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Relationship between IFN-τ Production by Bovines Embryos Derived *Ex Vivo* and Completely Produced *In Vitro*

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1. Introduction

Interferon-tau (IFN-τ) is secreted by the mononuclear cells of the primitive extra-embryonic trophoblast (Farin et al. 1989; Guillomot et al. 1990) which will become the main part of the future placenta, in ruminants. This cytokine is constitutively produced during the short period of the conceptus periimplantation (Martal et al. 1979; Godkin et al. 1982; Hansen et al. 1988; Charlier et al. 1989). It plays an essential role for maternal recognition of pregnancy, particularly allowing the maintenance of the *corpus luteum* and its progesterone secretion (Spencer et al. 2004). Indeed, intrauterine injections of recombinant IFN-τ extend the progesterone secretion by inhibiting pulsatile uterine secretion of luteolytic prostaglandin F-2α (Martal et al. 1990, 1998; Ott et al. 1993; Meyer et al. 1995). IFN-τ downregulates the expression of endometrial oxytocin receptors concentrations (Spencer and Bazer 1996; Mann et al. 1999). In early pregnancy, IFN-τ constitutes therefore a major signal in ruminants. Thus, it plays a role of both cytokine and reproductive paracrine hormone (Roberts et al. 1992; Bazer et al. 1994; Martal et al. 1997). In addition, IFN-τ exhibits potent antiviral and antiproliferative activities (Fillon et al. 1991; Pontzer et al. 1991, 1997; Bazer et al. 1994; Derreuddre et al. 1996).

The regulation of IFN-τ secretion remains poorly understood (Martal et al. 1998; Yamagushi et al. 2001; Demmers et al. 2001; Ezashi et al. 2001; Stewart et al. 2002; Spencer and Bazer 2002). Some growth factors and cytokines are implicated in the positive control of IFN-τ secretion such as Insulin-like Growth Factor (IGF-I) and IGF-II (Ko et al. 1991), Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) and Interleukine-3 (Imakawa et al. 1993, 1995; Emond et al. 2004). Indeed, these factors are secreted by trophoblast and endometrium in early pregnancy (Mathialagan et al. 1992; Vogiagis et al. 1997; Martal et al. 1997, 2002).

The induction of IFN-τ expression appeared genetically determined because IFN-τ secretion has been evidenced from hatched blastocysts completely produced *in vitro* (Hernández-Ledezma et al. 1992, 1993; Stojkovic et al. 1995). Therefore, the uterine environment is not necessary for the induction of IFN-τ expression but it could play an essential role in the control of IFN-τ secretion. Paternal genotype was a significant determinant of the embryo's ability to develop the blastocyst stage and of subsequent IFN-τ secretion (Kubish et al. 2001a). Besides, there is some evidence that the presence of other blastocysts could increase IFN-τ secretion (Larson and Kubish 1999).

Generally, the evaluation of the development potential of embryos today still depends on subjective morphological examination (Lindner and Wright, 1983; Buttler and Biggers 1989; Shamsuddin et al. 1992, Massip et al. 1995). However, the development stage and quality of the embryos significantly influence the IFN-τ secretion. Some authors have proposed that the produced amount of IFN-τ could be a useful objective indicator of embryo quality (Hernandez-Ledezma et al. 1992, 1993). But others, considering that the age of blastocysts formation *in vitro* exhibits significant effects on the IFN-τ production, have suggested a negative relationship between early IFN-τ production and blastocysts competence for embryonic development (Kubish et al. 1998, 2004).

The aim of this study was therefore to compare the IFN-τ secretion after hatching in bovine blastocysts of good homogeneous quality wether they are produced *in vivo* or completely *in vitro.*

2. Materials and methods

2.1 Production of *in vitro* bovine blastocysts

2.1.1 *In vitro* maturation of oocytes

Unless otherwise indicated, all chemicals in this study were purchased from Sigma-Aldrich, (Saint Quentin. Fallavier, France). Ovaries from Prim Holstein cows were quickly collected after death in a local slaughter-house and transferred to the laboratory into a saline solution with 0.9% (w/v) NaCl at approximately 35°C. The largest interval between animal killing and oocyte conditioning was 3 hours. Cumulus-oocyte complexes (COCs) were recovered by aspiration of follicles of 2-8 mm in diameter using a 18 gauge needle under vacuum pressure of approximately 50 mm Hg. The COCs were collected into Hepes-buffered tissue culture medium 199 (TCM 199 ref. M-7528) supplemented with 0.4% (w/v) BSA (Cohn Fraction V, ref. 9647). Before *in vitro* maturation, COCs were assessed morphologically: only those which displaid a compact and non-atretic *cumulus oophorus-corona radiata* with an oocyte exhibiting homogeneous cytoplasm were chosen for further *in vitro* culture. Selected COCs were washed thoroughly in TCM 199 (ref. M-4530) plus 10% (v/v) fetal calf serum (FCS, Life Technologies, Inc., Grand Island, NY USA). The maturation of about 60 COCs batches were achieved in 500µl TCM199 (ref. M-4530) supplemented with 10% FCS, and 10ng per ml EGF (ref. E-4127) with 100 µg/ml gentamicin (ref. G-1264), for 24 h at 39°C under humidified 5% CO_2 in air.

2.1.2 *In vitro* fertilization

Spermatozoa were prepared from frozen-thawed semen of a sole bull (Prim Holstein breed, electronic number: 44 13 835058) that had been characterized as suitable for *in vitro* fertilization in our laboratory. The contents of two 0.25 ml straws (each containing approximately 10^6 *spermatozoa* per ml) were layered upon a Percoll discontinuous gradient

(Hasler et al. 1995). Motile *spermatozoa* were collected after centrifugation at approximately 700g for 30 min, at room temperature. Then they washed in Hepes-buffered Tyrode's albumin lactate pyruvate medium (Talp) (Parrish et al. 1986) and pelleted by centrifugation at approximately 200g for 10 min at room temperature. Meanwhile, COCs were transferred to another four-wells dish containing 250µl of *in vitro* fertilization Talp supplemented with 0.01 mmol/l heparin (ref. H-3149), 0.2 mmol/l penicillamine (ref. P-4875), 0.1 mmol/l hypotaurine, 0.1 mmol/l epinephrine (ref. E-4250) and 6mg/ml fatty acid-free BSA. (ref. A-8806) Insemination was performed to get the final concentration of 2 x 10^6 *spermatozoa*/ml to fertilize the oocytes. Plates were incubated 5% CO_2 in humidified air at 39°C.

2.1.3 *In vitro* culture

After 20 hours, the *cumulus* cells were removed from the presumptive zygotes by intermittent gentle shaking for 2 min. Presumptive zygotes were then washed 4 times in synthetic oviduct fluid medium SOF (Tervit et al. 1972) according to Holm et al. (1999) containing 0.7 mM Na-pyruvate, 4.2 mM Na-lactate, 2.8 mM myo-inositol, 0.2 mM glutamine, 0.3 mM citrate, 30 ml/l essential amino acids mixture, 10 ml/l non-essential amino acids, 50 µg/ml gentamycin and 10% (v/v) fetal calf serum (SOF-FCS). All media were passed through a 0.2 µm membrane filter and were equilibrated overnight in an incubator at 39°C in 5% O_2 in humidified air. Presumptive zygotes were cultured in groups of 10-12 in 20µl droplets of SOF-FCS, culture was performed in a humidified atmosphere of 5% CO_2, 5% O_2 and 90% N_2 at 39 C. Cleavage was examined once between days 2 and 3, embryo development rate was evaluated on day 7 post insemination. On day 7, excellent and good quality blastocysts of grade 1 (according to the International Embryo Transfer Society standards-IETS, Stringfellow and Seidel, 1990) were selected for this study.

2.2 Production of *in vivo* bovine blastocysts

The oestrous cycles of french Prim Holstein donor cows were synchronised with an implant of 3mg Norgestomet-3.8 mg valerate oestradiol (Crestar® INTERVET S.A.- Holland) for 9 days. The oestrous reference was observed 48-68 hours after removing the implant. The cows were superovulated 8 to 12 days after oestrous with p-FSH (Stimufol® Merial SAS - France). A total dose of 450µg p-FSH was administered (8 i.m. injections with decreasing doses for 4 days) and an analogue of $PGF_{2\alpha}$ (500 µg i.m.; Cloprostenol Estrumate® Schering Plough Vet. S.A.) was injected at the moment of the 5th p-FSH injection. Two artificial inseminations (IA) were performed 12 and 24 hours after starting oestrous (day 0). The frozen-thawed semen for IA of embryos donor cows was the same that used for the production of *in vitro* bovine blastocysts. Generally, the *in vivo* embryos have a small chronological delay in their development in comparison with the *in vitro* produced embryos, because of the lack of precision in the moment of fertilization. For this reason the cows were collected at day seven and a half after IA. Embryos were recovered by uterine flushing with PBS (phosphate buffer saline) supplemented with 0.04% (w/v) BSA fraction V (same previous reference). Then embryos were washed 4 times in PBS supplemented with 0.4% (w/v) BSA fraction V. The viability of embryos was estimated according to IETS. Only excellent and good quality blastocysts grade I were selected for this study.

2.3 Determination of IFN-τ antiviral activity

Embryo culture media were assayed for antiviral activity by use of a cytopathic effect titration assay that used a bovine kidney cell line designated Madin and Darby Bovine

Kidney (MDBK cells) challenged with vesicular stomatitis virus. The extent of cell protection against viral lysis was compared with that of a diluted human INF-α (Alpha therapeutics Corporation Los Angeles, CA) used as a standard. This standard possesses 8.8 International Reference Units of activity per laboratory unit (u) when compared to human INF-α (Leukocyte IFN standard: GA-23-902-530 reference sample, obtained from NIH Bethesda MD, USA.)

The sensitivity of the IFN-τ assay was 0.012 u /100µl. Titrated IFN were expressed in laboratory units/embryo/24h, where one unit was equivalent to that amount of IFN that protected 50% of the MDBK cells monolayer from lysis upon exposure to the cytopathic effects of the virus. Immunoneutralization assays were performed with polyclonal antisera raised against IFN-τ. Briefly, IFN samples were serially diluted in a given dilution of anti-INF antisera (L'Haridon 1991). The excess of immunoneutralization results was determined from the IFN-τ activity compared with that of untreated controls.

2.4 Determination of the numbers of inner mass (ICM) and trophectoderm (TE) cells of blastocysts produced *in vitro* and *ex vivo.*

Equal numbers of hatched blastocysts (per treatment group, completely *in vitro* and *ex vivo* origin's) on day 10 after fertilizations (72h-cultured in individual droplets), were subjected to differential cell staining with fluorocrome using a modification of the procedure described by Stojkovic et al. (1997). Briefly, hatched blastocysts were washed several times in 0.1M phosphate-buffered saline (PBS, pH 7.2) containing 0,2% BSA. Hatched blastocysts were incubated in a 1:10 dilution in PBS of antiserum raised in rabbit against recombinant ovine IFN-τ (Martal et al. 1998) for 45 min at 39ºC in a humidified atmosphere of 5% CO_2 in air. Subsequently, embryos were washed five times in PBS supplemented with 5% (v/v) guinea-pig complement (ref. 72122 Bio-Mérieux S.A.) and 50µg/ml propidium iodide (ref. P4170) for 45 min at 39ºC in a humidified atmosphere of 5% CO_2. Blastocysts were then washed in PBS and placed in cold absolute ethanol containing 25µg/ml fluorochrome bisbenzimide (ref. B-2883) 30 min at 4°C. Finally embryos were washed in absolute ethanol, mounted in undiluted glycerol. Specific fluorescence was examined by confocal microscope (Zeiss, Paris, France) with mercury lamp under transmittance illumination and an UV exitation filter of 365 nm and a barrier filter of 420nm.

2.5 Experimental design

The aim of this experiment was to compare IFN-τ production of blastocysts *in vitro* culture from days 7 to 12 according to their origin (from completely *in vitro* and *in vivo*) and their quality. For this purpose, excellent and good quality blastocysts, developed over a stage of 7 days of *in vitro* and *in vivo* origin were sorted and cultured in individual droppers in 50µl of SOF- FCS for 5 days under paraffin oil. Plates were incubated in 5% CO_2, 5% O_2 and 90% N_2 at 39 °C in humidified air at 39°C. During the time of *in vitro* culture each equal volume of fresh medium was then added back to each culture every 24 hours and kept refrigerated at 4°C, and the blastocysts morphologically evaluated as indicated by Lindner and Wright (1983). Briefly, embryos were evaluated and graded by morphological criteria as follows: excellent (spherical, symmetrical with cells of uniform size, color and texture), good (a few imperfections such as few extruded blastomeres, irregular shape and presence of vesicles, >50% of extruded dark cells, cells of different size), fair (several imperfections, several extruded blastomeres, high percentage of extruded dark cells, too many cells of different size.)

2.6 Statistical analysis

Data on embryos origin (*in vitro* and *ex vivo*) and the quantity of IFN-τ released in a 24-h culture period and quality embryos, from day 7(Day 1 was the day of insemination) today 12, were evaluated in a 3 X 3 factorial arrangement in a complete randomized block design. The general linear models procedure (SAS Institute Inc.) was used by analysis of variance and regression coefficient least squares means. Differences were considered significant at $p<0.05$ (Little et al. 1996).

3. Results

3.1 Obtaining homogeneous expanded blastocysts of *in vitro* and *in vivo* origins on day 7

The results of the processes to obtain derived *ex vivo* and completely produced *in vitro* embryos are described in table 1. In five replicates, a total of 559 oocytes was chosen for production of completely *in vitro* bovine blastocysts. The average percentage of cleavage was 83% and the average blastocyst per oocyte rate of these trials was 43% (range 29-53%). Only day 7 grade I (excellent and good quality) blastocysts (n=46, about 29% of the total blastocysts on day 7) were removed and cultured in individual droppers. For production of *in vivo* bovine blastocysts, a total of 11 cows were treated. An average of 7.9 embryos were recovered per cow on day 7, only grade I (excellent and good quality) blastocysts (n=40 about 46% of total embryos) were recovered and cultured in individual droplets.

In vitro	Oocytes n	Cleavage day 2 % (n)	Day 7				Day 8			Total % (n)
			Blastocysts % (n)	Expanded Blastocysts % (n)	Total % (n)	**Embryos quality I used**	Blastocysts % (n)	Expanded Blastocysts % (n)	Total % (n)	
	559	83 (464)	17 (98)	8 (44)	25 (142)	**44**	6 (32)	17 (94)	23 (126)	48 (268)

In vivo	Superovulated cows n	Recovered embryos % (n)	Recovered embryos on day 7				
			Morulas % (n)	Blastocysts % (n)	Expanded Blastocysts % (n)	Degenerated Embryos % (n)	**Embryos quality I used**
	11	100 (87)	18 (16)	28 (24)	32 (28)	22 (I9)	**40**

Table 1. Description of the processes to obtain bovine *ex vivo* and completely produced *in vitro* embryos.

3.2 Effect of embryos origin on the IFN-τ production (Table 2).

On day 8, after 24h-culture,96% blastocysts (n=46) completely produced *in vitro* (group A) and 100% *ex vivo* blastocysts (group B) (n=40) secreted <54 pM IFN-τ. After 48h-culture, 41% group A (n=19) had an average IFN-τ production of 143 ± 24 pM versus 85 ± 12 pM for group B (53%, n= 21) ($p< 0.01$). The rest of group A (57%, n=26) and those of group B (47%, n= 19) both produced <54 pM IFN-τ.

On day 10, after 72 h-culture 63% group A (n=29) exhibits an average IFN-τ production of 491 ± 128 pM versus 216 ± 37 pM for group B (58%, n=23) (NS). The rest of group A (19%

n=9) and those of group B (13%, n=5) both secreted <54 pM IFN-τ. After 96 h-culture 57% group A (n= 26) had an average IFN-τ production of 499 ± 135 pM versus 353 ± 93 pM for group B (53%, n=21) (NS). The rest of group A (6% n=3) and those of group B (8%, n=3) both produced <54 pM IFN-τ.

Time culture (hours)	***In vitro* (Group A)**		***Ex vivo* (Group B)**	
	% (n)	IFN-τ concentration mean ± sem (pM)	% (n)	IFN-τ concentration mean ± sem (pM)
24	96(44) 4 (2)	< 54 93 ± 0	100 (40)	<54
48	41 (19) 57 (26)	143 ± 24 b < 54	53 (21) 47(19)	85 ± 12 a < 54
72	63 (29) 19 (9)	491 ± 128 a <54	58 (23) 13 (5)	216 ± 37 a < 54
96	57 (26) 6 (3)	499 ± 135 a < 54	53 (21) 8 (3)	353 ± 93 a < 54
120	48 (22) 13 (6)	559 ± 136 a < 54	50 (20) 10 (4)	333 ± 75 a < 54

Table 2. Comparative secretion of IFN-τ by bovine hatched blastocysts derived *ex vivo* and completely produced in vitro after 24h of individual culture in medium droplets from days 7 to 12.
Different superscripts within columns (a vs b) indicate significant differences ($p<0.05$).

The average of accumulated IFN-τ production per embryo from days 7 to 10 (72 hours) of group A was 550±129 pM IFN-τ (n=38) versus group B 277±41pM IFN-τ (n=28) ($p<0.05$) figure 1. At this stage of culture, on day 10 after fertilization, the morphological aspects of completely produced *in vitro* and *ex vivo* embryos were comparable as it is illustrated in the photographies 1 and 2 after examination by confocal microscope. The cellular counts of the group A embryos showed 50±17 cells (n=6) for inner cell mass(ICM) and 62±14 (n=4) for those of group B. The trophoblast cells were 369±135 (n=6) for group A and 393±98 (n=4) for group B, respectively. At this stage, the number of total embryonic cells were 418±152 for group A and 455±112 for group B, respectively. No statistically significant difference of the cells number between the groups A and B were observed, suggesting that the difference of IFN-τ production between them did not come from difference of embryonic cells growth.

Between days 7 to 11 (96 hours), the production of IFN-τ increased in 1100±20 pM IFN-τ (n=29) for group A versus 670±117 pM IFN-τ (n=24) for group B, ($p<0.05$). Between days 7 to 12 (120 hours) of *in vitro* culture, it exhibits 1691±290 pM IFN-τ (n=28) for group A versus 982±182 pM IFN-τ (n=24) for group B ($p<0.05$), figure 1.

According to the linear regression analysis, the IFN-τ production increases during the time of culture independently of the embryos origins ($r = 0.13$ for group A, $r = 0.23$ for group B; $p > 0.05$).

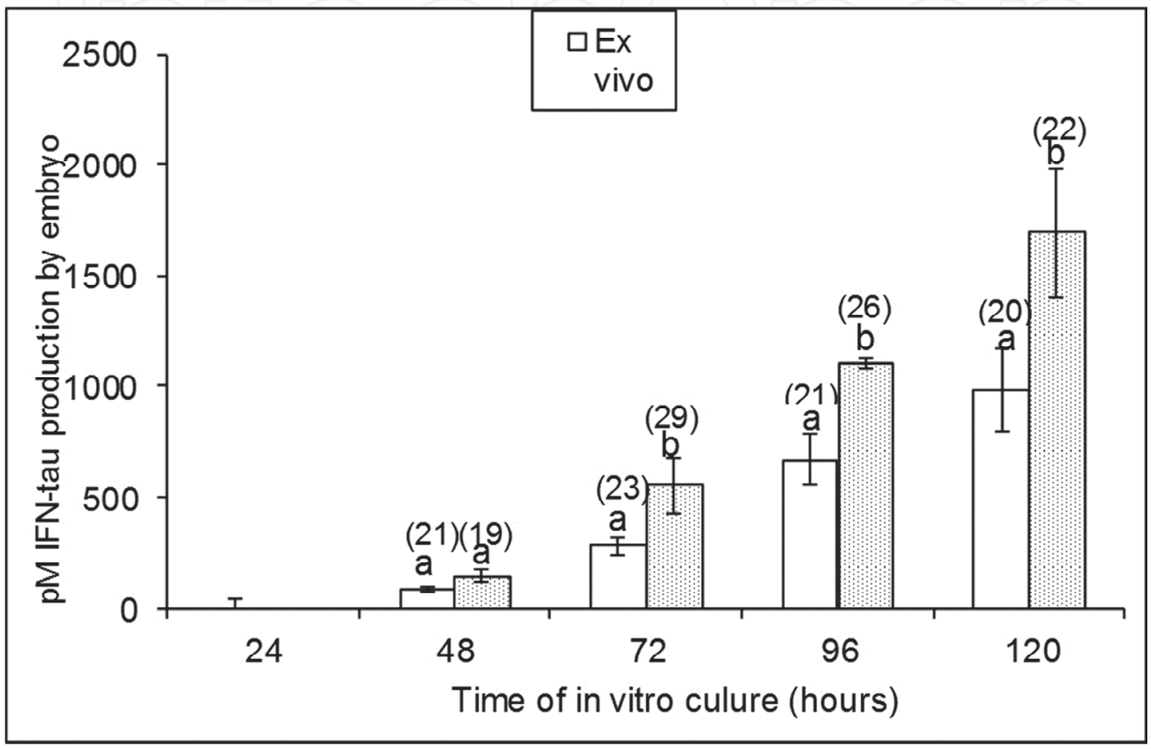


Fig. 1. Compared means of accumulated IFN-τ productions per blastocyst derived completely produced in vitro(group A) and *ex vivo* (group B), cultured individually from days 7 to12.

Least Squares Means indicate values with different superscripts (a vs b) are significantly different ($p<0.05$). (n): number of embryos.

3.3 Relationship between embryo quality and accumulated IFN-τ production by embryo, independently of its origin

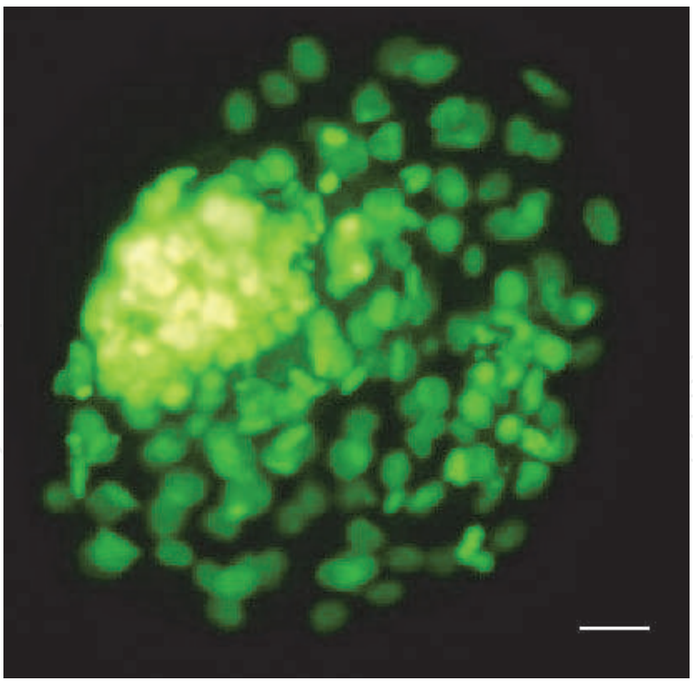
The average IFN-τ production from days 7 to 10 (72h-culture) in relationship with embryo quality did not show significant differences, ($p>0.05$): for quality I (excellent) the average IFN-τ production was 534±138 pM IFN-τ (n=21), for quality I (good) 389±101 pM IFN-τ (n=29) and for quality II (fair) 328±108 pM (n=5). The average IFN-τ production from days 7 to 11 (96h-culture) in relationship with embryo quality did not show significant differences ($p>0.05$); 1040±216 pM IFN-τ (n=17); 870±158 pM (n=33) and 507±262 pM (n=5),respectively. But the average IFN-τ production from days 7 to 12 (120h-culture) in relationship with embryo quality shows significant differences between excellent quality (1815±453 pM, n=10) or good quality (1356±200 pM , n=29) with fair quality (360±188 pM, n=4), ($p<0.02$), Table 3.

The logistic regression analysis of the relationship between the embryo quality and IFN-τ production showed predicted probabilities from observed responses: Concordance: 60.3% and Discordance: 23.9%.

Embryo quality	Time of culture (hours)						
	0 h	72 h.		96 h.		120 h.	
	n (%)	n (%)	IFN-τ concentration mean ± sem (pM)	n (%)	IFN-τ concentration mean ± sem (pM)	n (%)	IFN-τ concentration mean ± sem (pM)
Q1(Ex.)	81 (70)	24 (21)	534 ± 138a	20 (17)	1040 ± 216a	10 (12)	1815 ± 453 a
Q1(G.)	19 (16)	34 (29)	389 ± 101a	38 (33)	870 ± 158a	29 (34)	1356 ± 200ab
Q II	-	6 (5)	328 ± 108a	6 (5)	507± 262a	4 (5)	360 ± 188 b

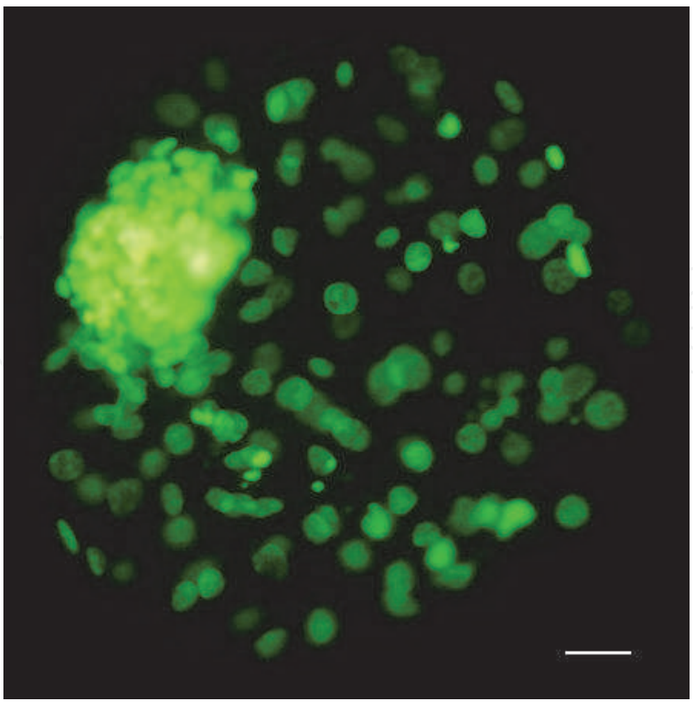
QI (Ex.): excellent quality grade I; QI (G.): good quality grade I; QII: fair quality grade II. Least Squares Means indicate values with different superscripts (a vs b) are significantly different ($p<0.05$).

Table 3. Relationship between embryo quality with accumulated IFN-τ production after culture individually of blastocysts whatever their *in vitro* or *in vivo* origins, from days 7 to 12.



Bar corresponds to 50µm.

Photograph 1. Blastocyst of ex vivo origin on day 10 (from day 7 and cultured during 3 days individually in droplet). Staining was by immunochemistry with ainti-IFN-τ immmunserum and propidium iodide - bisbenzimide. The nuclei of ICM and TE cells are fluorescent.



Bar corresponds to 50µm.

Photograph 2. Blastocyst of completely in vitro origin, was cultured 10 days 7 individually in droplet. Staining was by immunochemistry is like in photograph 1.

4. Discussion and conclusion

Our average results for producing bovine blastocysts *in vitro* can be compared with different reports, (Greve et al, 1993 Brackett and Zuelke 1993; Holm et al, 1999; Thompson 1997). Nowadays, after years of development, these techniques have reached a level of stability. The analysis of IFN-τ production by *in vitro* blastocysts confirms earlier studies (Kubish et al. 2004, 1998; Hernández-Ledezma et al. 1993; Stojkovic et al. 1995, 1999) and strongly suggests that expression of IFN-τ is constitutive and partly non dependent on factors from the uterine environment. Nevertheless, it is known that IFN-τ expression and secretion level might be modulated *in vivo* by growth factors and cytokines (Ko et al. 1991; Imakawa et al. 1993; Martal et al. 1998; Spencer and Bazer 2002; Miyazaki et al. 2002; Emond et al. 2004).

The IFN-τ production on day 8, after 24 hours of *in vitro* culture, was below 54pM and therefore undetectable in both *ex vivo* and completely produced *in vitro*; The IFN-τ is first produced by bovine conceptuses soon after the blastocysts expand and just prior to the time that the zona pellucida ruptures and hatching occurs (Hernández –Ledezma et al. 1992). It was only detectable from day 9 in both cases. These results have no relationship with the results reported by Kubish et al. (1998). In our study we only used very homogeneous blastocysts of good quality. We reported the production generated every 24 hours from day 7 to 12. Nevertheless, the accumulated production for each embryo agrees with values obtained by Kubish et al., (1998, 2004); Stojkovic et al., (1999); Hernandez- Ledezma et al., (1993).

To compare well the IFN-τ production of *ex vivo* and completely produced *in vitro* embryos in *in vitro* culture from day 7 to 12, in the present study only excellent and good quality

expanded blastocysts were chosen on day 7 after fertilization. Thus, a homogeneous production would be expected, however, significant differences in the average production of IFN-τ were found in relationship with embryo origin, *ex vivo* and completely produced *in vitro*, between day 8 and 9, after 48h of culture and in IFN-τ production for embryo accumulated, which showed that the IFN-τ production was greater for completely produced *in vitro* embryos even on day 9.

In vitro, modulation of the IFN-τ production could be induced on one hand by the conditions of the culture associated to the presence of biological factors contained in the fetal calf serum, and in the other hand by intrinsic causes of the embryo. Those reasons could explain the individual variability of IFN-τ production between the embryos. Our data showed high ranges of standard deviation for *in vitro* and *ex vivo* embryos and were in agreement with the results of Kubish et al. (1998) who also obtained ample ranges in the standard deviation; they also observed that the amount of IFN-τ produced was independent of the quality score of embryos received. Larson and Kubisch (1999) report that the presence of more blastocysts in the same medium can increase the IFN-τ secretion, nevertheless the nature of most of these factors of variation are badly known. In *in-vivo* conditions, the production of IFN-τ is modulated by the uterine environment. This production seems to be strongly correlated to the progesterone seric concentration, which would suggest that more elevated rates of progesterone would be favorable to the conceptus environment (Kerbler et al. 1997; Spencer et al. 2004). Indeed, progesterone stimulates the production of many others uterine factors than the IFN (Mann et al. 1999; Martal, 2002.). Several growth factors or citokynes have been involved in the secretion of IFN: IGF-I and IGF II (Ko et al. 1991), GM-CSF (Imakawa et al. 1995; Emond et al. 2004), this one is known as a powerful growth factor for the trophoblastic cells. It is possible that the variations in IFN-τ production between blastocysts at any of the stages of development is the result of genetic factors; others have previously reported that the fact sire genotype appears to influence IFN-τ secretion (Kubish et al. 2001); for this study, the frozen-thawed semen for IA of donor cows was the same as production of *in vitro* bovine. More over, according to Kubish et al. (2001), the batch of ovaries for production of *in vitro* bovine blastocysts, does not take into account the composition of breeds or ages of cows slaughter, these conditions may influence the variation in IFN-τ production.

However, their early-forming blastocysts were generally considered more developmentally competent than those which formed late, and these last authors suggested a possible negative relationship between early IFN-τ production and competence. In fact, this hypothesis has not been verified in the present study, possibly because of the homogeneity of the embryos chosen on the good appearance on day 7 according to the usual morphological criteria. Embryo quality is usually based on series of subjective visual assessments of morphologic parameters evaluation which may include embryo shape, size, cellular integrity, appearance of the cytoplasm and nucleus and other often intangible criteria (Lindner and Wright, 1983; Shamsuddin et al. 1992, Massip et al. 1995). In this study, the embryos which maintained in good and excellent quality had a better production of IFN-τ compared with those which turned to fair quality. This can be explained by a higher number of degenerating cells; in others the present study shows that the embryo quality is associated with IFN-τ production and confirms earlier studies (Kubish et al. 1998; Hernández-Ledezma et al; 1993; Stojkovic et al. 1995 and 1999).

Finally this study leads to the conclusion that significant differences in the production of IFN-τ were found in relationship with embryo origin, *ex vivo* and completely produced *in*

vitro. The detectable interferon production in the precocious stage on days 7 to 8 reflects the degree of embryonic development, but the amount of produced interferon has neither a positive nor negative effect on the future of the embryo viability

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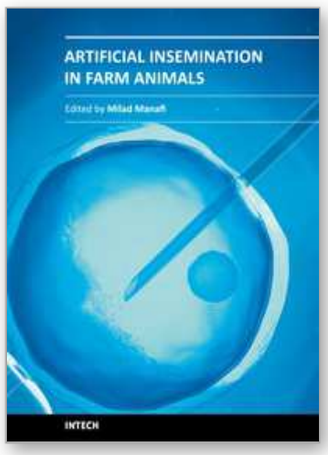

Artificial Insemination in Farm Animals

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Artificial insemination is used instead of natural mating for reproduction purposes and its chief priority is that the desirable characteristics of a bull or other male livestock animal can be passed on more quickly and to more progeny than if that animal is mated with females in a natural fashion. This book contains under one cover 16 chapters of concise, up-to-date information on artificial insemination in buffalos, ewes, pigs, swine, sheep, goats, pigs and dogs. Cryopreservation effect on sperm quality and fertility, new method and diagnostic test in semen analysis, management factors affecting fertility after cervical insemination, factors of non-infectious nature affecting the fertility, fatty acids effects on reproductive performance of ruminants, particularities of bovine artificial insemination, sperm preparation techniques and reproductive endocrinology diseases are described. This book will explain the advantages and disadvantages of using AI, the various methodologies used in different species, and how AI can be used to improve reproductive efficiency in farm animals.

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