—Full Paper—

Effect of Recombinant Bovine Somatotropin (rbST) on Cytoplasmic Maturation of Bovine Oocytes and Their Developmental Competence *In Vitro*

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Abstract. The objectives of this study were to evaluate the effects of recombinant bovine somatotropin (rbST) on the nuclear and cytoplasmic maturation of bovine oocytes and their further developmental competence to blastocysts *in vitro*. We analyzed the mitochondrial activity and concentration of intracellular stored calcium ($[Ca^{2+}]_{is}$) in matured oocytes and the morphology and chromatin status of produced embryos after *in vitro* fertilization. Cumulus-oocyte complexes were incubated in TCM 199 containing 10% fetal calf serum (control medium 1: CM 1) or 10% estrus cow serum (control medium 2: CM 2). The culture medium of the treatment groups was modified by supplementation of the control medium with 10 ng/ml rbST (CM 1A and CM 2A), 10^6 /ml granulosa cells (CM 1B and CM 2B), or 10 ng/ml rbST plus 10^6 /ml granulosa cells (CM 1C and CM 2C). No differences were observed in the percentages of oocytes reaching metaphase II between the groups. However, the proportion of blastocysts was highest in treatment groups CM 1C and CM 2C ($P < 0.05$). The type of serum did not alter the positive effect of rbST on the developmental competence of embryos. The fluorescence intensity of metabolically active mitochondria measured by intensity per oocyte (Em 570) after MitoTracker CMTM Ros Orange labeling was significantly increased in oocytes matured in the presence of 10 ng/ml rbST and granulosa cells (309.21 vs. $119.97 \mu A$; $P < 0.01$). In parallel, the concentration of $[Ca^{2+}]_{is}$ in oocytes, determined using fluorophore chlortetracycline, was significantly decreased (0.85 ± 0.02 vs. 0.97 ± 0.03 AU; $P < 0.05$). Based on these results, we concluded that rbST, in interaction with granulosa cells stimulates the oxidative activity of ooplasmic mitochondria and decreases the content of $[Ca^{2+}]_{is}$ in oocytes. These facts support the hypothesis that somatotropin influences the developmental competence of bovine oocytes during maturation *in vitro*, and this effect can be modulated by granulosa cells.

Key words: Bovine, Calcium, Embryo, Mitochondrial activity, Oocyte maturation, Recombinant bovine somatotropin (rbST)

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A considerable amount of literature has been published on the possibility of using *in vitro* maturation (IVM) and *in vitro* fertilization (IVF)

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technologies for the aims of embryo transfer and cell and genetic engineering [1]. Basic research on oocyte maturation would provide a source of high quality oocytes for reproductive cell technologies and benefit the commercial embryo transfer industry. Embryo development is strongly

influenced by events occurring during oocyte maturation. The developmental potential of bovine oocytes is related to many factors, such as the morphology of cumulus-oocyte complexes, the diameter of follicles, and the culture system [2]. One important factor in the complex of fertility events is the growth hormone, somatotropin (ST) [3, 4], which influences the regulation of growth and differentiation of various cell types and control of the metabolic activity of organs and tissues. Addition of ST during IVM of bovine cumulus-oocyte complexes accelerates nuclear maturation, induces cumulus expansion, and promotes subsequent embryonic development [5]. Izadyar *et al.* [6] showed that supplementation with 100 and 1,000 ng/ml ST during maturation *in vitro* significantly enhanced subsequent cleavage rate and embryonic development. Growth hormones (GHs) like ST during IVM increase cumulus expansion by promotion of cell proliferation and inhibition of apoptosis [7]. It has also been found that bovine cumulus cells, mural granulosa, and oocytes express mRNA for growth hormone receptor (GHR), but the stimulatory effect of GH on bovine oocyte maturation is dependent on cumulus cells and is not mediated by insulin-like growth factor (IGF)-I [8]. GH supplementation during IVM enhances the migration of cortical granules and sperm aster formation [6], leading previous researchers to conclude that the promotory effect of GH on the developmental competence of the oocyte is due to a higher fertilization rate as a consequence of improved cytoplasmic maturation.

The cytoplasm of the oocyte may play a crucial role in assembling the correct metabolic machinery for production of sufficient energy for cellular function during maturation, cleavage, and blastocyst formation. Development of organelle- and ion-specific probes now permits studies of compartmentalization and spatial organization within the oocyte and embryo. Reorganization of mitochondria and changes in their activity during maturation are important parameters for oocyte cytoplasmic maturation [9, 10]. Mitochondria provide energy for the intensive metabolism of oocytes during final maturation [11]. Oocyte apoptosis is influenced by alterations in the biochemical and ultrastructural properties of mitochondria [12]. Van Blerkom *et al.* [13] proposed that high-polarized pericortical mitochondria may have a function in the

acquisition of oocyte competence and in the regulation of early developmental processes. This may be associated with elevated metabolism or intracellular signalling through calcium-induced calcium release pathways. Calcium is an important intracellular messenger, and calcium ions regulate many key intracellular events. Because mitochondria synthesize ATP and serve as an internal source of Ca^{2+} , it is possible that mitochondria redistribute and aggregate within the oocyte to concentrate ATP and Ca^{2+} at sites of high demand [14, 15].

To explain the stimulatory effect of ST on oocyte maturation, we focused our interest on the alterations of mitochondrial activity and Ca^{2+} concentration. A better understanding of the metabolic characteristics of oocytes matured in the presence of rbST and the consequence of alterations in these parameters on developmental competence is needed and could give an answer to the question regarding which mechanisms are involved in the stimulation effect of ST on maturation of oocytes. Therefore, the aims of the present study were to (i) determine the influence of recombinant bovine ST (rbST) on the developmental competence of bovine oocytes (experiment 1), (ii) determine the influence of rbST on the ongoing embryonic quality of oocytes on days 2 and 5 (experiment 2), and (iii) evaluate the effects of rbST on the cytoplasmic quality of oocytes after IVM (experiment 3).

Materials and Methods

Recovery of oocytes and preparation of granulosa cells

Ovaries from dairy cows were obtained at a slaughterhouse and transported within 2 h to the laboratory in sterile PBS maintained at 35 to 37 C. The cumulus-oocyte complexes (COCs) were recovered by slicing the surfaces of ovaries. The oocytes were collected in TCM 199 with 10% fetal calf serum (FCS). Only oocytes having complete, compact, multilayered cumulus and homogeneous ooplasm were used for culture. Before cultivation, the oocytes were washed twice in TCM 199 with 10% FCS.

Granulosa cells (GC) were collected from the medium from which COCs were dissected from follicles (3 to 5 mm in diameter) and centrifuged twice for 5 min at 500 g. The final pellet of granulosa cells was resuspended in maturation

medium. Co-culture of oocytes and granulosa cells was carried out in Petri dishes in 2 ml of medium at 38.5 C in an atmosphere of 5% CO₂ for 24 h.

Oocyte maturation

Selected COCs were matured for 24 h at 38.5 C in different treatment groups based on following culture systems.

Control medium 1 (CM 1) contained TCM 199 with Earle's salts, L-glutamine, 25 mM HEPES supplemented with 10% (v/v) heat-treated FCS, and trace amounts of antibiotics (50 IU/ml penicillin and 50 µg/ml streptomycin).

CM 1 + rbST (CM 1A) contained TCM 199 with Earle's salts, L-glutamine, 25 mM HEPES supplemented with 10% (v/v) heat-treated FCS, trace amounts of antibiotics (50 IU/ml penicillin and 50 µg/ml streptomycin), and 10 ng/ml rbST.

CM 1 + GC (CM 1B) contained TCM 199 with Earle's salts, L-glutamine, 25 mM HEPES supplemented with 10% (v/v) heat-treated FCS, trace amounts of antibiotics (50 IU/ml penicillin, 50 µg/ml streptomycin), and 1×10^6 /ml granulosa cells.

CM 1 + rbST + GC (CM 1C) contained TCM 199 with Earle's salts, L-glutamine, 25 mM HEPES supplemented with 10% (v/v) heat-treated FCS, trace amounts of antibiotics (50 IU/ml penicillin and 50 µg/ml streptomycin), 10 ng/ml rbST and 1×10^6 /ml granulosa cells.

Control medium 2 (CM 2) contained TCM 199 with Earle's salts, L-glutamine, 25 mM HEPES supplemented with 10% (v/v) heat-treated ECS (estrous cow serum), and trace amounts of antibiotics (50 IU/ml penicillin and 50 µg/ml streptomycin).

CM 2 + rbST (CM 2A) contained TCM 199 with Earle's salts, L-glutamine, 25 mM HEPES supplemented with 10% (v/v) heat-treated ECS, trace amounts of antibiotics (50 IU/ml penicillin and 50 µg/ml streptomycin) and 10 ng/ml rbST.

CM 2 + GC (CM 2B) contained TCM 199 with Earle's salts, L-glutamine, 25 mM HEPES supplemented with 10% (v/v) heat-treated ECS, trace amounts of antibiotics (50 IU/ml penicillin and 50 µg/ml streptomycin), and 1×10^6 /ml granulosa cells.

CM 2 + rbST + GC (CM 2C) contained TCM 199 with Earle's salts, L-glutamine, 25 mM HEPES supplemented with 10% (v/v) heat-treated ECS, trace amounts of antibiotics (50 IU/ml penicillin

and 50 µg/ml streptomycin), 1×10^6 /ml granulosa cells and 10 ng/ml rbST.

The concentration of rbST used during IVM (10 ng/ml) was found to produce the optimal blastocyst rate in previous studies in which we compared 5, 10, 50 and 100 ng/ml rbST (unpublished data). All maturation media were adjusted to pH 7.3.

In vitro fertilization and subsequent embryo culture in vitro

After IVM, oocytes were partially denuded by gentle pipetting to remove the loosely attached expanded cumulus cells and were transferred in groups of 5 oocytes to fertilization droplets. Fertilization was carried out in 46 µl microdroplets of modified Tyrode's medium. A volume of 2 to 5 µl of the sperm suspension was added to each fertilization droplet to give a final concentration of approximately 1.0 to 1.5×10^6 motile sperm/ml. Fertilization was carried out for 24 h at 38.5 C under 5% CO₂ in 100% humidified air.

Evaluation of oocytes and embryos

Nuclear maturation of the oocytes was evaluated using the cytogenetic method described by Tarkowski [16]. Oocytes matured over 24 h were placed in 0.9% sodium citrate in water for 10 min, stripped free of cumulus cells using a dissecting needle, fixed in methanol-acetic acid (3:1), stained with 5% Giemsa solution for 10 min at room temperature, and examined under a light microscope at $\times 900$ magnification for determination of nuclear status. The procedure to estimate the chromatin status of embryos on day 2 (2- to 4-cell stages) and day 5 (morulae) was the same as described for oocytes; however, before analysis, the embryos were also incubated in 0.1% hyaluronidase for 20 min. Embryos with bi- or multinuclear blastomeres, blastomeres including pycnotic chromatin, and blastomeres without chromatin were classified as not intact.

Intracellular stored calcium [Ca²⁺]_{is} assay

The levels of [Ca²⁺]_{is} in the oocytes were estimated on the basis of previously described measurements of the fluorescence intensity of calcium-chlortetracycline (CTC) complexes on irradiation [17, 18]. The fluorescence intensity was recorded under a luminescent microscope (LUMAM-I-1, LOMO, St. Petersburg, Russia) equipped with a

special photoelectronic multiplier (FEU-39A), an excitation filter (FS1; 6-mm thick), a green analysis filter, an interference light filter (passing maximum wavelength of 520 nm), and a mercury lamp. The excitation wavelength was 380 to 400 nm, and the emission wavelength was 520 nm (slit=10 nm). Measurement was performed within 10 sec. Chlortetracycline solutions were prepared in saline (150 mM NaCl, pH 7.0) immediately prior to the experiments. The oocytes were placed in special quartz wells with saline containing 40 μ M CTC and were incubated for 5 min in a water bath (37 C). The diameter of the probe used was constant (1 mm), and the diameter of the areas assayed was 100 μ m. Irradiation was focused at the center of the oocytes, and the intensity of cell fluorescence was measured as an electric signal. The values of the background emission were subtracted from the obtained magnitudes. The levels of $[Ca^{2+}]_{is}$ were expressed in arbitrary units (AU) of the intensity of oocyte fluorescence.

Fluorescence labelling of mitochondria and measurement of fluorescence intensity

The *in vitro* matured COCs were incubated for 30 min in PBS containing 3% bovine serum albumin (BSA) and 200 nM MitoTracker Orange CMTM Ros (Molecular Probes, Eugene, OR, USA) under culture conditions [10]. Cell-permeant MitoTracker Orange-fluorescent tetramethylrosamine (M-7510) probe is readily sequestered by actively respiring organelles only depending upon their oxidative activity. Then, the cumulus cells were removed as described above, and the oocytes were washed 3 times in prewarmed PBS without BSA. The oocytes were fixed for 15 min at 37 C using freshly prepared 2% paraformaldehyde in Hank's balanced salt solution. The M-7510 probe contains a thiol-reactive chloromethyl moiety and can react with accessible thiol groups on peptides and proteins to form an aldehyde-fixable fluorescent conjugate that is well-retained after cell fixation over a period of six weeks. After fixation, the oocytes were washed 3 times in PBS, mounted on slides under coverslips, and stored in the refrigerator prior to evaluation by fluorescence microscopy.

An epifluorescence microscope (Jenalumar; Carl Zeiss, Jena, Germany) was used for all experiments. Emission wavelengths were separated by a 540 nm dichroic mirror followed by further filtering through a 570 nm long pass filter (red emission).

The mitochondrial aggregation pattern of the bovine oocytes was characterized by observation (up to 500 $\times$ magnification) of labeled mitochondria that were oxidatively active. The aggregation patterns were mainly classified as fine (small pixels of fluorescence intensity throughout the cytoplasm), or aggregated (larger linear aggregations of fluorescence intensity).

The fluorescence intensity (μ A) was measured using a Photometry System P 100 (Nikon, Tokyo, Japan). Microscope adjustments and photomultiplier settings were kept constant for all experiments. Oocytes were positioned in the plane of focus, and the area of measurement was adapted to the size of the oocyte. The emission intensity/oocyte data was reduced by compensation for the background fluorescence.

Statistics

The data was evaluated by *Chi*-square test, and all results with $P < 0.05$ were considered to be significant. Three replicates were performed in all experiments. Differences in the intensity of fluorescence of complex membrane-bound calcium-chlortetracycline were tested by ANOVA. Statistical analysis of the fluorescence intensity of mitochondria was conducted using the SAS System for Windows (release 8.02).

Results

Experiment 1

To determine the effect of rbST on the developmental competence of bovine oocytes, we compared the proportions of matured oocytes, i.e. metaphase II stage oocytes, cleaved embryos, and blastocysts after IVM in eight different treatment groups ($n=1,506$ oocytes). The data concerning rbST influence on bovine oocyte maturation in the different culture systems is shown in Tables 1 and 2. There were no differences in the percentages of oocytes reaching the metaphase II stage in all groups of experiments. As compared with the controls, supplementation with rbST or granulosa cells alone led to an increase in development to the blastocyst stage, but the highest level of blastocysts was observed after oocyte maturation with rbST and granulosa cells together ($P < 0.05$). The positive effect of rbST on the developmental competence of oocytes did not depend on the type of serum used

Table 1. Effect of rbST and granulosa cells (GC) on the developmental competence of oocytes matured in TCM 199 with FCS (CM 1) (n=792 oocytes, 3 replicates)

Culture system	Matured-M II % (n)	Cleavage % (n)	Developed to blastocyst % (n)
Control CM 1	75.8 (75/99)	35.9 (32/89) ^a	10.1 (9/89) ^a
CM 1 + rbST	77.7 (80/103)	48.5 (47/97) ^a	24.7 (24/97) ^b
CM 1 + GC	75.2 (82/109)	48.9 (48/98) ^a	25.5 (25/98) ^b
CM 1 + rbST + GC	78.4 (80/102)	69.5 (66/95) ^b	40.0 (38/95) ^c

^{a,b} P<0.05 (within columns).**Table 2.** Effect of rbST and granulosa cells (GC) on the developmental competence of oocytes matured in TCM 199 with ECS (CM 2) (n=722 oocytes, 3 replicates)

Culture system	Matured-M II % (n)	Cleavage % (n)	Developed to blastocyst % (n)
Control CM 2	73.2 (71/97)	37.7 (26/69) ^a	11.6 (8/69) ^a
CM 2 + rbST	76.2 (77/101)	50.6 (44/87) ^a	20.7 (18/87) ^a
CM 2 + GC	79.6 (78/98)	47.9 (45/94) ^a	20.2 (19/94) ^a
CM 2 + rbST + GC	83.9 (78/93)	67.5 (56/83) ^b	36.1 (30/83) ^b

^{a,b} P<0.05 (within columns).

during IVM.

Experiment 2

Since we did not obtain any significant differences in the quality of the culture systems in experiment 1 using FCS (CM 1) or ECS (CM 2), all subsequent investigations were done with only one culture system supplemented with FCS and 1×10^6 granulosa cells/ml.

Analysis of cytogenetic and morphological parameters revealed that rbST had an influence on subsequent embryo quality.

Figure 1 summarizes the data concerning the effect of rbST during IVM on subsequent embryo quality. The proportions of intact 2- to 4-cell embryos on day 2 and the proportion of intact morulae on day 5 were higher in the group of embryos originating from oocytes cultured with rbST ($P<0.05$).

Experiment 3

As in the second experiment, we used TCM 199 + 10% FCS and 1×10^6 granulosa cells/ml for maturation of oocytes in the presence or absence of 10 ng/ml rbST. For evaluation of the status of mitochondria and the level of $[Ca^{2+}]_{is}$, only intact oocytes with morphological signs of maturation (cumulus expansion, extrusion of a polar body) were used after IVM. The level of $[Ca^{2+}]_{is}$ in oocytes, indicated by the fluorescence intensity of

Fig 1. Embryo quality on days 2 and 5 after IVF of *in vitro*-matured oocytes in TCM 199 with FCS (CM 1) + GC, with or without 10 ng/ml rbST (n=347 embryos). ^{a,b} P<0.05.

calcium-CTC-complexes/oocyte, was decreased under the influence of rbST (0.85 AU<0.96 AU; $P<0.05$) (Table 3).

The data in Table 4 demonstrates that the fluorescence intensity of the oocytes prelabeled by vital mitochondrial specific probe CMTM Ros and measured by fluorescence intensity at 570 nm emission/oocyte was significantly increased in oocytes matured in the presence of 10 ng/ml rbST compared with those matured in the absence of rbST (309.2 and 120 μ A, respectively; $P<0.05$). In parallel, there was a slight tendency ($P=0.1$) for a higher proportion of oocytes with aggregated mitochondria after IVM with rbST.

Table 3. Levels of intracellular stored calcium (Ca^{2+})_{is} in bovine oocytes after IVM in TCM 199 + FCS (CM 1) + GC, with or without 10 ng/ml rbST

Treatment group	No. of oocytes	Fluorescence intensity of the calcium-CTC complexes in oocytes (AU $\pm$ SEM/oocyte)
CM 1 + GC	63	0.97 $\pm$ 0.03 ^a
CM 1 + GC + rbST	65	0.85 $\pm$ 0.02 ^b

^{a,b} $P < 0.05$.

Discussion

It has been shown that growth hormones are involved in the processes of sexual differentiation, pubertal maturation, gonadal steroidogenesis, gametogenesis and ovulation [4]. Boland *et al.* [19] demonstrated that the effects of bST are related to the beneficial effect of IGF-I on the preovulatory follicle and on oocytes with a possible positive effect on subsequent CL function. Treatment of superovulated donor cows with GH at the time of insemination decreases the number of unfertilized oocytes and increases the percentage of transferable embryos. As was shown in an *in vitro* model, both GH and IGF-I stimulate embryonic development in cattle, and the effects of GH may likely involve IGF-I-independent mechanisms [20].

Expression of GHR gene, the presence of the receptor in preimplantation bovine embryos, and the beneficial effect of GH on cleavage, blastocyst formation, and hatchability of embryos was shown by Izadyar *et al.* [5, 6]. The stimulatory effect of rbST during IVM on the development of bovine oocytes after IVF was also confirmed in our study. Moreover, we found that this effect is amplified in the presence of granulosa cells independent of the type of serum.

Bovine embryos derived *in vitro* are recognized as being of lower quality than those obtained following superovulation, artificial insemination, and non-surgical recovery [21]. *In vitro*-produced embryos have abnormalities in metabolism and a high incidence of chromosome abnormalities [22–24]. The level of apoptosis is decreased by rbST in bovine blastocysts derived from *in vitro*-matured and fertilized oocytes [7]. In analysis of the morphology and chromatin status of *in vitro*-produced embryos on days 2 and 5, rbST decreased

Table 4. Fluorescence intensity/oocyte and mitochondrial aggregation based on vital labeling of metabolic active mitochondria in bovine oocytes after IVM in TCM 199 + FCS (CM 1) + GC, with or without 10 ng/ml rbST (n=73 oocytes)

Treatment group	Fluorescence intensity/oocyte ($\mu\text{A} \pm \text{SEM}$)	Oocytes with mitochondria in aggregation % (n)
CM 1 + GC	119.97 $\pm$ 28.7 ^a	15.4 (6/39)
CM 1 + GC + rbST	309.21 $\pm$ 46.8 ^b	32.4 (11/34)

^{a,b} $P < 0.05$.

the proportion of embryos with morphological and chromatin abnormalities. In comparison with the experimental group, we found a higher proportion of degenerated embryos with bi- and multinuclear blastomeres, anuclear cells, pycnotic nuclei, and abnormal cytokinesis in the control group. Therefore, it is evident that the embryos obtained from *in vitro*-matured oocytes in the presence of rbST and granulosa cells had higher potential for continued development to blastocysts. This raises the question, which intracellular mechanisms could be involved in the rbST-derived effects on cytoplasmic oocyte maturation? Several studies have been carried out to study factors affecting the developmental competence of the oocyte [25].

In our study, we used two markers for evaluation of cytoplasmic maturation of bovine oocytes co-cultured with granulosa cells; we compared the mitochondrial activity and the content of calcium in intracellular depots of oocytes matured in the presence or absence of rbST. The central role of mitochondria in metabolism and bioenergetics is well defined. It has been shown that the level of mitochondrial activity can be used for assessment of the functional status and quality of oocytes in pigs, cattle, and horses [10, 11, 26, 27]. In the present study, the oxidative activity of mitochondria in oocytes matured in the presence of rbST was significantly increased in comparison with the control group. The reason for the increase in fluorescence intensity of labeled mitochondria during maturation is likely an increase of respiratory activity to provide ATP for activation and preimplantation embryo development. Findings concerning increasing levels of fluorescence intensity of labeled mitochondria during porcine and bovine oocyte maturation [10, 28] positively correlate with the results of other

authors [11, 29] who found increased levels of ATP content/oocyte during IVM.

Calcium is an important intracellular messenger, and calcium ions regulate many key intracellular events. It is known that calcium-dependent signalling pathways are involved in the regulation of mammalian oocyte meiotic maturation [30, 31]. A relationship between calcium stores and developmental competence has also been described for *in vitro*-matured bovine oocytes by He *et al.* [32], who used these parameters to evaluate differences between calf and cow oocytes. Thus, the plasma membrane Ca^{2+} currents of immature oocytes and intracellular Ca^{2+} stores in mature oocytes are good markers for the developmental potential of oocytes. Previously, we showed that normal oocytes have low levels of $[\text{Ca}^{2+}]_{\text{is}}$ immediately after removal from follicles in comparison with oocytes with signs of degeneration [33]. Moreover, we suggested a relationship between the NADH concentration and calcium from intracellular stores in bovine oocytes. In the present study, we found that supplementation with rbST during IVM decreased the level of intracellular calcium stores in oocytes after maturation. An increase in mitochondrial activity and calcium exit from intracellular stores led to activation of metabolism in matured oocytes.

Izadyar *et al.* [8] showed that the stimulatory effect of growth hormone on *in vitro* maturation of bovine oocytes is exerted through cumulus cells

and is not mediated by IGF-I. Addition of rbST during maturation of bovine oocytes decreased the level of pycnotic cells in cumulus cells [34]. The results of our study indicate a possible influence of bovine follicular granulosa cells on somatotropin-related effects on the cytoplasmic maturation and developmental competence of oocytes *in vitro*.

In conclusion, the results of the present study clearly demonstrate that rbST supplementation together with follicular somatic cells (granulosa cells) has a beneficial effect on the cytoplasmic quality of bovine oocytes and their subsequent development to the blastocyst stage after IVF. In oocytes matured in the presence of rbST and somatic cells, increased levels of respiratory activity were associated with decreased levels of calcium from intracellular stores. Granulosa cells under the influence of somatotropin may produce a stimulatory substance(s) that promotes the potential of bovine oocytes for development to the blastocyst stages. Therefore, possible mechanisms of somatotropin-mediated effects on developmental competence of bovine oocytes are the subject of ongoing experiments.

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