °C under an atmosphere of 5% CO_2 in air with maximum humidity in four-well dishes with 50 COCs and 500 μL of Tyrode′s albumin lactate pyruvate medium per well. Semen from the same tested bull was used for all experiments. Approximately 20h post insemination (pi), presumptive zygotes were denuded by gentle vortexing and washed four times in PBS and once in Synthetic Oviductal Fluid (SOF) [29] medium before being randomly assigned to treatment. Groups of 25 to 30 zygotes were placed in 25 μL droplets overlaid with mineral oil in SOF medium with 3 mg/mL BSA and supplemented with 0, 10 or 100 ng/mL human recombinant leptin until Day 9 pi. For the FCS/FCS group, BSA was substituted by 10% FCS. The dishes were incubated in an atmosphere of 5% CO_2, 5% O_2 and 90% N_2 at 39°C. Cleavage was assessed at 48 hpi and blastocyst development was recorded on Days 6, 7, 8 and 9 pi (Day 0 = day of IVF) and was expressed as a fraction of the total number of presumptive zygotes cultured. Day 7 blastocysts were snap frozen in liquid nitrogen and stored at -80 °C for transcript abundance analysis. The remaining embryos were maintained *in vitro* until Day 9 to record embryo development.

2.3. RNA extraction, reverse transcription, and quantification of mRNA transcript abundance

Poly (A) RNA was prepared as previously described [30] from three groups of 5 blastocysts of each experimental group using the Dynabeads mRNA Direct Extraction KIT (Dynal Biotech, Oslo, Norway) following the manufacturer's instructions with minor modifications. Immediately after mRNA extraction, the RT reaction was carried out following the manufacturer's instructions (Bioline, Ecogen, Madrid, Spain) using poly (T) primer, random primers and Moloney murine leukaemia virus reverse transcriptase enzyme in a total volume of 40 μL to prime the RT reaction and to produce cDNA. Tubes were heated to 70 °C for 5 min to denature the secondary RNA structure and then the RT mix was completed with the addition of 100 units of Superscript RT enzyme. They were then incubated at 42 °C for 60 min to allow the reverse transcription of RNA, followed by 70 °C for 10 min to denature the RT enzyme.

The quantification of all mRNA transcripts was carried out by real-time qRT-PCR. For qRT-PCR, 3 groups of cDNA per experimental group were used with two repetitions for all genes of interest. Experiments were conducted to contrast relative levels of each transcript and histone *H2a.z* that has been previously tested as a stable reference gene [30], in every sample. PCR was performed by adding a 2 μL (4 μL for *LEPR*) aliquot of each sample to the PCR mix containing the specific primers to amplify the housekeeping gene, *H2a.z*, long isoform of leptin receptor (*LEPR*) [12] and several transcripts which have been associated with aspects of embryo quality including glucose metabolism (solute carrier family 2 (facilitated glucose transporter) member 1, *SLC2A1*) [31], apoptosis (Bcl-2-associated X protein, *BAX*) [32] and pregnancy outcome (aldo-keto reductase family 1 member B1, *AKR1B1*) and placenta-specific 8 (*PLAC8*) [33]. Primer sequences and the approximate sizes of the amplified fragments of all transcripts are shown in Table 1. PCR conditions were optimized to achieve efficiencies close to 1 and then the comparative cycle threshold (CT) method was used to quantify expression levels as described in [34]. Quantification was normalized to the endogenous control, *H2a.z*. Fluorescence was acquired in each cycle to determine the threshold cycle or the cycle during the log-linear phase of the reaction at which fluorescence

Table 1
Details of primers used for qRT-PCR.

Gene	Primer sequence (5′–3′)	Fragment size, bp	Gene bank accession no.
H2A.Z	AGGACGACTAGCCATGGACGTGTG CCACCACCAGCAATTGTAGCCTTG	212	NM_016750
LEPR	ACACCAGCATGATGCAGATC TCTGTAGTTGCTGGCACCAT	315	AB199589
AKR1B1	CGTGATCCCCAAGTCAGTGA AATCCCTGTGGGAGGCACA	152	M314631
PLAC8	CGGTGTTCCAGAGGTTTTTCC AAGATGCCAGTCTGCCAGTCA	163	NM_016619
BAX	CGGTGTTCCAGAGGTTTTTCC AAGATGCCAGTCTGCCAGTCA	163	NM_016619

increased above background for each sample. Within this region of the amplification curve, a difference of one cycle is equivalent to doubling of the amplified PCR product. According to the comparative CT method, the ΔCT value was determined by subtracting the *H2a.z* CT value for each sample from each gene CT value of the sample. Calculation of ΔΔCT involved using the highest sample ΔCT value (i.e., the sample with the lowest target expression) as an arbitrary constant to subtract from all other ΔCT sample values. Fold changes in the relative gene expression of the target were determined using the formula $2^{-\Delta\Delta CT}$.

2.4. Statistical Analysis

Data were analyzed using the SPSS program for Windows (SPSS 15.0, Inc, Chicago, IL, USA) and SigmaStat software package (Jandel Scientific, San Rafael, CA, USA). Differences in embryo development (cleavage rate and blastocyst yield) and mRNA expression between experimental groups were analyzed using one-way repeated-measures ANOVA with arcsine transformation. All the results are expressed as the mean ± SEM. and statistical significance was accepted for $P \leq 0.05$.

3. Results

3.1. Effect of leptin supplementation on embryo development in vitro

Oocyte maturation in the presence of 100 ng/mL leptin resulted in a reduction in cleavage rate ($P < 0.005$) and blastocyst yield ($P = 0.05$) compared to the control group and oocytes IVM with 10 ng/mL leptin (Table 2).

Similarly, embryo culture in the presence of 100 ng/mL leptin reduced cleavage rate, although not significantly compared to the control. No significant effect of leptin during IVC on blastocyst yield was observed.

When both oocyte maturation and embryo culture took place in the presence of 100 ng/mL leptin cleavage rate was reduced ($P < 0.005$) and blastocyst yield was depressed compared to the control group ($P = 0.05$), the 10/10 leptin ($P < 0.005$) and the FCS/FCS groups ($P < 0.005$). The presence of 10 ng/mL leptin in both IVM and IVC increased Day 6 blastocyst yield over that obtained with the other leptin-treatments ($P < 0.005$). No significant differences in the number of Day 7, Day 8, Day 9 blastocysts among the other leptin-supplemented groups were observed.

Table 2
Effect of leptin during IVM/ IVC period on development of bovine embryos *in vitro*.

Leptin (ng/ml) IVM/IVC	n	Cleavage rate n (%)	Blastocyst yield per day n (%)				Total blastocysts n (%)
			Day 6	Day 7	Day 8	Day 9	
0/0	281	215 (76.5)^{a,b}	10 (3.5)^{c,d}	34 (12.1)	32 (11.3)	4 (1.4)	80 (28.4)^{a,b,c,*}
10/0	123	100 (81.3)^{a}	8 (6.5)^{c}	15 (12.2)	10 (8.1)	1 (0.8)	34 (27.6)^{a,bc,*}
100/0	180	115 (63.8)^{c}	3 (1.6)^{c,d}	11 (6.1)	14 (7.7)	4 (2.2)	32 (17.7)^{c,*}
0/10	147	120 (81.6)^{a}	1 (0.6)^{d}	20 (13.6)	11 (7.4)	5 (3.4)	37 (25.1)^{a,b,c}
0/100	116	79 (68.1)^{b,c}	3 (2.5)^{c,d}	12 (10.3)	14 (12.0)	5 (4.3)	34 (29.3)^{a,b}
10/10	156	127 (81.4)^{a}	19 (12.1)^{b}	22 (14.1)	13 (8.3)	2 (1.2)	56 (35.9)^{a}
100/100	202	131 (64.8)^{c}	1 (0.5)^{d}	14 (6.9)	17 (8.4)	8 (3.9)	40 (19.8)^{b,c,*}
FCS/FCS	138	109 (78.9)^{a}	24 (17.3)^{a}	17 (12.3)	7 (5.0)	1 (0.7)	49 (35.5)^{a}

N, Total number of oocytes for IVM/IVF/IVC; ^{a,b,c,d} $P < 0.005$; * $P = 0.05$.

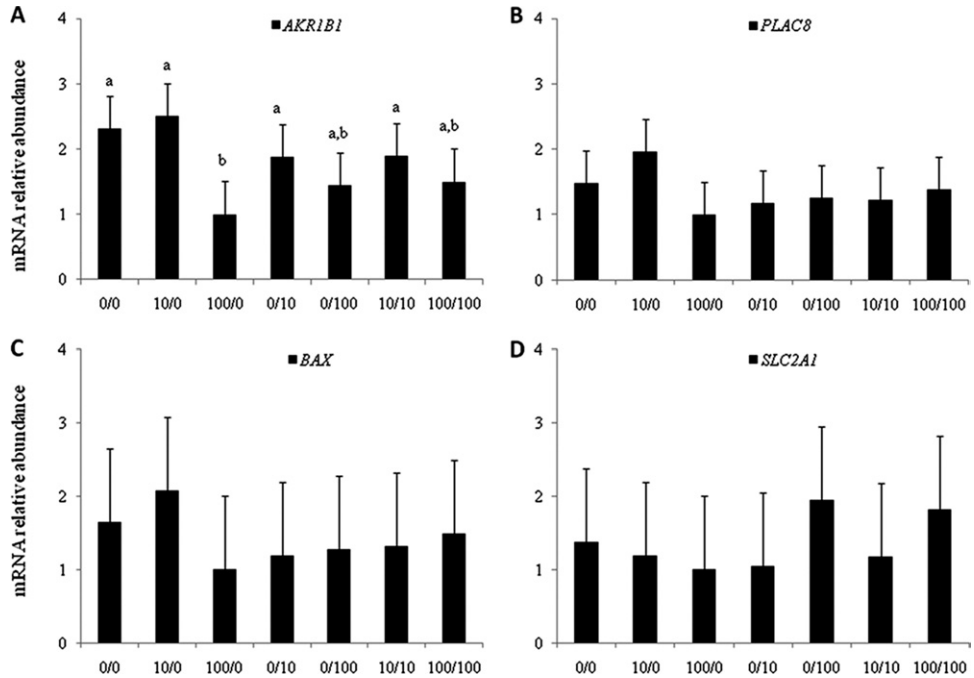

Fig. 1. Effect of leptin during IVM/IVC on the abundance of A) *AKR1B1,* B) *PLAC8,* C) *BAX* and D) *SLC2A1* mRNA in Day 7 blastocysts. Error bars represent SEM. Means in rows with different letters differ: $^{a,b}(P < 0.005)$.

3.2. Effect of leptin supplementation on transcript abundance of quality-related genes in blastocysts

The relative abundance of the transcripts studied is shown in Figure 1. The expression of *AKR1B1* was lower in blastocysts from COCs matured with 100 ng/mL leptin (group 100/0 lep) compared to 0/0 lep and 10/0 lep groups ($P < 0.005$). There was no evidence of any leptin-induced alteration during oocyte maturation or embryo culture in the relative transcript abundance of *SLC2A1, BAX* and *PLAC8* in Day 7 blastocysts.

3.3. Effect of leptin supplementation on LEPR mRNA relative abundance in blastocysts

The differences in *LEPR* expression in Day 7 blastocysts among all experimental groups are depicted in Figure 2. *LEPR* expression was lower in the group matured with 10 ng/mL leptin and cultured without leptin (group 10/0 lep) compared to the rest of the maturation treatments ($P < 0.05$, Figure 2A). Regarding the effect of leptin during IVC, the opposite situation was found in the group matured without leptin and

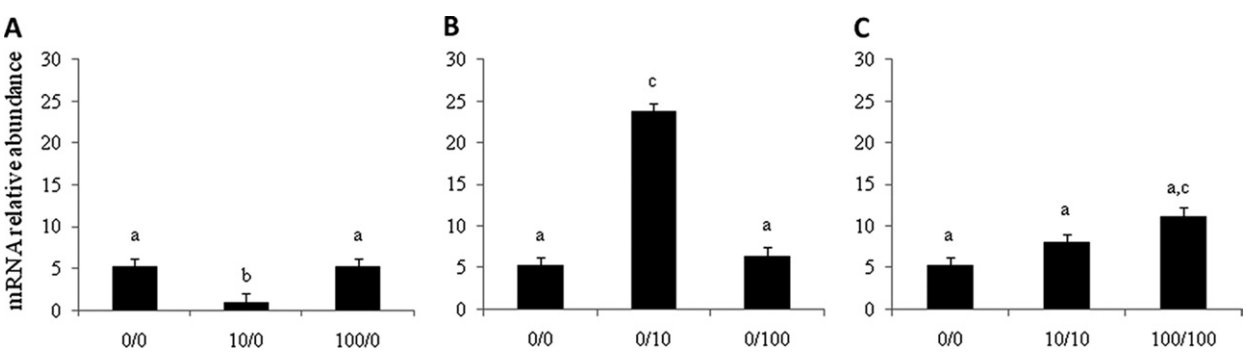

Fig. 2. Abundance of *LEPR* mRNA in Day 7 blastocysts. A) Effect of leptin during IVM on the expression of *LEPR* in blastocysts. B) Effect of leptin during IVC on the expression of *LEPR* in blastocysts. C) Effect of leptin during IVM/IVC period on the expression of *LEPR* in blastocysts. Error bars represent SEM. Means in rows with different letters differ: $^{a,b,c}(P < 0.05)$.

cultured with 10 ng/mL leptin, which displayed higher abundance than all other groups ($P < 0.05$, Figure 2B). No differences in expression of *LEPR* were found when different concentrations of leptin were added during both IVM and IVC period (Figure 2C) and among the rest of the groups studied (Figure 2A, B and C).

4. Discussion

This study demonstrates that high leptin concentrations, usually associated with obesity or related pathologies, are detrimental for preimplantation embryo development. However, in those embryos that reach the blastocyst stage, leptin did not alter the relative abundance of genes related to glucose intake, apoptosis and implantation.

During maturation, the oocyte acquires the developmental competence, which allows successful fertilization and the subsequent embryonic development. Previous work has shown that addition of 10 ng/mL leptin plus gonadotropins during IVM stimulates meiotic progression and enhances the developmental competence of the matured oocyte, which results in more and better quality bovine blastocysts [14]. Leptin appears to modulate Follicle Stimulating Hormone-mediated steroidogenesis by granulosa cells [35–37]. Thus, oocyte maturation may be improved [38–40]. In the current study, neither blastocyst yield nor gene expression of genes related to embryo quality was improved when 10 ng/mL leptin was added to gonadotropin-free IVM media compared to oocytes matured without leptin. Therefore, the absence of leptin does not seem to be a determinant factor that reduces oocyte quality in bovine. Previous results have suggested that low Body Condition Score may hamper fertility in cattle through other metabolic factors, such as the increase of non-esterified fatty acid concentration in follicular fluid affecting oocyte quality [41,42]. In contrast, our results indicate that higher leptin concentrations, frequently associated with obese-related pathologies [43–45], seems to be detrimental by itself on oocyte competence exerting an inhibitory effect on cleavage and blastocyst yield *in vitro*, as it was previously shown in other species [46–48]. Such an effect is cumulus-cell mediated as has been previously shown in cattle [12]. The establishment of an optimal concentration of leptin during IVM for embryo survival is controversial, even in the same species. Some studies have suggested that leptin only may exert its effect when it is added *in vitro* at very high concentrations (500–1000 ng/mL), improving cytoplasmic maturation in cattle [12]. However, these results contrast with the findings of other authors, who reported that much lower leptin concentrations (1–100 ng/mL) resulted in an increase in developmental competence [14,46,49]. Also, the relationship between intra-follicular leptin and embryo quality is controversial in women: whereas some reports established an association between both parameters [15,50] other have not found a statistical correlation [16,17,51]. Although underlying mechanisms of leptin action could be similar, these conflicting results may reflect the different thresholds existing among oocytes/embryos from different strains or species with respect to leptin sensitivity, the culture conditions or the type of exogenous leptin used.

It has been reported that during embryo culture leptin enhances blastocyst formation *in vitro* in different species [23,52,53] through an autocrine/paracrine pathway. However, leptin increases apoptosis at high concentrations [54]. In the bovine model, the effect of leptin supplementation during IVC had not been addressed before. Under our experimental conditions, leptin does not affect embryo development, in agreement with previous reports in other species [54–56]. It has been reported that exposure of preimplantation bovine embryos to hyperlipidemic conditions, as occur during the post-fertilization period, results in reduced embryo quality and developmental potential [57]. So, negative effects of metabolic disorders during post-fertilization period on embryo development and quality may be not mediated by leptin in bovine.

Previous studies have shown that the post-fertilization culture environment, rather than oocyte maturation, is the main period responsible for determining embryo quality, as assessed by cryotolerance and transcript abundance of candidate genes [28,58]. However, in the current study, IVC with leptin did not affect relative transcript abundance of the selected quality-related genes *PLAC8*, *AKR1B1*, *SLC2A1* and *BAX* in Day 7 blastocysts. Although *AKR1B1* expression has been negatively linked with embryo quality, while the lower expression of *AKR1B1* in the embryos matured with 100 ng/ml leptin in the current study seems do not reflect the developmental potential of the embryos [33]. Its downregulation may reflect transcriptome alterations and it is possible that other mechanisms driven directly or indirectly by leptin may have influenced *AKR1B1* transcription. In this sense, glucose metabolism is a suitable candidate for an indirect effect, as *AKR1B1* has been implicated in diabetic complications [59].

The long *LEPR* isoform mediates most of the leptin signaling [60,61]. Corroborating previous results in cat-

tle [14] and in other species [9,23,56] we detected mRNA expression of the long isoform of *LEPR* in the bovine blastocyst in all experimental groups. This indicates that, independent of leptin concentrations, all embryos synthesize *LEPR* mRNA and may be sensitive to this hormone. Changes in the relative mRNA abundance of *LEPR* in blastocysts only occured when leptin concentration increased or decreased between 0–10 ng/mL range and *vice versa* from IVM to IVC period. This situation suggests that the embryo may be reacting to precise dynamic changes of leptin concentrations and, as a consequence, to transitory variations in the metabolic status or in the BMI during the periconception period. The finding of leptin and leptin receptor mRNA expression in preimplantation embryos and in endometrial epithelia cell cultures and the regulation of its secretion by the human embryo suggests that leptin may be involved in the molecular mechanism of the implantation process [7]. Changes in *LEPR* gene expression have also been shown in corpus luteum, endometrium and placental trophoblast during the luteal phase and early pregnancy [62]. However, the changes in mRNA expression levels of *LEPR* are not reflected in blastocyst yield in this study. The changes observed are part of molecular mechanisms that could be modifying the sensitivity of the embryo to leptin concentrations with consequences not at the blastocyst stage, but later in the implantation process or fetal development [62,63].

The portion of embryos developing to blastocysts by Day 6 in the 10/10 leptin group was significantly higher than in the other groups, indicating a possible increase of their developmental potential [64]. Similar observations have also been reported in other species, such as in mice and sheep, where physiological concentrations of leptin tended to accelerate blastocyst formation [23]. This may be due to the effect of leptin in promoting cell proliferation throughout early embryo development [9,23,46]. In the current study, although physiological leptin concentrations appear to accelerate blastocyst development, it does not significantly improve cleavage rate and total blastocysts compared to those cultured without leptin. In contrast, higher leptin concentrations significantly reduced the cleavage and total blastocyst rate compared to those cultured without leptin or with 10 ng/mL. The presence of high leptin concentrations during oocyte maturation may reduce the developmental capacity of oocytes to support early embryonic development during the interval between fertilization and the maternal-embryonic transition. In this period, maternal RNAs support embryo function and polarization are driven by post-transcriptional processes applied to a preexisting mRNA pool in the oocyte [65,66]. However, embryo development continues after maternal-embryonic transition when its transcriptional activity is switched on. Such development tends to be significantly impaired if high leptin supplementation is maintained also during embryo culture. Therefore, both the high leptin concentration and the exposure time appear to be important for embryo survival [23]. Current work shows that leptin modulates oocyte competence and blastocyst development. However, the presence or absence of leptin did not influence the expression profile of some genes that are related to embryo quality, so leptin itself does not seem to be essential for *in vitro* bovine embryo survival and quality [54,67]. In agreement with our results, other authors [68,69] also postulated that the reproductive failure in *ob/ob* leptin deficient mice was not at the oocyte or embryo level, since they were able to undergo ovulation, fertilization and embryo development *in vivo*, as we have demonstrated *in vitro*. In contrast, Malik et al [70] showed that leptin was necessary for normal preimplantation and implantation processes *in vivo*. Leptin probably does not act alone for this; it interacts coordinately with other hormones (e.g; growth hormone, insulin or ghrelin), growth factors (e.g; VEGF, IGF-I) or drugs used in the ovarian stimulation protocols of fertility treatments (e.g; gonadotropins) modulating the ovarian responsiveness and the function of corpus luteum [71] and thus, the oocyte and embryo quality [26]. But the interactions of leptin with other hormonal regulators of reproduction, oocytes and embryos need to be clarified in future investigations.

5. Conclusion

In conclusion, physiological concentrations of leptin modulate bovine oocyte maturation and preimplantation embryo development but do not appear to be essential for such processes *in vitro*. It does not affect expression of the genes studied in blastocysts related to pregnancy outcome, glucose intake and apoptosis. However, our findings suggest that exposure to high concentrations of leptin can be detrimental to bovine embryo survival.

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