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Early development of ruminant embryos, autonomous process or the result of a positive dialog with surrounding maternal tissues?

Pascal Mermillod^{1,3}, Barbara Schmalz¹, Christine Perreau¹, Guillaume Tsikis¹, Emmanuelle Martinot¹, Amanda Cordova¹ & Yann Locatelli^{1,2}

ABSTRACT

Background: After artificial insemination and multiple ovulation and embryo transfer (MOET), *in vitro* production of embryos (IVP) represents the third generation of techniques aimed at a better control of animal reproduction. This technique involves four major steps: oocyte collection, oocyte *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* development of the resulting embryos (IVD). These different steps are now well established in domestic ruminant species (cattle, sheep and goat) although the variability of the number and quality of the oocytes collected and the low viability of frozen – thawed *in vitro* produced embryos still limit the large-scale use of this promising technology. Beyond the potential use of IVP in breeding schemes, this technique is also required for the establishment of new biotechnologies such as cloning and transgenesis. Additionally, the knowledge of oocyte and embryo physiology acquired through IVP techniques may stimulate the further development of other techniques such as marker assisted and genomic selection of preimplantation embryos and also benefit to assisted procreation in human being. This paper will discuss the possible function of maternal environment in the regulation of early development and the consequences of these functions for IVP, in view to improve IVP embryos viability.

Review: Comparisons between *in vivo* and *in vitro* produced embryos pointed out several differences in morphology, metabolism and gene expression. IVP embryos have a modified lipid metabolism, resulting in increased triglycerids accumulation, translating into different density. This altered lipid metabolism may account for differences in membrane structure and increased sensitivity to oxidative stress, resulting in lower cryoresistance of these IVP embryos. The identification of modified metabolic pathways leading to these lipidic disorders will provide clues for modification of culture conditions in view to restore normal lipid metabolism through appropriate precursors supplementation of the media. The natural embryo environment from fertilization to blastocyst stage is the oviduct. *In vivo*, oviduct epithelial cells provide ideal development support by regulating physico-chemical embryo microenvironment. Under *in vitro* conditions, the lack oviduct support may result in embryo exposure to toxic metabolites and oxidative stress. In addition, *in vitro* developing embryos may lack oviduct originated embryotrophic factors that regulate and stimulate early development *in vivo*. The use of co culture systems to mimic natural embryo environment *in vitro* may allow to improve embryo development, restore normal metabolic parameters, and increase embryo viability and cryoresistance. In addition, such co culture systems involving oviduct epithelial cells will help to identify critical development parameters and to point out potential embryotrophic factors.

Conclusion: *In vitro* embryo production is a promising technique for improvement of selection schemes and diffusion of genetic gain through safe exchanges of embryos. To allow a larger use of this technology, improvements should be obtained in management of oocyte collection and *in vitro* treatment to improve its quality and in embryo *in vitro* development systems. A better knowledge of interactions between the developing embryo and maternal environment will allow to improve *in vitro* systems to produce high viability embryos.

Keywords: Ruminants, embryo, oviduct, development, freezing

¹Institut National de la Recherche Agronomique (INRA), UMR6175, Physiologie de la Reproduction et des Comportements, Nouzilly, France.

² Muséum National d'Histoire Naturelle (MNHN), Parc de la Haute Touche, Obterre, France.

³ Corresponding author: INRA, Physiologie de la Reproduction et des Comportements, 37380 Nouzilly, France. (Pascal.Mermillod@tours.inra.fr), + 33 2 47 42 79 20 ; +33 2 47 42 77 43.

I INTRODUCTION

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I INTRODUCTION

The first attempts of early development of ruminant embryos *in vitro* resulted in a systematic block at the 8-16 Cell stage [72]. This stage of development corresponds to the maternal to zygotic transition (MZT) of gene expression in ruminant embryos [64]. The first successes of ruminant embryo development up to the blastocyst stage *in vitro* have been obtained by using co culture systems involving oviduct epithelial cells [10,16]. Further evolution of this technique involved use of new media especially designed to support early embryo development based on the composition of oviduct fluid, such as SOF medium [14] and bovine oviduct medium for embryo culture (BOME) [39] or sequential media fitted to changing embryo requirements [32]. However, it is generally accepted that *in vitro* produced blastocysts are less resistant to cryopreservation procedures than *in vivo* derived embryos [45,58]. In addition, a better knowledge of embryo – maternal interactions would provide interesting clues for deciphering early reproductive physiology and design improved methods for embryo *in vitro* production (IVP) in mammals [52]. Recent studies comparing bovine oocyte maturation, fertilization and embryo culture *in vivo* vs. *in vitro* have demonstrated that the origin of the oocyte is the main factor affecting blastocyst yield while the post-fertilization culture environment is critical in determining blastocyst quality, measured in terms of cryotolerance and relative transcript abundance whatever the origin of the oocyte [57,59]. Early development is often considered as an autonomous process, regulated by the embryo itself (maternal transcripts and embryo transcription activity). However, it is clear that embryo environment could act through specific sensitive windows to modulate embryo metabolism, gene expression pattern and morphology through epigenetically regulated mechanisms [68]. These data underline the key effect of maternal microenvironment on the success of early embryo development in terms of viability of these embryos. In addition, early embryonic mortality may explain up to 40% of reproductive failure [24], highlighting the need for a better knowledge of early embryo development regulation. This paper will review some aspects of the molecular and functional dialog between cleaving embryo and maternal surrounding, leading to efficient development and full embryo viability.

II OVIDUCT EFFECT ON EARLY DEVELOPMENT

The oviduct appears to play a crucial role in different aspects of early reproduction control like gametes final preparation and transport, fertilization, early embryo development [25]. The oviduct may regulate early development through several mechanisms: regulating metabolites in oviduct fluid to fit embryo requirements and metabolism capacities, protect embryos against oxidative stress by removing toxic compounds from the medium (negative action), regulate embryo cell proliferation through the secretion (positive action) of growth factors [4]. *In vivo* studies of these activities are difficult due to low accessibility of the oviduct (surgery requirement) and to the low quantity of biological material available. The use of *in vitro* models is required to allow the study of oviduct cell activities. These models rely on co culture of early embryos with oviduct cells. To avoid any bias, it is important to make sure that *in vitro* systems reflect more or less the physiological situation. Therefore, the origin of oviducts as well as the culture methods should be carefully designed to develop pertinent co culture systems [73].

Oviduct cell culture

Anatomically, the oviduct is a tube with a virtual lumen bordered by an epithelial cell layer, composed of ciliated and secreting cells. This epithelium is surrounded by conjunctive tissue and smooth muscle cells. Bovine oviduct epithelial cells (Boec) could be collected after enzymatic digestion using trypsin or collagenase. Alternatively, Boec could be collected mechanically, by scrapping the inner layer after longitudinal dissection of the oviduct or by gentle squeezing of the whole organ. Mechanical methods are less detrimental to sensitive structures like cilia and allow to collect larger numbers of cells [66]. Whereas enzymatic Boec collection provides mainly individual cell suspensions, mechanical methods allow to collect large sheets of epithelium. Once in culture, these sheets rapidly form vesicles bordered by a single layer of epithelial cells. The apical pole of the cells being outside of the vesicles, the cilia movements make the vesicles turning and moving in the

medium. These vesicles could be used for embryo co culture [63] but the use of monolayers allow a better standardization of the co culture conditions and easier access to functional study of OEC activities. Within few days, in the presence of serum, these vesicles attach to the culture support and cells proliferate to reach confluence within 5 to 7 days [48,67]. Numerous traditional media could be used to culture Boec (TCM-199, DMEM/F12, RPMI1640,...). Although TCM-199 is frequently used, media more fitted to embryo needs, like SOF, could be used alternatively. In our laboratory, we usually use TCM-199 medium supplemented with 10% FCS to initiate Boec cultures and drive them to confluence and then replace it with SOF supplemented with 5% FCS 24h before using them for embryo development support [60].

The origin of the oviduct (stage of the female cycle) could influence the cell differentiation status and molecular activities [3]. Even the side of the oviduct (ipsi- vs. Contralateral of previous ovulation) as well as the oviduct anatomical region (isthmus vs. Ampulla) could influence protein secretion pattern [2]. For example, oviduct specific glycoprotein (OGP), one of the major oviduct secreted proteins (OSP) is expressed by oviduct cells from ovulation and decreases after 3 to 4 days *in vivo* and it is more expressed in the ampulla, as compared to isthmus [6]. However, once in culture, cell dedifferentiation may at least partially equalize these differences in molecular activities [62]. The use of specific culture systems like coated culture inserts, favouring cell attachment and polarization, could help to maintain oviduct cell morphological and functional differentiation in culture [8]. As shown in Figure 1, Boec secreted proteins are more or less stable during the time period at which cells are generally used for supporting embryo development in co culture (day 5 to 13), although some slight variations between secreted protein patterns could be observed. It is interesting to note that proteins found in conditioned medium (lanes S5 and S13) do not correspond to major cellular proteins (lane Cell), indicating that they are not the result of passive release after cellular death.

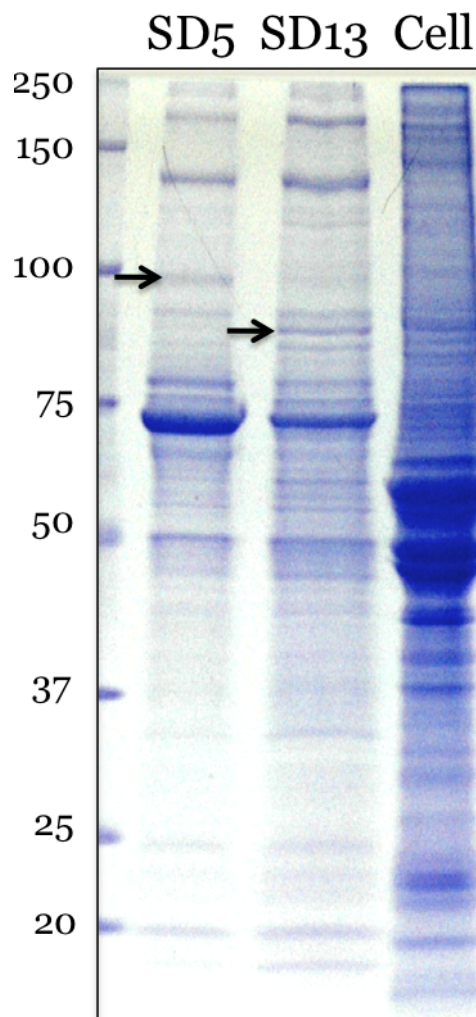


Figure 1. SDS-PAGE analysis of bOEC conditioned media collected after 5 (SD5) or 13 (SD13) days of culture, compared to cell lysate obtained on Day 13. Conditioned media were concentrated 80 fold by centrifugation on Vivaspin 5 kD cut off membranes concentrators (Sartorius, Paris, France) before analysis. The gel was stained with Coomassie brilliant blue. Arrows indicate differential protein bands between Day 5 and Day 13. Molecular weight markers are indicated on the left (kDa).

Oviduct – embryos co culture

It has been well established that the environment encountered by the embryo during its early development influences its gene expression at the blastocyst stage [43,51]. These differences translate into modification of several embryo parameters and may result in long-term alterations of embryo and offspring physiology [13,69]. Maternal environment induces higher cell number and allocation between trophectoderm and inner cell mass, modified embryo gene expression pattern and embryo metabolism. All together, these modifications result in higher viability and improved cryoresistance of the IVP embryos [57,59].

In our experiments (Table 1), bovine IVP embryos co cultured in 500 µl wells with Boec resulted in unaffected cleavage rate at 2 days pi, whereas the development to the blastocyst stage was improved in co culture as compared with embryos developed in medium alone in the same volume. This effect was particularly marked for embryos developing in TCM-199 medium. However, the level of development of co cultured embryos did not reach the rate observed for embryos developed in SOF oil overlaid droplets. In addition, co culture improved the total embryo cell number in both media (Table 1), as well as embryo *in vitro* survival after vitrification and thawing (Table 2). We observed similar development results for goat IVP embryos co cultured with goat OEC [60] or with Boec (not shown), indicating that the effect of oviduct cells is not species specific.

Table 1. Effect of culture system on the development of IVM/IVF bovine zygotes. Embryos were cultured by groups of 25 in oil overlaid droplets of SOF supplemented with 5% FCS under 5% O₂, 5% CO₂ and 90% N₂ at 38.8°C (Control) or in 4-well plates with 500 µl of SOF or TCM-199 medium with 5% FCS under 20% O₂, 5% CO₂ and 75% N₂ at 38.8°C in the presence (SOF Boec, TCM Boec) or absence (SOF, TCM) of bovine oviduct epithelial cells monolayers (Unpublished personal data).

Treatment	N	Cleavage rate Day 2 pi % (n)	Blastocyst yield		Cell Number Mean ±SEM(n)
			Day 7 pi%* (n)	Day 8 pi%* (n)	
Control	413	73 (303)	37 ^a (113)	51 ^a (155)	126.2 ^a ± 7.8 (34)
SOF	378	72 (273)	15 ^b (40)	27 ^b (74)	107.4 ^{a±} 12.4 (23)
SOF Boec	385	79 (306)	22 ^c (67)	41 ^c (127)	133.5 ^a ± 7.6 (67)
TCM	387	73 (281)	1 ^d (4)	10 ^d (27)	70.3 ^b ± 9.6 (8)
TCM Boec	389	73 (284)	16 ^{bc} (46)	28 ^b (80)	122.5 ^{a±} 11.1 (30)

* % of cleaved embryos

a, b, c, d Different superscripts within columns indicate statistical differences (P<0.05, chi-square test).

N: Total number of cumulus oocyte complexes, 7 replicates.

Boec: Bovine oviduct epithelial cells.

pi: Post insemination.

Table 2. Effect of culture system on the *in vitro* survival of vitrified bovine IVP embryos. IVP embryos were developed by groups of 25 in oil overlaid droplets of SOF supplemented with 5% FCS under 5% O₂, 5% CO₂ and 90% N₂ at 38.8°C (Control) or in 4-well plates with 500 µl of SOF or TCM-199 medium with 5% FCS under 20% O₂, 5% CO₂ and 75% N₂ at 38.8°C in the presence (SOF Boec, TCM Boec) or absence (SOF, TCM) of bovine oviduct epithelial cells monolayers. At 7 days post insemination, blastocysts were vitrified – thawed and placed in culture in 4-well plates in 500 µl of SOF supplemented with 5% FCS for 48h. Survival (reexpansion and/or hatching) was evaluated at different times of post thawing culture (5, 24 and 48h) (Unpublished personal data).

Treatment	Thawed embryos (n)	Survival rate post-thawing			
		5h% (n)	24h% (n)	48h% (n)	48h*% (n)
Control	67	43 ^{ax} (29)	18 ^{bx} (12)	9 ^{bx} (6)	10 ^{bx} (3)
SOF	32	22 ^{ay} (7)	22 ^{ax} (7)	0 ^{by}	0 ^{by}
SOF Boec	51	59 ^{az} (30)	59 ^{az} (30)	39 ^{az} (20)	30 ^{az} (9)
TCM	10	17 ^{ay} (2)	0 ^{by}	0 ^{by}	0 ^{by}
TCM Boec	33	67 ^{az} (22)	67 ^{az} (22)	54 ^{az} (18)	41 ^{az} (9)

* % of hatched embryos/number of blastocysts 5h after thawing.

Within lines, values with different letters (a–c) differ significantly (P < 0.05, Chi-square).

Within columns, values with different letters (x–z) differ significantly (P < 0.05, Chi-square).

One of the possible effect of somatic cells in co culture is the regulation of gas partial pressure in the medium and particularly the diminution of oxygen [14,42]. In our co culture system (Table 3), we observed that the rate of embryo development to the blastocyst stage was significantly reduced under 20% O₂ and that this effect was suppressed in co culture. In addition, the rate of development in co culture under 20% O₂ in SOF or TCM-199 was higher than the development in the same media under 5% O₂ without co culture, indicating that Boec exert other effects in addition to the control of oxygen level.

Table 3. Effect of culture system on the development of IVM/IVF bovine zygotes. Embryos were cultured by groups of 25 in oil overlaid droplets of SOF supplemented with 5% FCS (Control) or in 4-well plates with 500 µl of SOF or TCM-199 medium with 5% FCS in the presence of 5% O₂, 5% CO₂ and 90% N₂ (group 5% O₂). In group 20% O₂, embryos were cultured in the same media in the presence or absence of bovine oviduct epithelial cells monolayers under 20% O₂, 5% CO₂, 75% N₂ atmosphere (Unpublished personal data).

	Treatment	n	No. of cleaved oocytes Day 2 pi % (n)	Blastocyst yield		No. of Hatched blastocyst Day 8 pi % (n)
				Day 7 pi%* (n)	Day 8 pi%* (n)	
5% O ₂	Control	171	59 (101)	22 ^a (22)	30 ^{ac} (30)	30 ^{ac} (34)
	SOF	161	65 (104)	19 ^a (20)	19 ^a (20)	40 ^{ac} (23)
	TCM	158	73 (116)	5 ^b (6)	10 ^b (11)	0 ^b
20% O ₂	SOF	137	65 (89)	6 ^b (5)	6 ^b (5)	0 ^b
	TCM	170	63 (107)	4 ^b (4)	4 ^b (4)	0 ^b
	SOF Boec	187	67 (126)	24 ^a (30)	35 ^c (44)	43 ^a (19)
	TCM Boec	185	66 (122)	17 ^a (21)	27 ^{ac} (33)	23 ^c (7)

* % of cleaved/embryos

a, b, c, d Different superscripts within columns indicate statistical differences (P<0.05, Chi-square).

n: Total number of cumulus oocytes complexes, 3 replicates.

Boec: Bovine oviduct epithelial cells.

pi: Post insemination.

We used the goat model to confirm increased viability of vitrified – thawed IVP embryos after OEC co culture [60]. Results are represented in Table 4. Vitrified – thawed IVP embryos displayed a very low survival (9% of kids born) as compared with fresh ones (62% of kids born). Survival of IVP embryos was increased after Goec co culture (33% of kids born) but remained significantly lower compared to fresh ones, indicating that co culture tend to restore *in vivo*-like development conditions but that the method remains to be improved. Any further improvement of IVP technique in terms of quality, viability and cryoresistance of the embryos produced will require a better knowledge of the embryo maternal communication regulating early development [52].

Table 4. Effect of culture system on the viability of goat IVP embryos. Embryos were developed in SOF supplemented with 5% FCS with our without goat oviduct epithelial cells support. They were then vitrified at the blastocyst stage (Day 7 pi), two embryos per straw [20]. Straws were thawed and their content was transferred to synchronized recipients. Pregnancy rate was evaluated at Days 34 and 90 by echography and kiddings were recorded. Modified from [60].

Treatment	Recip. N	Emb. n	Pregnant Day 34n (%)	PregnantDay 90n (%)	Kidding n (%)	Kids born n (%)
SOF Fresh	13	26	12 ^a (92)	12 ^a (92)	12 ^a (92)	16 ^a (62)
SOF vit.	29	58	6 ^b (21)	4 ^b (14)	4 ^b (14)	5 ^b (9)
gOEC vit.	18	36	13 ^a (72)	10 ^c (56)	10 ^c (56)	12 ^c (33)

a,b,c Values with different superscripts in the same column are significantly different (P<0.05, Chi-square).

gOEC: goat oviduct epithelial cells.

pi: Post insemination.

Oviduct secreted proteins

The knowledge of embryo – maternal communication in the oviduct relies on the identification of oviduct secreted proteins (OSP). Many OSP have been identified already [18,28]. In our co culture system, we investigated the level of expression in oviduct cells at different culture times of transcripts encoding for some of these proteins involved in different mechanism that can explain early development support. The expression of these genes were compared in freshly collected cells (F0), at Day 5 of culture, representing the beginning of confluence and the moment of co culture initiation and at Day 13, representing the end of co culture, for cells cultured in SOF or TCM-199 with or without developing embryos.

We quantified the mRNA two antioxidant enzymes: Copper, Zinc Superoxide Dismutase (Cu,Zn-SOD) and Phospholipid Hydroperoxide Glutathione Peroxidase (GPx-4). Indeed, Cu,Zn-SOD scavenges superoxide radicals and GPx-4 reduces lipid hydroperoxides and H₂O₂ the by-product of Cu,Zn-SOD action [34,44,53]. As shown in Figure 2, Boec expressed continuously Cu,Zn-SOD during all the culture period. This result is in accordance with those obtained in vivo showing constant Cu,Zn-SOD Mrna level all along the oviduct and a constant enzymatic activity throughout the oestrus cycle [33]. GPx-4 transcript has been detected throughout the culture with a significant increase at confluence (Day 5) followed by a steady level up to the end of the culture (Day 13). GPx-4 is probably stimulated by oxidative stress due to high oxygen concentration (20%) during initial Boec culture. Indeed, oxygen tension in the oviduct is approximately 5-6 % [44]. GPX-4 is the only GPx family member with the ability to reduce lipid hydroperoxides bound to cell membrane. GPx-4 mRNA expression in bovine oviduct is up-regulated at the periovulatory period [34] which is characterized by increased lipid synthesis [22]. Then, in our experiments, the beneficial effect of Boec on blastocyst rate and quality may be at least partly explained by detoxification of highly diffusible ROS by Boec secreted SOD and GPx-4 antioxidant enzymes. However, blastocyst rates were always higher in Boec co-culture system even under a reduced oxygen concentration, suggesting that Boec may have other ways to support blastocyst formation. This additional effect could be mediated by growth factors.

In our experiments, control embryos cultured in reduced volume of SOF (1 µl per embryo) showed higher blastocyst and hatching rates as well as cell number per embryo as compared with those produced in a larger volume of SOF (500 µl). These results are consistent with those obtained in mice by [53] showing that embryos cultured singly had a lower development rate as compared with those cultured in groups in the same volume of medium (25µl). They also reported that the development of embryos cultured singly was markedly improved by the addition of growth factors (EGF, TGF-α and TGF-β), indicating that these growth factors may mediate the cooperation between embryos. A similar collaboration between developing embryos was also reported in cattle [12]. Growth factors secretion may at least partly account for the positive effect of Boec observed in our study. In view to test this hypothesis, we quantified the Mrna of some growth factors known to have a role in embryo development such as insulin-like growth factors IGF-I and -II, transforming growth factor-α and -β1 (TGF-α and TGF-α1), and basic fibroblast growth factor (FGF2).

TGF-α mRNA significantly decreased throughout the culture and particularly after 13 days of culture whatever the medium. This decrease was partly abolished in the presence of embryos. TGF-α expression was found in the inner cell mass cells and antisense oligonucleotides against EGF receptor attenuated embryo cell proliferation [5]. Therefore, TGF-α produced by inner cell mass may stimulate trophectoderm proliferation through EGF receptors in a paracrine manner. Oviduct produced TGF-α may enhance this paracrine mechanism.

FGF2 and TGF-α1 expression in Boec cultured during 13 days with or without embryos did not differ. FGF2 is an activator of protein synthesis and a potent mitogen [35]. The exposure of bovine embryos to media containing FGF2 increased in vitro development from the morulae and early blastocyst stages but not at earlier stages [36]. Furthermore, basic fibroblast growth factor (bFGF), a related growth factor is a maternal transcript in the bovine oocyte. Maternal bFGF transcripts are detectable in cleaved embryos up to the eight-cell stage [70]. Therefore, embryos may lack autocrine FGF family factors from the morula stage and oviduct secretion may then replace this autocrine production. Transforming Growth Factor β stimulates cell proliferation and induces a variety of other cellular effects [27]. TGF-β1 has been reported to improve early development in the mouse, in conjunction with EGF [53] and in the bovine [41]. In bovine embryos, Mrna for both TGF beta type I and II receptors were detected throughout preimplantation development [61]. All together, these results suggest both autocrine and paracrine activities for this growth factor.

The insulin-like family of growth factors is made up of insulin-like growth factors (IGF)-I and -II, IGF binding proteins (IGFBP) and IGF receptors [27]. In our study, IGF-I and IGF-II transcripts have been detected throughout the

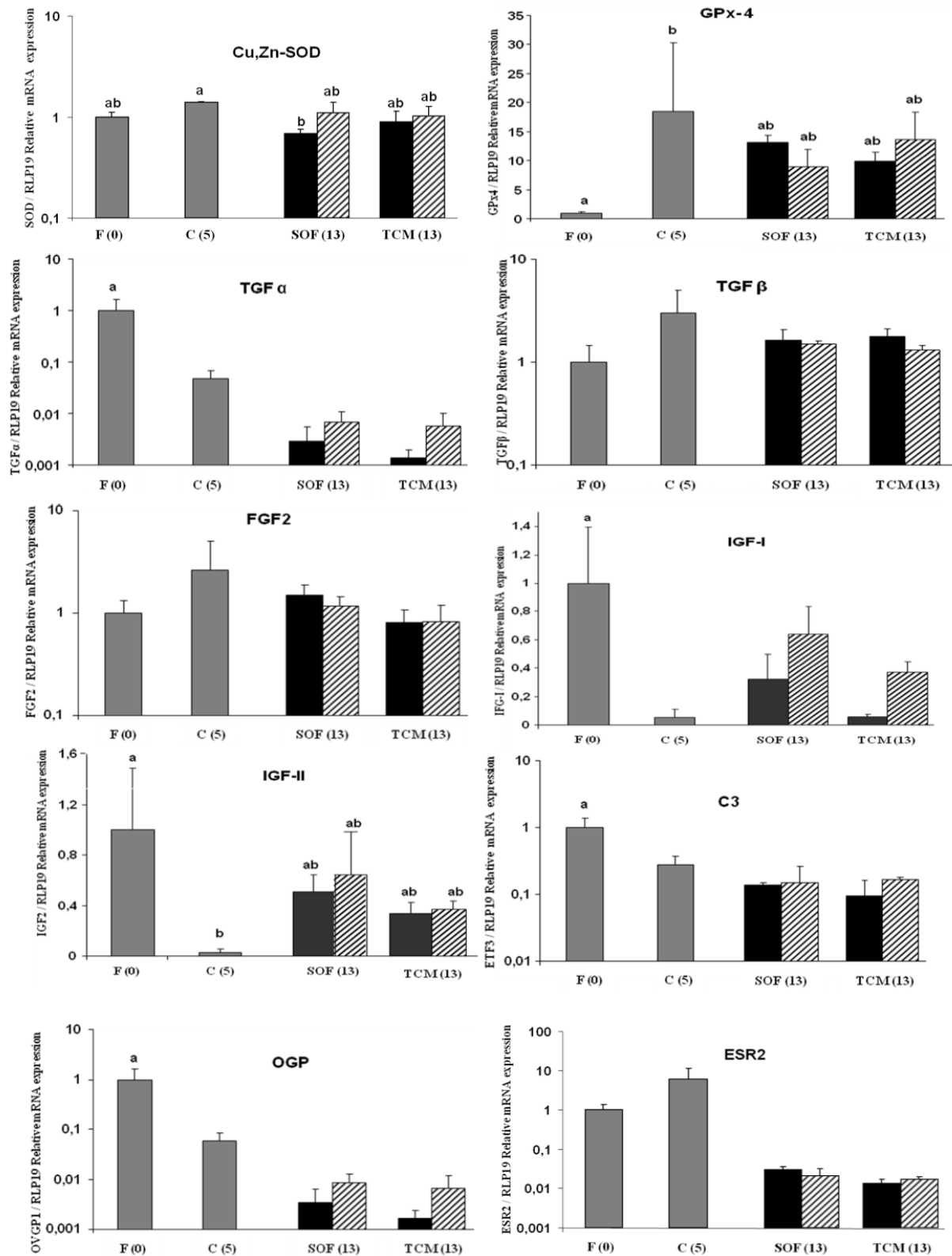


Figure 2. RT-qPCR measure of several marker genes expression in bOEC during culture period. Transcript abundance was determined at different culture times: F(0) for freshly isolated cells, C(5) for cells at confluence on Day 5, SOF (13) for cells cultured during 13 days in SOF, TCM (13) for cells cultured during 13 days in TCM-199 in the presence (striped bars) or absence (black bars) of embryos cultured from Day 5 to Day 13 on bOEC monolayer. BOEC isolated from three individuals were analysed. Different superscript letters indicate significant differences between sampling time points ($P < 0.05$). The level of expression is represented relatively to RPL19 housekeeping gene and to level observed in F(0).

culture with a significant decrease at Day 5, indicating that when cells were in proliferation, transcriptional activity for these two genes was low. In contrast, when cells reached confluence, transcriptional levels were increased again up to the end of culture. Although it was not significant, it seems that in TCM-199, the presence of embryos stimulated IGF-I mRNA expression. These results are in agreement with previous data showing that IGF-I was stimulating the *in vitro* development of bovine embryos [46], as well as blastocyst formation and cell proliferation in the inner cell mass of human embryos [40]. IGF-I stimulated uptake of both amino acids and glucose and antibodies directed against IGF-I receptor completely inhibited these effects in human [40] and in bovine [47], suggesting that preimplantation development *in vitro* is stimulated by IGF-I. Furthermore, [54] and [75], failed to detect IGF-I transcript in mouse and bovine embryos, indicating that this growth factor is probably produced by maternal environment *in vivo*. In a previous study, [56] has shown a significantly higher IGF-II transcript level in *in vivo* derived blastocyst as compared with *in vitro* produced ones. Then, it is possible to hypothesize that in our co-culture system, IGF-II has been implicated in the improvement of blastocyst rates, in SOF and in TCM.

Complement C3 and OGP mRNA levels decreased significantly between culture initiation and confluence and were maintained at a steady level thereafter, with a tendency of stimulation of transcription in the presence of embryos for OGP. C3 protein is expressed in the porcine [7] and human [38] oviduct. Previous studies have demonstrated that C3 enhanced trophoblast development, blastocyst size and hatching rate in mouse [74]. OGP has been shown to increase sperm capacitation and its ability to fertilize bovine oocytes [29], the cleavage and blastocyst rates in ovine [31] but no effect was found when OGP was added after IVF [23].

Finally, C3-deficient mice are fertile and can produce offspring with normal appearance [71] and null mutation of OGP gene did not affect mice fertility [1] signifying that the role of these genes on embryo development is not crucial in rodent reproduction.

Other authors [55] found the same down regulation for OGP transcript suggesting a clear dedifferentiation of primary bovine oviduct cells *in vitro* in a static culture system. The addition of hX Γ increased bovine embryonic development in co-culture with bovine oviduct epithelial cells but not in medium alone. This effect was abolished when OGP or LH receptor synthesis were inhibited [49]. Recently, OGP has been shown to take part in sperm-oocyte interaction prior to fertilization by modulating zona pellucid hardening [9]. C3 expression is estradiol regulated in pig oviduct [7]. In our co-culture system, Boec were not treated by exogenous hormones. Then, the decrease of C3 and OGP expression between fresh cells and confluence may be explained by the lack of hormonal stimulation. OGP expression has been reported to be stimulated also by estradiol in pig oviduct [6] and in Boec [65]. Steroid receptors are expressed throughout culture of oviduct cells in our system, indicating that these cells could be responsive to such stimulus from exogenous origin or from the embryos.

In our study, osteopontin mRNA is expressed throughout culture and increased significantly after confluence. The incubation of *in vitro* matured porcine oocytes with osteopontin during fertilization and development increased blastocyst rate and decreased apoptosis and fragmentation [21]. Although osteopontin have obvious effects on gametes [19], it may also directly affect zygote and blastocyst formation [50] possibly by reducing apoptosis to enhances embryo quality which translates into higher cell count and improved cryotolerance. Further examinations will be carried out to establish direct impact of osteopontin on embryo development and cryoresistance.

These data indicate that Boec cultures display a gene expression profile compatible with the regulation of embryo early development through different mechanisms of growth factors stimulation. This observation is in agreement with the ability of Boec conditioned media to support embryo development in the absence of cells and with the fact that this activity could be suppressed by decomplexation treatment or by ultrafiltration through a 10 kDa ultrafiltration membrane [48].

III GAMETES – EMBRYO REGULATED OVIDUCT ACTIVITIES

Despite numerous studies of the effect of oviduct cells on early embryo development, only few data are available on the ability of embryos to modulate oviduct activities. *In vivo* studies are difficult given the scarce biological material available. In addition, the embryo may exert a local action, specifically on immediately surrounding cells, increasing the difficulty of any possible approaches.

The oviduct is a highly regulated milieu. At ovulation, the released of follicular fluid with high steroids contents together with the changing hormonal status of the female are influencing oviduct physiology. In addition,

spermatozoa have been shown to influence oviduct cell transcription activity in mice [11], prostaglandin secretion in cattle [30] and protein secretion in pig [17]. *Cumulus* oocyte complexes may also modulate oviduct cells activities [15] or modulate the effect of developing embryo on oviduct cells gene expression activity [26].

The observation of gene expression differences between ipsi- vs. contralateral oviduct seems to indicate that oviduct is sensitive to the presence of gametes and/or embryos. However, E2 sensitive genes (OGP, GPX4) specifically stimulated by steroids release at ovulation may explain partly the differences observed between oviducts containing oocyte – embryo or not. However, specific action of the presence of embryos on oviduct gene expression patterns have been observed in mice [37].

Suitable *in vitro* models will allow the development of high throughput transcriptomic and proteomic approaches that will help to decipher oviduct – early embryo interactions and to progressively build an integrated model of this dialog that will help to develop more suited methods of embryo development *in vitro*.

IV CONCLUSIONS

Increasing lines of evidence suggest a molecular communication between early developing embryo and maternal tissues in the oviduct. The functional significance of this dialog remains to be fully established. *In vitro* systems may mimic these interactions and allow the use of up-to-date potent molecular tools to identify oviduct – embryo communication actors and functions. This new knowledge of early development regulation will open the way for increased comprehension of basic mechanisms of cleavage and differentiation regulation in early embryo and provide new tools for improvement of reproductive technologies such as IVP or cloning.

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