Relationship between growth hormone concentrations in bovine oocytes and follicular fluid and oocyte developmental competence

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In the last few years, several works suggest that Growth Hormone (GH) is involved in follicular development and oocyte maturation. These actions may reflect endocrine roles of pituitary GH and also account for local autocrine or paracrine activities of GH produced in reproductive tissue. This study was aimed to verify whether the developmental competence of bovine female gametes might be related to ovarian GH. We evaluated the localisation and distribution of GH in the cumulus oocytes complexes (COCs) and the concentration of GH in the oocytes and in the follicular fluids (FF) from ovaries classified on the basis of the follicles number. Oocytes retrieved from ovaries with more than 10 follicles of 2 to 5 mm in diameter (High ovaries, Hi) show higher rate of maturation and blastocyst formation than those retrieved from ovaries with less than 10 follicles (Low ovaries, Lo). At the same time we measured Estrogen (E_2) and Progesterone (P_4) concentrations in FF, to relate oocytes quality, GH concentration and follicle health. GH localization in COCs and oocytes was performed by indirect immunofluorescence and its concentration within the ooplasm was evaluated by microspectrophotometer analysis. GH, E_2 and P_4 concentrations in FF were measured by an Enzyme Linked ImmunoSorbent assay (ELISA). We observed a positive, diffuse signal at cytoplasmic level in most of the cumulus cells, with no differences between COCs collected from Hi and Lo ovaries. On the contrary, GH level was significantly higher in the oocytes collected from Lo ovaries than in those recovered from Hi ovaries. Finally we found that also GH level in the FF was inversely related to the oocytes developmental capability. We suggest that the increase of GH in the oocytes and in the FF derived from Lo ovaries might be interpreted as attempt of the follicular environment to improve ovarian activity and in turn oocytes developmental competence in a autocrine-paracrine manner. Moreover, E_2, and P_4 levels in FF suggest that, in our model, atresia processes are also involved in oocyte developmental capability and that the highest level of GH may represent a local reaction to these phenomena.

Key words: Cow, growth hormone, oocyte developmental competence.

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There is much evidence to suggest that the intrinsic quality of the oocyte is the key factor determining the proportion of oocyte developing to the blastocyst stage (Krisher, 2004).

Quality, or developmental competence, is acquired during folliculogenesis as the oocytes grow and their companion somatic cells differentiate (Eppig *et al.*, 1994).

Gonadotropins are known to be the major regulators of gonadal function. However, it is now well known that growth factors and metabolic hormones, also locally produced, play important roles (Hunter *et al.*, 2004). Several studies suggest that growth hormone (GH) is directly involved, alone or in combination with FSH and LH, in follicular development and oocyte maturation, both *in vivo* and *in vitro* (Bevers and Izadyar, 2002; Hull and Harvey, 2001; Kaise *et al.*, 2001).

Although GH actions may reflect endocrine roles of pituitary GH, they may also account for the local autocrine or paracrine actions of GH produced in reproductive tissue. The GH receptor and its mRNA have been localized in the ovary of various species: rat (Carlsson *et al.*, 1993; Zhao *et al.*, 2002); human (Sharara and Nieman, 1994); sheep (Eckery *et al.*, 1997); cow (Izadyar *et al.*, 1997; Kolle *et al.*, 1998). GH gene expression and protein localization have been described in cumulus oocyte complexes (COCs) and in the ovary of the rat (Zhao *et al.*, 2002) and cow (Izadyar *et al.*, 1999). Moreover, GH is also present in the follicular fluid of cows (Borromeo *et al.*, 1996; 1998) and women (Mendoza *et al.*, 1999). Recently, it has been demonstrated that oocyte developmental competence is directly influenced by follicular fluid composition (Iwata *et al.*, 2006; Nicholas *et al.*, 2005).

Many other factors affect oocyte quality in bovine species during follicular growth; these include the diet (Gong, 2002), season (Silva *et al.*, 2006), hormonal stimulation (Sirard *et al.*, 2006), size (Ali *et al.*, 2004; Lonergan *et al.*, 1994) and health of fol-

licles (Mermillod *et al.*, 1999).

In a previous study, we demonstrated that bovine oocyte quality is also related to the number of follicles located in the originating ovary; in particular, we observed that oocytes recovered from ovaries with a follicle > 10 mm in diameter or more than 10 follicles of 2 to 5 mm in diameter show higher rates of maturation and blastocyst formation than those collected from ovaries with less than ten follicles of 2 to 5 mm and no follicles > 10 mm in diameter (Gandolfi *et al.*, 1997).

To verify whether the developmental competence of bovine female gametes is related to ovarian GH, we determined the concentration of GH in the oocytes and in the follicular fluids, starting from ovaries classified on the basis of the follicle number.

During follicular development, granulosa cells are the major source of follicular estrogen (E_2) and theca and granulosa cells together determine the intrafollicular concentration of progesterone (P_4) and testosterone (T) (Bigelow and Fortune, 1998; McNatty *et al.*, 1984; Soboleva *et al.*, 2000). Changes in concentrations of steroids occur in cattle when follicles mature or become atretic and are usually related to a certain size or stage of follicular development (Ginther *et al.* 2001; Ginther *et al.*, 1996). In that sense, the E_2/P_4 ratio may indicate the degree of atresia (De los Reyes *et al.*, 2006; Price *et al.*, 1995). For this purpose, we measured E_2 and P_4 concentrations in follicular fluid, to relate oocyte quality, GH concentration and follicle health.

Materials and Methods

Chemicals and reagents

Chemicals and reagents were purchased from Sigma Chemical Company (St. Louis, MO, USA), unless otherwise stated.

Oocyte and follicular fluid collection

Bovine ovaries were obtained from a local abattoir and transported to the laboratory within 2 h in sterile saline (9 g NaCl/L) maintained at 32-34°C. All subsequent procedures were conducted at a temperature of 36-38°C. As previously described (Gandolfi, *et al.*, 1997), ovaries were classified into two categories as follows: low efficiency (Lo), with less than 10 follicles between 2-5 mm in diameter and no follicles ≥ 10 mm; high efficiency (Hi), with more than 10 follicles between 2-5 mm in diameter. In both categories, the presence or the absence of a corpus luteum was not taken into account.

Cumulus oocyte complexes (COCs) and follicular fluid were aspirated from all 2-5 mm follicles of Lo and Hi ovaries with a 18-gauge needle mounted on an aspiration pump (COOK-IFV, Australia) with a vacuum pressure of -28 mm/Hg. After COCs removal, follicular fluid was centrifuged for 10 min at 500 g and supernatant stored at -20°C until assayed.

In both categories, COCs were examined under a stereomicroscope, and only complexes medium-brown in colour, with five or more complete layers of cumulus cells and a finely granulated homogenous ooplasm were used for *in vitro* embryo production (IVP).

In vitro maturation

Selected COCs were then washed twice in M199 supplemented with 20 mM HEPES and 0.4% of BSA (HM199) and twice in the maturation medium. The whole procedure was performed in approximately 30 min.

The basic maturation medium (bMM) was TCM-199 supplemented with 0.68 mM L-glutamine, 0.2 mM sodium pyruvate, 25 mM NaHCO3, 0.4% fatty acid free bovine serum albumin, 0.1 IU/mL human recombinant FSH (hrFSH, Gonal-F, Serono, Rome, Italy). Groups of about 30 COCs were matured in 500 μL of bMM for 24 h in four-well dishes (NUNC, VWR International, Milan, Italy) at 38.5° C under 5% CO_2 in humidified air.

In vitro fertilization

After maturation, oocytes were fertilized as previously described (Luciano *et al.*, 1999). Briefly, the contents of a straw of cryopreserved bull spermatozoa (CIZ, S. Miniato Pisa, Italy) was thawed and cells separated on a 45-90% Percoll gradient. Sperms were counted and diluted to a final concentration of 1×10^6 spermatozoa/mL of fertilization medium, which was TALP supplemented with 0.6% (w/v) fatty acid free BSA, 10 μg/mL heparin, 20 μM penicillamine, 1 μM epinephrine, and 100 μM hypotaurine. Groups of 30-34 COC were cultured in 300 μL of fertilisation medium and incubated for 18 h in four-well dishes at 38.5° C under 5% CO_2 in humidified air.

Embryo culture

The embryo culture medium consisted of synthet-

ic oviduct fluid (SOF) (Tervit *et al.*, 1972), modified as previously described (Luciano *et al.*, 2005). Briefly, SOF was buffered with 25 mM $NaHCO_3$, supplemented with MEM essential and non-essential amino acids, 0.72 mM sodium pyruvate, 2.74 mM myo-inositol, 0.34 mM sodium citrate and 5% calf serum (SOF-C). Embryo rinse and manipulation medium was SOF supplemented with 0.3% (w/v) BSA fraction V, MEM essential and non-essential amino acids, 0.72 mM sodium pyruvate and buffered with 10 mM HEPES and 5 mM $NaHCO_3$ (SOF-R). After fertilization, presumptive zygotes were vortexed for 2 min in 500 μL of SOF-R, rinsed twice and then transferred in groups of 30-34 t0 400 μL of SOF-C under 400 μL of mineral oil. Incubation was performed at 38.5°C in a humidified gas mixture composed of 5% CO_2, 5% O_2 and 90% N_2., Cleavage and blastocyst rate were assessed, respectively 48 h and 186 h post insemination. At the end of the culture, blastocysts were fixed in 60% methanol in PBS, and cell nuclei were counted under fluorescence microscopy after staining with 0.5 mg/mL propidium iodide.

Hormone assays in follicular fluid

GH concentrations in follicular fluid were measured by an enzyme-linked Immunosorbent assay (ELISA), based on polyclonal antibodies specific for bovine GH, as previously described (Borromeo *et al.*, 1996). The assay was validated for use with ovarian fluids. The sensitivity of GH was 0.25 ng/mL. The intra- and inter-assay coefficients of variation ranged between 2.6 to 5.1% and 8.5 to 12.7%, respectively.

E_2 and P_4 were measured in unextracted samples of follicular fluid using EIA (Neogen Corporation, Lexington, KY, USA). The sensitivity limits were 0.04 ng/mL of E_2 and 0.4 ng/mL of P_4. The intra- and inter-assay coefficients of variation were 4.8 to 7.2% and 5.4 to 9.6% for E_2, respectively, and 5.2 to 8.4% and 6.2 to 10.1% for P_4, respectively.

Localization of GH in the oocytes and COCs

In order to visualize the distribution and the localization of GH within the oocyte and the cumulus cells, 40 COCs were isolated from Hi and Lo ovaries, respectively. Twenty COCs from each group were immediately fixed intact after their aspiration from the ovary; from the remaining samples, cumulus cells and zona pellucida were removed (Modina, *et al.* 2004). All the samples were processed as whole mount and analyzed with an indirect immunofluorescence protocol.

The cumulus cells were separated in a defined saline medium [(0.1% (w/v) polyvinyl plcohol (PVA) in PBS-PBS/PVA)] by vortexing for 2 min at 35 Hz; zona pellucida was dissolved by digestion with 0.5% (w/v) pronase E in PBS at 39°C for 1-3 min. After several washes in PBS/PVA, oocytes were treated with a blocking solution [(1% (w/v) BSA, 0.3% (w/v), Triton X-100 and 10% (v/v) normal donkey serum in PBS)] for 30 min at room temperature (RT).

Oocytes were incubated overnight at 4°C with the same anti-bovine GH antibodies used for ELISA, which were previously shown to be suitable for immunoistochemistry (Borromeo, *et al*. 1998), diluted 1:50 in 1% (w/v) BSA, 0.1%(v/v) Triton X-100 and subsequently washed several times in PBS/PVA. Finally, samples were incubated with FITC donkey anti-rabbit IgG secondary antibody (Jackson ImmunoResearch laboratories, Inc.) diluted 1:300 in PBS/PVA for 30 min at RT.

Nuclear status was evaluated after incubation in 0.01% (w/v) 4'-6-diamidino-2-phenylindole (DAPI) for 10 min at RT.

COCs and denuded oocytes were washed in PBS/PVA for 1h at RT, transferred onto glass slides in 20 μL of Fluoro-Guard (Biorad-Missisauga, ON, Canada) mounting medium and observed with a conventional epifluorescence microscope (Nikon, Eclipse E 600, Japan).

Negative controls were performed by replacing GH antibodies with either PBS or normal rabbit serum. A group of oocytes were incubated with GH antibodies saturated with GH proteins.

Microdensitometric quantification of GH

In order to quantify GH concentration within the oocytes, 141 and 91 denuded and dezoned oocytes collected from Hi and Lo ovaries respectively, were analysed as whole mount by indirect immunofluorescence, as described above. After GH localization, each sample was transferred onto a glass slide in 20 μL of Fluoro-Guard (Biorad-Missisauga, ON, Canada) mounting medium in the center of a tapering to produce a fixed space about 200 μm thick and evaluated as single cells with a Universal Microspectrophotometer System equipped for epifluorescence analysis (Zeiss, Milan, Italy). Values of fluorescent intensity were expressed as arbitrary units.

Table 1. *In vitro* embryo development of COCs isolated from Lo and Hi ovaries.

Ovaries	*Oocytes (n)*	*Cleaved (%)*	*Percentage of blastocysts over cleaved oocytes*	*Percentage of blastocysts over total oocytes*	*Blastocysts Cell number*
Hi	128	93.7±0.8*	35.4±2.1*	33.2±1.9*	96.8±5.9
Lo	112	88.9±1.3	9.4±1.5	8.3±1.2	85.8±9.1

*The data are expressed as means ± SEM. *, p<0.05.vs Lo.*

Table 2. Follicular fluid concentration of GH, P_4 and E_2 in 2-5 mm follicles from Hi and Lo ovaries.

Ovaries	*Follicles (n)*	*GH (ng/mL)*	*P_4 (ng/mL)*	*E_2 (ng/mL)*
Hi	45	6.64±0.88	32.21±7.49	9.23±3.39
Lo	50	15.25±2.06*	68.09±15.4*	1.98±0.66*

*The data are expressed as means ± SEM; *, p<0.01.vs Hi.*

Table 3. Micro-densitometric quantification of bGH immunofluorescence of single denuded and dezoned oocytes derived from different classes.

Ovaries (n)	*Oocytes Units*	*Arbitrary*
Hi	141	2.089±0.14*
Lo	91	2.889±0.32

*The data are expressed as means ± SEM; n, number of samples ; *, pp< 0.05.vs Lo.*

Statistical analysis

Experiments were replicated at least 3 times. Results are expressed as mean ± SEM. Data were analyzed using one-way ANOVA followed by Scheffe's test for multiple comparisons (SuperANOVA, Abacus Concepts, CA, USA). Probabilities of less than 0.05 were considered statistically significant.

Results

In vitro embryo production

The percentage of COCs selected by morphological criteria suitable for IVP procedures from Lo ovaries was not different from the percentage of those isolated from Hi ovaries, as previously demonstrated (Gandolfi, *et al*. 1997). As expected from the selection criteria used in these experiments, oocytes isolated from Hi ovaries (i.e. yielding oocytes with high developmental competence) did indeed show a higher developmental potential than those from Lo ovaries (i.e. yielding oocytes with low developmental competence). No significant differences were evident in blastocyst cell number (Table 1).

Hormone assays in follicular fluid

As indicated in Table 2, GH and P_4 concentrations were significantly higher in follicular fluid aspirated from Lo ovaries than in the follicular fluid isolated from Hi ovaries ($p<0.01$).

On the contrary, E_2 concentration was significantly higher in follicular fluid aspirated from Hi ovaries than in the follicular fluid isolated from Lo ovaries ($p<0.01$).

Localization and microdensitometric quantification of GH

In all the COCs examined, most of the cumulus cells showed a positive, diffuse signal at the cytoplasmic level (Figure 1). No differences were found between COCs of Hi and Lo ovaries.

Denuded oocytes showed a positive uniform GH signal within the cytoplasm, with an intensity ranging from strong to weak (Figure 2b, c). Nuclear staining confirmed that all the oocytes were in the germinal vesicle stage, showing different degrees of chromatin organization (Lodde, *et al*. 2007) (Figure 2c, f, i). All the controls were negative, indicating the specificity of the anti-bovine GH antibody (Figure 1h; Figure 2h).

Microdensitometric analysis showed that oocytes harvested from Lo ovaries had a significantly higher GH concentration than those collected from Hi ovaries (Table 3).

Discussion

The quality of the oocyte, defined as the competence to yield a blastocyst following *in vitro* maturation, fertilization and culture (Lonergan *et al*., 2003) represents the most important factor that limits the application of assisted reproductive technologies in domestic animals (Merton *et al*., 2003). Physiologically, only naturally selected follicles

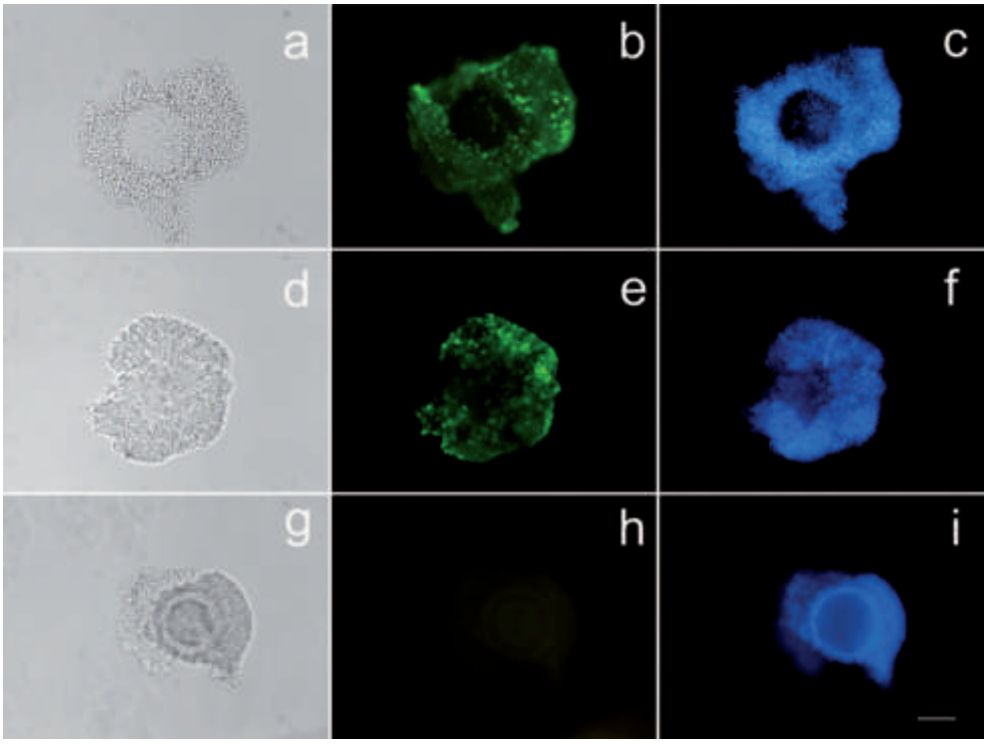

Figure 1. Immunolocalization of GH in representative cumulus oocyte complexes analysed as a whole mount. GH localization in COCs derived from Hi ovary (b) and Lo ovary (e); control (h); nuclear staining with DAPI (c, f, i); bright fields (a, d, g). Bar=100 µm.

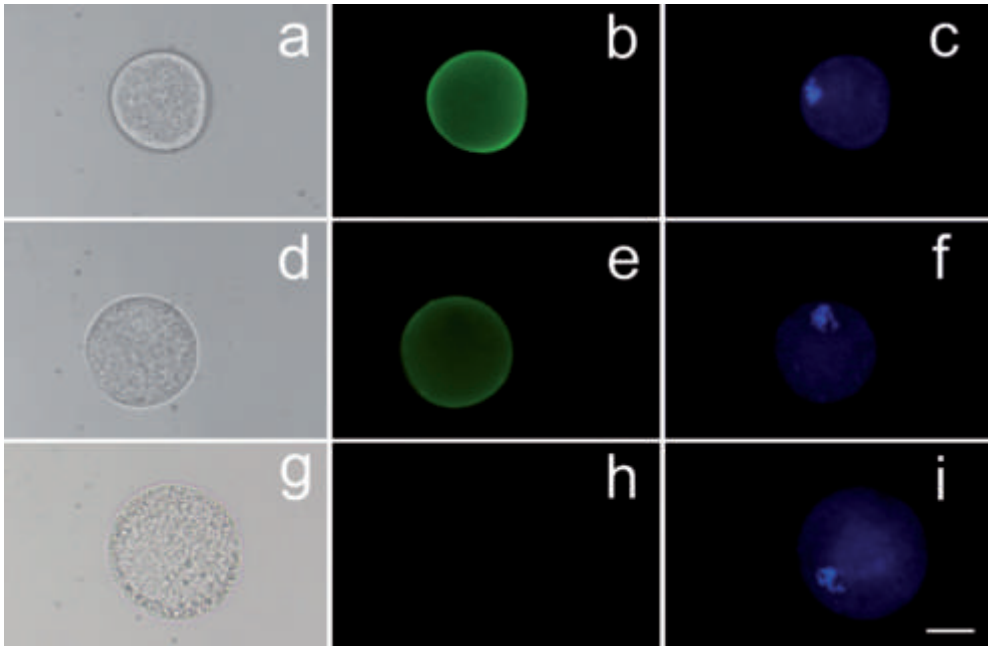

Figure 2. Immunolocalization of GH in representative denuded and dezoned oocytes analysed as a whole mount. Intense GH signal in oocyte derived from Hi ovary (b); weak GH signal in oocyte derived from Lo ovary (e); control (h); nuclear staining with DAPI (c, f., i); bright field (a, d, g). Bar=50 µm.

come to ovulation; on the contrary, embryo technologies use oocytes from follicles at different sizes and stages of follicular growth. The heterogeneity of these oocytes can lead to suboptimal embryo production compared to the natural environment situation (Sirard, *et al*. 2006).

In the present study, we confirmed that in cow, oocyte developmental competence is linked to the morphology of the ovary (Gandolfi *et al*., 1997), and we show for the first time that it also can be related to different amounts of GH concentrations, both within the oocytes and in the follicular fluid. We also found that P_4 concentrations were significantly higher in follicular fluids collected from Lo ovaries than in those collected from Hi ovaries. These data suggest that in our model, atresia

processes may also be involved in oocyte developmental capability (Borromeo *et al.*, 1998), even if in small antral follicles the E_2/P_4 ratio is controversial because the ability to respond to LH and produce androgen precedes the ability of granulosa cells to aromatize estrogen to androgen (McNatty *et al.*, 1984; Roberts and Skinner, 1990). A previous study, which indicated that in pigs, cyclical growth and atresia of ovarian follicles result in changes in overall oocyte quality (Shimada and Terada 2002), support our hypothesis; and the observation that in follicular fluid P_4 concentrations were consistently higher than E_2 concentrations, agrees with the recent work of De los Reyes *et al.* (2006).

GH localization in bovine COCs and ovary was previously reported by Izadyar *et al.* (1999). These authors observed that at the onset of *in vitro* maturation, COCs obtained from follicles 2 to 8 mm in diameter showed strong and moderate GH immunoreactivity in the cumulus cells and oocytes, respectively, while the expression of GH mRNA was found only in the oocytes, indicating active synthesis of the hormone at this site. Similarly, we found a positive signal for GH both in oocytes and cumulus cells. However, we generally observed a positive, diffuse signal at the cytoplasmic level in most of the cumulus cells, with no differences between the two groups of COCs considered. On the contrary, although all the oocytes displayed a positive, uniform signal within the cytoplasm, various grades of intensity were detected, suggesting different levels of secretion and/or storage. The present study has been characterized by different approaches and objectives. First of all Izadyar *et al.* (1999) localized GH proteins on COC sections with avidin-biotin peroxidase staining and the intensity of each immunoreaction was evaluated by light microscopy and scored arbitrarily as negative, moderate or strong. We used a more defined technical approach, since we analysed COCs and denuded oocytes as whole mount by indirect immunofluorescence and the intensity within the ooplasm of each single oocyte was validated by microspectrophotometric analysis. Secondarily, in the study described above, Izadyar *et al.* (1999) analysed a pool of COCs selected only on the basis of the presence of a multilayered compact cumulus investment, without taking into account the originating gonad. In our experimental model, before COC collection, we determined the morphology of each ovary to associate GH concentrations and oocyte quality to the morphological appearance of the ovary.

We thus found that the intracytoplasmic concentration of GH within the oocytes reflected GH concentration in follicular fluid and that a high level of the hormone is inversely related to the developmental capability. Conversely, Mendoza *et al.* (1999) demonstrated that in women, elevated concentrations of GH in follicular fluid were associated with rapid cleavage, good morphology of cleaving embryos and high embryo implantation potential.

However this result is only apparently in contrast with our data since Mendoza *et al.* (1999) considered follicular fluids from pre-ovulatory follicles (>18 mm in diameter) and from patients subjected to ovarian stimulation with FSH and HMG. Stimulatory treatments, in fact, induce dynamic changes in follicular fluids as individual cells of the follicles respond to the administration of gonadotropins by secreting different hormones, such as GH, growth factors, and cytokines that, either directly or indirectly, influence oocyte viability and developmental potential (Mendoza *et al.* 2002). Our experimental conditions are completely different, since the morphology of the ovaries collected from the slaughterhouse indicated that cows were not submitted to hormonal treatments; moreover, we analyzed COCs aspirated from growing middle antral follicles.

Although results obtained in our previous report suggest that systemic factors, such as age, are not likely to be implicated in the diversities between COCs from different ovaries (Gandolfi *et al.*, 1997), the morphology of Lo ovaries could be compared to those of women during the decline of fertility. In women, in fact, the basis of ovarian ageing is ovarian follicle depletion, and the number and the quality of remaining oocytes are closely related (Nikolaou and Templeton 2004; Nikolaou 2004) The causes of oocyte quality deterioration are not completely known; however, the qualitative decline of the remaining gametes is characterized by fewer granulosa cells per follicles, which have diminished function, indicated by less *in vitro* steroid and glycoprotein production and by decreased mitosis and increased apoptosis (Seifer *et al.*, 1996) and by mitochondrial damage (Takeuchi *et al.*, 2005).

Starting from these data, we assumed that the increase of GH concentration in oocytes and follicular fluids collected from Lo ovaries might be interpreted as an attempt of the follicular environment

to improve ovarian activity and oocyte developmental competence in an autocrine-paracrine manner.

In accordance with this hypothesis, several pieces of evidence suggest that, in the cow, GH has a promotory effect both *in vitro* (Izadyar *et al.*, 1998; Kuzmina *et al.*, 2006; Moreira *et al.*, 2002; Pozzobon *et al.*, 2005) and *in vivo* on developmental competence (Moreira *et al.*, 2002), leading to speculate that an early rise of GH in small antral follicles located in Lo ovaries is beneficial for oocyte quality by enhancing, or acting in synergy with gonadotropin in developmental processes. By a similar approach, we can also consider the capacity of the hormone to stimulate oxidative activity of ooplasmic mitochondria in women (Kuzmina, et al. 2006) and to increase cumulus expansion by promotion of cell proliferation and inhibition of apoptosis (Kolle, *et al*. 2003), and to enhance oocyte quality by accelerating and coordinating cytoplasmic and nuclear maturation (Izadyar, *et al*. 1997; 1998) during *in vitro* maturation of bovine oocytes.

The increase of follicular GH concentration in Lo ovaries as a reaction to the atresia processes may also be considered. *In vivo* studies in fact suggest that GH stimulates growth and prevents atresia in small follicles, which develop and undergo atresia throughout the oestrous cycle. For instance, GH administration increases the number of small follicles in cattle (Gong *et al.*, 1991) and horses (Cochran *et al.*, 1999) and GH and gonadotropins are required to prevent atresia in follicles >2 mm following hypophysectomy in sheep (Eckery *et al.*, 1997). To date, it is evident that the greatest amount of GH stored in the follicular environment of Lo ovaries is not enough to restore oocyte quality, even considering the P_4 level in the follicular fluids, confirming the general opinion that several factors influence, directly or indirectly, oocyte viability and developmental potential.

Finally, although literature is limited and restricted to specific cases, it cannot be excluded that in our model, ovarian GH may exert an inhibitory effect. It is well known that the activities of GH are progonadal at physiological concentration; in fact, an antigonadal activitiy at pharmacological concentrations and in patho-physiological excess has been demonstarted. Mice (Ghosh and Bartke, 1993), rodents (Bartke *et al.*, 1996) and pigs (Bartke *et al.*, 1996), transgenically expressing the GH gene, show a decrease of circulating gonadotropins that, in turn, decrease male and female fertility. As supposed by Hull and Harvey (2001), these actions are thought to reflect an endocrine role of pituitary GH and a complementary autocrine and paracrine role of GH produced within the ovary; the local production of the hormone within the ovary thus reflects an emergency mechanism to rapidly regulate or finely tune cellular functions that are normally regulated by pituitary GH. In conclusion, although this study provokes some new questions, it contributes to better understandjng the role of ovarian GH and the cellular mechanisms that modulate the achievement of oocyte developmental competence. The acquisition knowledge on this topic is of primary importance since until the processes involved in oocyte quality are elucidated, any effort to use assisted reproductive technologies in the treatment of human infertility or animal production for biomedical purposes will be insufficient at best.

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References

Ali A, Coenen K, Bousquet D, Sirard MA. Origin of bovine follicular fluid and its effect during in vitro maturation on the developmental competence of bovine oocytes. Theriogenology 2004;62:1596-606.

Bartke A, Chandrashekar V, Steger RW. Effects of growth hormone on neuroendocrine function. Acta Neurobiol Exp (Wars) 1996;56: 833-42.

Bevers M, Izadyar F. Role of growth hormone and growth hormone receptor in oocyte maturation. Mol Cell Endocrinol 2002;197:173-78.

Bigelow KL, Fortune JE. Characteristics of prolonged dominant versus control follicles: follicle cell numbers, steroidogenic capabilities, and messenger ribonucleic acid for steroidogenic enzymes. Biol Reprod 1998;58:1241-9.

Borromeo V, Berrini A, Bramani S, Sironi G, Finazzi M, Secchi C. Plasma levels of GH and PRL and concentrations in the fluids of bovine ovarian cysts and follicles. Theriogenology 1998;49:1377-87.

Borromeo V, Bramani S, Berrini A, Sironi G, Finazzi M, Cremonesi F, et al. Growth hormone but not prolactin concentrations in the fluid of bovine ovarian cysts are related to the cystic stage of luteinization. Theriogenology 1996;46:481-9.

Carlsson B, Nilsson A, Isaksson OG, Billig H. Growth hormone-receptor messenger RNA in the rat ovary: regulation and localization. Mol Cell Endocrinol 1993;95:59-66.

Cochran RA, Leonardi-Cattolica AA, Sullivan MR, Kincaid LA, Leise BS, Thompson DL et al. The effects of equine somatotropin (eST) on follicular development and circulating plasma hormone profiles in cyclic mares treated during different stages of the estrous cycle. Domest Anim Endocrinol 1999;16:57-67.

De los Reyes M, Villagran ML, Cepeda R, Duchens M, Parraguez V, Urquieta B. Histological characteristics and steroid concentration of ovarian follicles at different stages of development in pregnant and non-pregnant dairy cows. Vet Res Commun 2006;30:161-73.

Eckery DC, Moeller CL, Nett TM, Sawyer HR. Localization and quantification of binding sites for follicle-stimulating hormone, luteinizing hormone, growth hormone, and insulin-like growth factor I in sheep ovarian follicles. Biology of Reproduction 1997;57:507-13.

Eppig JJ, Schultz RM, O'Brien M, Chesnel F. Relationship between the developmental programs controlling nuclear and cytoplasmic maturation of mouse oocytes. Dev Biol 1994;164:1-9.

Gandolfi F, Luciano AM, Modina S, Ponzini A, Pocar P, Armstrong DT, et al. The in vitro developmental competence of bovine oocytes can be related to the morphology of the ovary. Theriogenology 1997;48:1153-60.

Ghosh PK, Bartke A. Effects of the expression of bovine growth hormone on the testes and male accessory reproductive glands in transgenic mice. Transgenic Res 1993;2:79-83.

Ginther OJ, Bergfelt DR, Beg MA, Kot K. Follicle Selection in Cattle: Relationships among Growth Rate, Diameter Ranking, and Capacity for Dominance. Biol Reprod 2001;65:345-50.

Ginther OJ, Wiltbank MC, Fricke PM, Gibbons JR, Kot K. Selection of the dominant follicle in cattle. Biol Reprod 1996;55:1187-94.

Gong JG. Influence of metabolic hormones and nutrition on ovarian follicle development in cattle: practical implications. Domest Anim Endocrinol 2002;23:229-41.

Gong JG, Bramley T, Webb R. The effect of recombinant bovine somatotropin on ovarian function in heifers: follicular populations and peripheral hormones. Biol Reprod 1991;45:941-9.

Hull KL, Harvey S. Growth hormone: roles in female reproduction. J Endocrinol 2001;168:1-23.

Hunter MG, Robinson RS, Mann GE, Webb R. Endocrine and paracrine control of follicular development and ovulation rate in farm species. Anim Reprod Sci 2004;82-83:461-77.

Iwata H, Inoue J, Kimura K, Kuge T, Kuwayama T, Monji Y. Comparison between the characteristics of follicular fluid and the developmental competence of bovine oocytes. Anim Reprod Sci 2006;91:215-23.

Izadyar F, Van Tol HT, Colenbrander B, Bevers MM. Stimulatory effect of growth hormone on in vitro maturation of bovine oocytes is exerted through cumulus cells and not mediated by IGF-I. Molecular Reproduction & Development 1997;47:175-80.

Izadyar F, Zeinstra E, Bevers MM. Follicle-stimulating hormone and growth hormone act differently on nuclear maturation while both enhance developmental competence of in vitro matured bovine oocytes. Molecular Reproduction & Development 1998;51:339-45.

Izadyar F, Zhao J, Van Tol HT, Colenbrander B, Bevers MM. Messenger RNA expression and protein localization of growth hormone in bovine ovarian tissue and in cumulus oocyte complexes (COCs) during in vitro maturation. Mol Reprod Dev 1999;53:398-406.

Kaise GG, Sinowatz F, Palma GA. Effects of growth hormone on female reproductive organs. Anat Histol Embryol 2001;30:265-71.

Kolle S, Sinowatz R, Boie G, Lincoln D. Developmental changes in the expression of the growth hormone receptor messenger ribonucleic acid and protein in the bovine ovary. Biol Reprod 1998;59:836-42.

Kolle S, Stojkovic M, Boie G, Wolf E, Sinowatz F. Growth hormone-related effects on apoptosis, mitosis, and expression of connexin 43 in bovine in vitro maturation cumulus-oocyte complexes. Biol Reprod 2003;68:1584-9.

Krisher RL. The effect of oocyte quality on development. J Anim Sci 2004;82 E-Suppl:E14-23.

Kuzmina TI, Alm H, Denisenko V, Tuchscherer A, Kanitz W, Torner H. Effect of Recombinant Bovine Somatotropin (rbST) on Cytoplasmic Maturation of Bovine Oocytes and Their Developmental Competence In Vitro. J Reprod Dev 2006;53:309-16.

Lodde V, Modina S, Galbusera C, Franciosi F, Luciano AM. Large-scale chromatin remodeling in germinal vesicle bovine oocytes: Interplay with gap junction functionality and developmental competence. Mol Reprod Dev 2007;74:740-9.

Lonergan P, Monaghan P, Rizos D, Boland MP, Gordon I. Effect of follicle size on bovine oocyte quality and developmental competence following maturation, fertilization, and culture in vitro. Mol Reprod Dev 1994;37:48-53.

Lonergan P, Rizos D, Gutierrez-Adan A, Fair T, Boland MP. Oocyte and embryo quality: effect of origin, culture conditions and gene expression patterns. Reprod Domest Anim 2003;38:259-67.

Luciano AM, Lodde V, Beretta MS, Colleoni S, Lauria A, Modina S. Developmental capability of denuded bovine oocyte in a Co-culture system with intact cumulus-oocyte complexes: Role of cumulus cells, cyclic adenosine 3',5'-monophosphate, and glutathione. Molecular Reproduction and Development 2005;71:389-97.

Luciano AM, Pocar P, Milanesi E, Modina S, Rieger D, Lauria A, et al. Effect of different level of intracelluar cAMP on the in vitro maturation of cattle oocytes and their subsequent development following in vitro fertilization. Mol Reprod Devel 1999;54:86-91.

McNatty KP, Heath DA, Henderson KM, Lun S, Hurst PR, Ellis LM, et al. Some aspects of thecal and granulosa cell function during follicular development in the bovine ovary. J Reprod Fertil 1984;72:39-53.

Mendoza C, Cremades N, Ruiz-Requena E, Martinez F, Ortega E, Bernabeu R, et al. Relationship between fertilization results after intracytoplasmic sperm injection, and intrafollicular steroid, pituitary hormone and cytokine concentrations. Hum Reprod 1999;14:628-35.

Mendoza C, Ruiz-Requena E, Ortega E, Cremades N, Martinez F, Bernabeu R, et al. Follicular fluid markers of oocyte developmental potential. Hum Reprod 2002;17:1017-22.

Mermillod P, Oussaid B, Cognie Y. Aspects of follicular and oocyte maturation that affect the developmental potential of embryos. J Reprod Fertil Suppl 1999;54:449-60.

Merton JS, de Roos AP, Mullaart E, de Ruigh L, Kaal L, Vos PL, et al. Factors affecting oocyte quality and quantity in commercial application of embryo technologies in the cattle breeding industry. Theriogenology 2003;59:651-74.

Modina S, Beretta M, Lodde V, Lauria A, Luciano AM. Cytoplasmic changes and developmental competence of bovine oocytes cryopreserved without cumulus cells. Eur J Histochem 2004;48:337-46.

Moreira F, Badinga L, Burnley C, Thatcher WW. Bovine somatotropin increases embryonic development in superovulated cows and improves post-transfer pregnancy rates when given to lactating recipient cows. Theriogenology 2002;57:1371-87.

Nicholas B, Alberio R, Fouladi-Nashta AA, Webb R. Relationship between low-molecular-weight insulin-like growth factor-binding proteins, caspase-3 activity, and oocyte quality. Biol Reprod 2005;72:796-804.

Nikolaou D, Templeton A. Early ovarian ageing. Eur J Obstet Gynecol Reprod Biol 2004;113:126-33.

Nikolaou DS. Poor response-oocyte quantity or quality? Fertil Steril 2004;81:1161-2; author reply 62-3.

Pozzobon SE, Lagares MA, Brum DS, Leivas FG, Rubin MI. Addition of recombinant human growth hormone to in vitro maturation medium of bovine oocytes. Reprod Domest Anim 2005;40:19-22.

Price CA, Carriere PD, Bhatia B, Groome NP. Comparison of hormonal and histological changes during follicular growth, as measured by ultrasonography, in cattle. J Reprod Fertil 1995;103:63-8.

Roberts AJ, Skinner MK. Estrogen regulation of thecal cell steroidogenesis and differentiation: thecal cell-granulosa cell interactions. Endocrinology 1990;127:2918-29.

Seifer DB, Gardiner AC, Ferreira KA, Peluso JJ. Apoptosis as a function of ovarian reserve in women undergoing in vitro fertilization. Fertil Steril 1996;66:593-8.

Sharara FI, Nieman LK. Identification and cellular localization of growth hormone receptor gene expression in the human ovary. J Clin Endocrinol Metab 1994;79:670-2.

Shimada M, Terada T. FSH and LH induce progesterone production and progesterone receptor synthesis in cumulus cells: a requirement for meiotic resumption in porcine oocytes. Mol Hum Reprod 2002;8:612-8.

Silva CC, Rosa HJ, Knight PG. Seasonal variations in the developmental competence of bovine oocytes matured in vitro. Vet Rec 2006;158:473-5.

Sirard MA, Richard F, Blondin P, Robert C. Contribution of the oocyte to embryo quality. Theriogenology 2006;65:126-36.

Soboleva TK, Peterson AJ, Pleasants AB, McNatty KP, Rhodes FM. A model of follicular development and ovulation in sheep and cattle. Anim Reprod Sci 2000;58:45-57.

Takeuchi T, Neri QV, Palermo GD. Construction and fertilization of reconstituted human oocytes. Reprod Biomed Online 2005;11:309-18.

Tervit H.R., Whittingham D, Rowson L. Successful culture in vitro of sheep and cattle ova. J Reprod Fertil 1972;30:493-97.

Zhao J, Taverne MA, van der Weijden GC, Bevers MM, van den Hurk R. Immunohistochemical localisation of growth hormone (GH), GH receptor (GHR), insulin-like growth factor I (IGF-I) and type I IGF-I receptor, and gene expression of GH and GHR in rat pre-antral follicles. Zygote 2002;10:85-94.