RELATIONSHIP BETWEEN EMBRYONIC INTERFERON (oTP) AND EMBRYO SURVIVAL IN SHEEP.

L. Bodin[1], P. Reinaud[2], D. Lajous[1], J.P. Poivey[1], G. Charpigny[2]
[1] INRA Station d'Amélioration Génétique des Animaux. BP21 - 31326 Castanet Tolosan - FRANCE
[2] INRA Station de Physiologie Animale, 78352 Jouy en Josas - FRANCE

SUMMARY

The objective of this study was to analyse the secretion of embryonic interferon (oTP) by ovine conceptus and to relate it with embryo survival of its dam. Two groups of Romanov ewes were selected for their high (H - n=32) or low (L - n=33) breeding value for embryo survival. Intra-uterine insemination were done; at day 10, 12 and 15 of pregnancy, uterine horns were flushed with PBS and embryos were incubated for 24 hours. oTP was assayed by radio-immunoassay in flushing and in medium culture. Total oTP content in flushings increased widely from day 10 (μ=1.8 ng ; se=0.4), to day 12 (μ=3.4 μg ; se=1.5) and day 15 (μ=72.8 μg ; se=6.1) of pregnancy, it was more related to the age than to the embryo size : that shows the very fast change of activity of the trophoblast cells. The H group presented more total oTP than the L group, the difference was significant on day 10 and vanished by day 15. On day 15 of pregnancy all flushings got similar amount of oTP suggesting the existence of a global regulation at the beginning of implantation. In vitro, the H embryos secreted also higher amount of oTP. On day 10 of pregnancy that was not explained by the size difference of the embryos. Variance analysis showed significant group and dam effects on oTP secreted by the Day 10 embryos, which could mean that maternal effect plays a role in the early development of the embryo and in its ability to secrete oTP.

INTRODUCTION

Genetics of embryo survival is much less advanced than that of other litter size components and in sheep very few results of genetic variability are reported. However it is known that most embryonic looses occur during the first three weeks of pregnancy which starts with the recognition of embryos by the mother. Trophoblastin, evidenced in sheep by Martal *et al.* (1979), is considered as the embryo signal which alters the sexual cycle and prevents the female to return to oestrus. This protein, also called ovine trophoblast protein (oTP) which belongs to the interferon (IFN-τ) family is secreted by the trophoblast of the conceptus from 10 days after conception and disappears at 21 days (Guillomot *et al.*, 1990 ; Roberts *et al.*, 1992). It is clear, that any significant dysfunction in the production of such a signal could be responsible for embryo looses. The question is to know if genetic variability of this function plays a role in embryo survival and what sort of genetic control is involved. The present study was aimed to characterise the oTP secretion and its relationship with embryo survival.

MATERIALS AND METHODS

The experiment was undertaken at the INRA experimental farm of Langlade (Toulouse, France) where a flock of 600 Romanov ewes are under selection for increasing ovulation rate and embryo survival since 1984 (Ricordeau *et al.*, 1986). Breeding values for ovulation rate (OR) and embryo survival (ES) are estimated by an animal model based on data recorded at the first and second mating (9 and 20 months old). Two groups of adult Romanov ewes were selected. They differed widely on their breeding value in embryo survival but had similar ovulation rate (High embryo survival H ; n=32 ewes - Low embryo survival L n=33 ewes). Ewes were synchronised (40mg FGA vaginal sponge inserted during 14 days and injection of 250 UI PMSG) and intra-uterine inseminated with fresh semen of an unique Romanov sire. In each group,

laparatomies were performed on day 10 (n=22) , 12 (n=22), and 15 (n=21) of pregnancy. Uterine horns were flushed with 50 ml of phosphate buffer saline (PBS). After removal of conceptuses, flushings were stored at -20°C until assay for oTP. Each conceptus upon microscopic examination was identified, measured and individually cultivated for 24 hours as described by Charpigny *et al.* (1988). Volume of culture milieu were 0.5, 2 and 15 ml minimum essential medium respectively for Day 10, Day 12 and Day 15 embryos. OTP was measured in uterine flushing and in culture medium by radio-immunoassay. Uterine flushing collected on day 10 of pregnancy were concentrated by ultra-filtration before assay. The sensitivity of the assay was 150 pg./ml at ID80, and the inter-assay variation coefficient was 12%. All data were analysed by means of the Statistical Analysis System. Difference between group were compared by a t-test and by least-squares analysis of variance using the General Linear Models procedure. The model contained the independent effect of group and ewe within group.

RESULTS

Breeding values for embryo survival was -3.59 for the L group (range -7.0 to -1.1) and 6.85 for the H group (range 4.4 to 13.2), which corresponds to 4 and 34% embryo looses during the two first pregnancy respectively for the H and L group. Ovulation rate controlled at embryo recovery was similar in both group (μ =4.00) but pregnancy rate, identified by observation of well developed embryos or oTP concentration in uterine flushing was lightly higher in H (0.66 ; n=21) than in L group (0.60 ; n=20). The ewes which showed non fertilised oocytes in uterine flushing and those for which no embryos were found were not taken into account. Six H and eight L ewes presented undeveloped embryo at recovery or oTP level significantly lower (but not 0) than the average for this stage, that corresponds to 22.2 and 28.6 % of the fertilised ewes. The number of developed embryos recovered was 13 and 25 on day 10 ; 9 and 19 on day 12 and 29 and 15 on day 15 of pregnancy respectively in the L and H group. The global recovery rate was 2.68 and 2.81 embryos per ewe and 0.60 and 0.57 embryos per corpus luteus in the L and H group.

Table 1. oTP recovered in uterine flushing on day 10, 12 and 15 of pregnancy in H and L group of ewes, and amount of oTP per embryo (mean and standard error).

	Group	Day 10 (ng)	Day 12 (µg)	Day 15 (µg)
Total oTP	L	0.98 (0.15)	1.6 (0.5)	73.9 (8.5)
	H	2.36 (0.62)	4.5 (2.3)	71.2 (9.0)
	Prob. > t	0.06	0.28	0.83
oTP/emb.	L	0.47 (0.11)	0.53 (0.13)	30.5 (4.5)
	H	1.20 (0.54)	1.1 (0.54)	42.3 (9.1)
	Prob. >t	0.22	0.36	0.22

There was a very large difference of oTP quantity in uterine horns flushed on day 10 (μ=1.8 ng ; se=0.4), 12 (μ=3.4 µg ; se=1.5) and 15 (μ=72.8 µg ; se=6.1) of pregnancy. In uterine flushings, total oTP and oTP per embryo were higher in the H than in the L group at each stage (table 1). This difference was significant on day 10 of pregnancy. There was no significant effect of the number of recovered embryo on the quantity of oTP in the uterine flushing on day 10, 12 and 15 of pregnancy. Moreover on day 15, when the secretion was high the amount of oTP by embryo was inversely proportional to the embryo number (β=-12µg/emb. ; R^2=0.49).

Capacity of conceptuses to synthesise oTP was not affected by the culture since only 3 among 56 incubated embryos did not secreted oTP. The significant difference of oTP secreted by embryos of the H group is displayed in table 2. Conceptuses from the H group secreted in vitro 4 times much oTP on day 10 and twice much on day 12 than those from the L group. On day 10 of pregnancy, the difference of the size

did not explain alone the higher oTP secretion of the H embryos. In contrast on day 12 of pregnancy, this significant difference was mainly due to the larger size of the H embryos.

Analysis of variance showed a significant dam within group effect on oTP secreted by individual embryo by day 10 of pregnancy. This effect disappeared by day 12.

Table 2. In vitro secretion of oTP during a 24 hours incubation period and embryo size for embryos collected on Day 10 and Day 12 of pregnancy (mean and standard error).

	Day 10			Day 12		
	oTP ng/24h	size mm²	oTP ng/24h/mm²	oTP ng/24h	size mm²	oTP ng/24h/mm²
L	2.90 (0.79)	0.86 (0.22)	4.25 (0.99)	392.8 (58)	16.7 (3.1)	31.6 (8.2)
H	11.7 (2.24)	1.38 (0.17)	8.94 (1.71)	964.6 (203)	27.2 (5.2)	39.5 (4.8)
P>t	0.001	0.08	0.02	0.02	0.11	0.41

DISCUSSION

For the first time the concentrations of oTP secreted in the uterine lumen by the conceptuses during three stages of early pregnancy is reported. Until now, nearly all data for oTP expression came from cultured blastocysts and there are conflicting results about the beginning of the synthesis of oTP. It has been reported that oTP is produced as early as day 8-9 of pregnancy by cultured blastocysts (Ashworth and Bazer, 1989 ; Martal *et al.*, 1987) whereas for non cultured blastocysts oTP could not be detected by immunohistofluorescence or by in situ hybridization on day 10 but only on day 11 of pregnancy (Guillomot *et al.*; 1990). Our results demonstrate a non equivocal production in vivo of oTP by Day 10 blastocysts.

A very important increase of oTP was observed between the 10th and the 15th day of pregnancy since uterine contents of oTP change from 1 ng to 70 µg. This increase reflects the important increase of the size of the conceptus when it is undergoing rapid expansion from a spherical to an elongated form. However the raise of oTP was more marked than the size of the trophoblast. For example a 2000-fold increase was observed in uterine oTP contents between day 10 and day 12 of pregnancy whereas trophoblast area increased only 20 times. Our data show a 100-fold increase in the protein secreted per trophoblast cell and suggest an increase in the mRNA transcripts for oTP. This is higher than the 5 to 10-fold increase reported by Farin *et al.* (1989). However we could overvalue protein synthesis per trophoblast mass because the quantities of oTP measured in uterine flushings represent the accumulation of the protein produced by the conceptus for a more long time for Day 12 than for Day 10 embryos. Data obtained from in vitro synthesis of oTP by Day 10 and Day 12 conceptuses indicate a 20 fold increase in oTP secretion per trophoblast mass.

An original observation is that the quantities of oTP measured in uterine flushings was conversely proportional to the number of embryos. This phenomenon was more evident as the pregnancy progresses. One explanation is that the size capacity of the uterus restricts the elongation of the trophoblasts so that the total trophoblast tissue tends to be identical whatever are the number of conceptuses.

In 11 % of the ewes we found neither non-fertilised oocytes nor degenerated embryos nor conceptuses while oTP was detected in abnormal concentration. That the concentrations of oTP were lower than the expected values allowed us to conclude that for these ewes embryonic mortality occurred between the 10th and 15th day of pregnancy.

Although the role of oTP for increasing pregnancy rate and embryo survival has been evidenced (Schalue *et al.*, 1991), this is the first report on a within breed relationship between embryonic interferon secretion and breeding value for embryo survival. In this Romanov flock, the ewes from the "High embryo survival" group had higher oTP contents in their uterine horns than the ewes from "Low embryo survival"

group. This difference was maximum on day 10, soon after the beginning of the secretion ; it was again significant on day 12 of pregnancy but not on day 15. When the conceptuses collected in uterine flushings were put in culture the differences were more significant between the 2 groups of ewes. Although the conceptuses of the L group tended to be smaller than those of the H group, the difference observed between group at day 10 was not due to a difference of trophoblast cell number but to a difference in activity of theses cells. However although the trophoblast size increased similarly in both group between day 10 and day 12, the difference of activity of trophoblastic cells vanished.

Generally, geneticists consider embryo survival as a trait of the dam and ignore any variation due to the embryo (Hanrahan, 1980 ; Hanrahan and Quirke, 1985, Bodin *et al.*, 1992). However, if we consider embryo survival as an embryo trait, the expression of its genome represents the direct effects of genes from the dam and sire and interactions between them. Moreover embryo survival can be subject to maternal effect through the uterine environment. The importance of each contribution depends on the stage at which looses occur. Results presented here, clearly show that before implantation conceptus genes play a role in its own survival. But the significant dam effect evidenced on day 10 of pregnancy means that maternal effect plays also a role even in very early embryo development and consequently in conceptus ability to secrete embryonic interferon. That also agrees with observations of Nephew *et al.* (1991).

REFERENCES

ASHWORTH, C.J., BAZER, F.W. (1989) *Biology of Reproduction*, **40** : 425-433

BODIN, L., HANRAHAN, J.P., POIVEY, J.P. (1992) *43rd Animal Meeting of the EAAP*. Madrid

CHARPIGNY, G., REINAUD, P., HUET, J., GUILLOMOT, M., CHARLIER, M., PERNOLLET, J.C., MARTAL, J. (1988) *FEBS Letters*, **228** : 12-16.

FARIN, C.E., IMAKAWA, K., ROBERTS, R.M. (1989) *Molecular Endocrinology*, **3**: 1099-1107

GUILLOMOT, M., MICHEL, C., GAYE, P., CHARLIER, M., TROJEAN, J., MARTAL, J. (1990) *Biology of the cell*, **68** : 205-211

HANRAHAN, J.P. (1980) *Poc. Aust. Soc. Anim. Prod.*, **13** : 405-408

HANRAHAN, J.P., QUIRKE, J.F. (1985) in *"Genetics of Reproduction in Sheep"*, pp 193-201, Ed. R.B. Land, D.W. Robinson, Butterworths, London.

MARTAL, J., LACROIX, M.C., LOUDES, C, SAUNIER, M., WINTENBERGER-TORRES, S. (1979) *Journal of Reproduction and Fertility*, **56** : 63-73.

MARTAL, J., CHARLIER, M., CHARPIGNY, G., CAMOUS, S., CHENE, N.,REINAUD, P., SADE, S., GUILLOMOT, M. (1987) *Livestock Production Science*, **17** : 193-210

NEPHEW, K.P., McCLURE, K.E., OTT, T.L., DUBOIS, D.H., BAZER, F.W., POPE, W.F. (1991) *Biology of Reproduction*, **44** : 536-539.

RICORDEAU, G., POIVEY, J.P., LAJOUS, D., EYCHENNE, F. (1986) *3rd Wrld. Cong. Genet. Appl. Livest. Prod., (Nebraska -1986)*, 90-95

ROBERTS, J. C., CROSS, LEAMAN, D. W. (1992) *Endocrine Reviews*, **13** (3), 432-452

SCHALUE-FRANCIS, T.K., FARIN, P.W., CROSS, J.C., KEISLER, D., ROBERTS, R.M. (1991) *Journal of Reproduction and Fertility*, **91** : 347-356.

ACKNOWLEDGEMENTS

This study was supported by the INRA AIP n° 91/4765 on Genetics of embryonic mortality. The authors thank F. Eychenne and the staff of the experimental farm, E. Lecloux as well as, J. Fallières and J. Miahle for their technical assistance.