Production of two species of interferon by Large White and Meishan pig conceptuses during the peri-attachment period

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Summary. Antiviral activities present in uterine flushings from pregnant Large White, Large White 'hyperprolific', and prolific Meishan gilts, between Days 8 and 20 of gestation were compared. Flushings (20 ml) from all gilts between Days 14 and 20 were positive in an in-vitro interferon (IFN) assay using vesicular stomatitis virus as a challenge infection. Highest antiviral activities (of up to 400 000–1 200 000 total Units/flushing) were obtained at Day 16 of gestation, i.e. clearly after the beginning of attachment. There was no major difference between breeds although, at Day 14, flushings from Meishan gilts yielded significantly higher titres than those from the other two, suggesting a correlation with the previously described earlier trophoblast elongation in Meishan gilts. Conceptus cultures contained antiviral activity, with values very close to those obtained *in vivo*, but the difference between breeds was not significant. Cultures from Day 20 on contained very little antiviral activity. The antiviral activity was associated with a mixture of at least two IFNs, one of which was IFN-alpha like, and the other was serologically identified as an IFN-gamma, that is an 'immune IFN', previously found to be secreted only by T lymphocytes. This finding may have implications for our understanding of the immunology of early pregnancy.

Keywords: pig; conceptus; attachment; antiviral activity; immune interferon

Introduction

Pregnancy in mammals remains an immunological enigma, since the maternal tissues tolerate the semi-allograft constituted by the implanting conceptus. Successful immunization of women that experience repeated abortions with paternal antigens, and experimentation on in-bred lines of mice that habitually abort (Chaouat *et al.*, 1983; Gill, 1983; Hunziker & Wegmann, 1986), have provided an experimental basis for several theories of the immunology of gestation. One hypothesis (Hunziker & Wegmann, 1986) states that a cascade of immune reactions, triggered by paternal antigens, favourably contributes to maternal tolerance of the fetus. Growing numbers of cytokines have been found in the reproductive tract during pregnancy in several animal species (Hunt, 1989), but their immune role(s) is not known.

An interferon-alpha (IFN- α) with potent antiviral activity is secreted during the peri-implantation period by sheep and cattle conceptuses. This IFN- α , produced by the trophoctoderm, is the same molecule as trophoblastin or ovine trophoblast protein-1 (oTP-1), a conceptus secretory protein previously shown to be antiluteolytic (Martal *et al.*, 1979; Heyman *et al.*, 1984; Bazer *et al.*,

1987; Helmer *et al.*, 1988; Vallet *et al.*, 1988; Pontzer *et al.*, 1988). The identity between oTP-1 (or bTP-1) and IFN- α , most probably IFN- α_{II} , is based upon clear-cut nucleotide and amino-acid sequence homologies (Imakawa *et al.*, 1987; Charpigny *et al.*, 1988; Stewart *et al.*, 1989; Charlier *et al.*, 1989; Imakawa *et al.*, 1989). That the only identified role for a conceptus-derived cytokine is not immunological may seem paradoxical, but suggests that such a cytokine may have multiple biological effects.

In ruminants, maternal recognition of pregnancy (the maintenance of corpus luteum function) depends upon synthesis of specific proteins by the conceptus, but it has been reported that pig conceptus secretory proteins have no significant effect on CL lifespan in cyclic gilts (Harney & Bazer, 1989). However, significant antiviral activity has been reported between Days 12 and 17 of gestation in uterine flushings of gilts and in pig conceptus-conditioned culture medium (Cross & Roberts, 1989; Mirando *et al.*, 1990). We have extended this study to include the comparison of 3 breeds of pigs, i.e. Large White controls, Large White hyperprolific (Large White^H), and Chinese Meishan. Meishan pigs are highly prolific. Therefore, study of relevant mechanism(s) could be valuable both for our understanding of the physiology and/or immunology of gestation, but also for future pig breeding improvement. Previous studies have indicated faster and more synchronous conceptus development between Days 8 and 14 in Meishan than in Large White gilts, and also higher embryo survival rates at Day 30 in Meishan gilts (Bazer *et al.*, 1987). Comparative data concerning antiviral activity in Meishan and European breeds of pigs are not available. The present study was to quantify, as far as possible, time-dependent and breed-dependent levels of antiviral activity produced by pig conceptuses in early pregnancy, and to identify as far as possible the species of interferon(s) produced.

Materials and Methods

Animals. Data were obtained from 45 pregnant Meishan, 27 Large White and 33 Large White Hyperprolific (Large White^H) gilts. The Large White^H genotype has been obtained by selection of sons of hyperprolific sows, on the basis of their prolificacy index. Large White^H gilts are characterized by a higher ovulation rate, which is counterbalanced, at the first parity, by a high embryonic mortality (Bolet *et al.*, 1986). All gilts had experienced 3 oestrous cycles before pregnancy. Detection of oestrus was made twice daily. Gilts were artificially inseminated with semen from boars of the same breed at 12 and 24 h after the onset of oestrous behaviour for both Large White and Large White^H gilts, and 24 and 36 h for Meishan, since the interval between oestrus and ovulation is longer by about 10 h in Meishan females (Terqui *et al.*, 1990). Day 0 of gestation was defined as the day of the first insemination. The fertility rates were 90% for Meishan gilts, and 78% for gilts of the two Large White genotypes.

Experimental design. In Exp. I, 52 gilts were assigned randomly on Days 8, 10, 11, 12 and 14 of gestation (with at least 2 and at most 6 gilts per genotype per day). Antiviral activities were measured in the uterine flushings which were kept frozen at -20°C for 6 months (Fig. 1). In Exp. II, data were obtained from 53 gilts: 48 were assigned randomly on Days 14, 16 and 20 of gestation for measuring antiviral activity in uterine flushings and in conceptus-conditioned culture medium (5 ± 1 gilts per genotype, per day). The antiviral assay was done within 2 weeks after sampling. In addition, 5 Meishan gilts were slaughtered on Days 17 (2), 19 (1), 22 (1) and 25 (1) of pregnancy for analysis of antiviral activity in conceptus culture medium.

Flushings. The reproductive tracts were obtained immediately after slaughter and put on ice until uterine horns were flushed as described previously (Geisert *et al.*, 1982a). Each uterine horn was independently flushed with 20 ml 0.9% (w/v) saline. Resulting fluids were clarified by centrifugation at 1700 g for 10 min. Supernatants were immediately frozen at -20°C until assayed for antiviral activity. Average volumes of uterine flushings were 14 ± 5 ml per uterine horn on Days 14–16 (there was no significant difference between Days 14 and 16), and 23 ± 8 ml at Day 20. On Day 20 of pregnancy, for the majority of gilts, it was impossible to recover implanted conceptuses by flushing; therefore the uterine horn was dissected to extract conceptuses. At Day 20, the numbers of conceptuses per gilt were counted but, for Days 8–16, the conceptuses were removed only after flushing the uterine horns.

Ovarian weight and the number of corpora lutea (CL) per ovary were determined. Lengths and weights of uteri were obtained after collecting uterine flushings. The width of one uterine horn, endometrial surface area and total endometrial weight were also measured as described previously (Bazer *et al.*, 1988). Weights of the gilts were also recorded before slaughter.

Conceptus cultures. Conceptuses were collected on different days of gestation for assay of secretion of antiviral activity in culture. About 0.5 g conceptus (wet weight) was transferred to 90-cm Petri dishes, in 12 ml Menezos' B2 medium (A.P.I. Systemes, Montalieu, France) without bovine serum albumin, supplemented with 5 mg glucose/ml,

0.2 IU insulin/ml, 0.05% polyvinyl-pyrrolidone (M_r 360 000; Sigma, St Louis, MO, USA), 0.1 mg aprotinin/ml, 100 IU nystatin/ml, 100 IU penicillin/ml, and 10 µg streptomycin/ml. Incubation was at 38°C in a controlled atmosphere of 5% CO₂-air mixture, with constant rocking. After a 24-h incubation, supernatants were centrifuged at 5000 g for 5 min, divided into aliquants, and stored at -70°C.

Antiviral activities in uterine flushings and in conceptus-conditioned culture medium were determined as previously described (La Bonnardire & Laude, 1981). Briefly, monolayers of Madin-Darby Bovine Kidney (MDBK) cells in microtitration plates were incubated overnight with serial 3-fold dilutions of fluids under study and standard IFN, in Minimum Essential Medium (MEM) plus 5% fetal calf serum (FCS). Supernatants were then withdrawn, and cells infected with Vesicular Stomatitis Virus, serotype Indiana, in MEM + 2% FCS (multiplicity of infection: 1 Plaque Forming Unit per 50 cells). After a further 24-h incubation, cells were stained with a solution of 1% violet crystal in 1:5 ethanol/water (v/v), when control infected cell sheets were 100% lysed. End-point protective dilutions were scored, and titres were calibrated with a laboratory reference IFN included in each assay. This reference IFN was assigned a titre of 1000 International Units/ml after parallel titrations with reference Human Interferon Alpha (Ref. Ga23-902-530, NIH, Bethesda, MD, USA) on MDBK cells. Titres in flushings were expressed both in IU/ml (Fig. 1) and in total IU per uterine horn (Table 2, results from Exp. II). The latter figures were calculated, on Days 14 and 16, as titres in IU/ml $\times$ 20 ml, and on Day 20 as titres in IU/ml $\times$ 30 ml (as volumes of Day-20 flushings are higher by 9-10 ml, see above: 'Flushings'). Standard deviations of geometric means (arithmetic mean of log IU/ml) were calculated using Student's table at the level 0.05.

Characterization of antiviral activity. For measuring IFN stability at acidic pH, a pool of Day-16 uterine flushings from Large White gilts was dialysed for 24 h against either PBS pH 7.4, or 0.2 M-glycine-HCl pH 2.0. Residual antiviral activity was determined as described above, in comparison with rPoIFN- α 1 and rPoIFN- γ submitted to the same treatment. *E. coli*-derived pig IFN- γ was a generous gift of Dr Martinod (Biovet-Ciba-Geigy, St Aubin, Switzerland).

The following cell lines and strains were used for measuring cross-species antiviral activity of uterine flushings and of conceptus culture medium. MM 10 (Muscle de Mouton) is a sheep cell line established at INRA (Jouy-en-Josas) from fetal lamb fibroblasts. RPa (Rein de Porc Adulte) is a strain of low passage, epithelial pig kidney cells isolated in the same laboratory from an adult pig, and was found, like other primary pig kidney cells, to be very susceptible to a direct toxic effect of IFN- α (Laude & La Bonnardire, 1984). Human Placental WISH cells and mouse mammary L929 cells were purchased from ATCC (Rockville, MD, USA). All these cells were grown and maintained in MEM + 10% FCS.

Seroneutralization of IFN activity present in flushings from Large White gilts was assayed by a constant antibody method (Kawade & Watanabe, 1984), in which 1:100 dilutions of antisera were applied to MDBK cells before serial 3-fold dilutions of IFN. The extent of neutralization was measured by the log(IU/ml) difference in activity between control flushings and the corresponding titre in the presence of antiserum. Sheep antiserum to human leucocyte IFN (natural IFN- α) was a gift of C. Chany (INSERM, Hpital St Vincent-de-Paul, Paris, France). Rabbit antiserum to recombinant pig IFN- γ was provided by B. Charley (Charley *et al.*, 1988). Rabbit antiserum to oTP-1 was raised at INRA (Jouy-en-Josas) against trophoblastin purified by ion-exchange chromatography (Charpigny *et al.*, 1988).

Results

Ovulation rates and embryo survival

Ovulation rates did not differ significantly between breeds and were 14.6 ± 2.1 (mean $\pm$ s.d.) for Meishan, 15.8 ± 2.0 for Large White, and 15.8 ± 2.7 for Large White^H gilts.

On Day 20 of gestation, embryonic survival was higher ($P < 0.01$) for Meishan (80%) than Large White (66%) and Large White^H (54%) gilts. The length, weight and width of the uterine horns as well as total endometrial surface area and calculated weight were greater ($P < 0.01$) for Large White and Large White^H gilts irrespective of day of gestation (data not shown).

Antiviral activity in flushings

Titres of IFN were measured in uterine flushings between Days 8 and 20 of gestation. Figure 1 shows time-dependent titres in uterine flushings from Meishan and Large White gilts obtained in Exps I and II. The duplication of Day-14 samples showed that samples from Exp. I had apparently lost much of their antiviral activity after 6 months. Titres became detectable on Day 12 for Meishan gilts, were maximum at Day 16, and progressively declined until Day 20 in the two breeds. The highest mean titres were obtained for Meishan gilts ($N = 5$), i.e. $10^{4.26} = 14\,590$ IU/ml.

Only data from Exp. II for Days 14, 16 and 20 were sufficient for statistical analysis (Table 1). On Day 14, flushings from Meishan gilts contained more IFN than did those from Large White

Fig. 1. Antiviral activity (MDBK cells + VSV) in uterine flushings on Days 8 to 20 of pregnancy in gilts of two genotypes, Meishan and Large White. Data from Exp. I (Days 8–14) and Exp. II (Days 14–20) are shown separately to take into account the lower IFN titres from Exp. I. Flushings with no antiviral activity were all scored as 10⁰. Each square (black or empty) is representative of several gilts, as follows: Meishan gilts were 6, 5 and 4 at Days 8, 10 and 12, respectively, and Large White gilts were 2, 5, 3 and 4 at Days 8, 10, 11 and 12, respectively. Corresponding values for Exp. II are given in Table 1.

($P < 0.05$) and Large White^H ($P < 0.001$) gilts. But at Day 20 titres for Large White^H gilts were higher than for Meishan ($P < 0.05$) and Large White ($P < 0.001$) gilts. Finally, the decrease in antiviral activities in flushings from Large White and Meishan gilts between Days 16 and 20 was significant ($P < 0.01$ and $P < 0.001$, respectively). Uterine flushings from all gilts, without exception, contained IFN activity. The antiviral activities were quite high, i.e. 10 000–20 000 IU/ml between Days 14 and 16 of gestation. The uterine flushing from one Meishan gilt contained 61 000 IU/ml, or a total of about 1 200 000 IU in that uterine horn.

Antiviral activity in conceptus culture medium

In Exp. II, conceptuses from 32 pregnant gilts, representing the 3 breeds, were cultured (Table 2). In 12-ml cultures, total activities secreted per conceptus between Days 14 and 20 ranged from

Table 1. Total antiviral activities ($\log_{10}$ units) in uterine flushings of three genotypes of gilts (Exp. II)

Genotype	Day of gestation		
	14	16	20
Meishan	5.18 $\pm$ 0.45 (5)	5.46 $\pm$ 0.51 (5)	4.53 $\pm$ 0.15 (6)
Large White	4.57 $\pm$ 0.55 (6)	5.09 $\pm$ 0.46 (5)	4.08 $\pm$ 0.82 (4)
Large White ^H	4.07 $\pm$ 0.43 (6)	4.96 $\pm$ 0.49 (6)	5.03 $\pm$ 0.3 (5)

Values are mean $\pm$ s.e.m. for the number of gilts in parentheses.

10 000 to 100 000 units, i.e. slightly less than total activities in uterine flushings. On Days 14, 16 and 20 (2–4 gilts per group) breed differences in activities were not detected. Nevertheless, unlike in uterine flushings, mean activities tended to be higher in Large White conceptus-conditioned culture medium. Titres were highest in culture medium containing Day-16 conceptuses (all 3 genotypes), then decreased to Day 20. As for uterine flushings, the decrease in titres at Day 20 compared to Days 14 and 16 was significant ($P < 0.05$) for Meishan and Large White conceptuses. Conceptuses from Meishan gilts slaughtered on Days 22 (3 conceptuses) and 25 ($N = 2$) were cultured, but less than 100 units of IFN activity were detected, compared with 100 000 units of activity on Day 16.

Table 2. Total antiviral activities ($\log_{10}$ units) in conceptus-conditioned culture medium after 24 h (Exp. II)

Genotype	Day of gestation						
	14	16	17	19	20	22	25
Meishan	4.71 $\pm$ 0.69 (3)	4.95 $\pm$ 0.6 (3)	4.15 (2)	4.2 (1)	3.6 $\pm$ 0.00 (3)	1.93* (1)	1.8† (1)
Large White	5.06 $\pm$ 1.4 (3)	5.31 $\pm$ 0.61 (3)	nd	nd	4.06 $\pm$ 0.78 (3)	nd	nd
Large White ^H	4.2 (2)	4.56 $\pm$ 0.42 (4)	nd	nd	3.88 $\pm$ 2.05 (3)	nd	nd

Values are mean $\pm$ s.e.m. for the number of gilts in parentheses; nd = not determined.

*Average of 3 conceptuses from the same gilt.

†Average of 2 conceptuses from the same gilt.

Characterization of interferon

The measure of relative antiviral activities on a panel of cells from various species is one means to characterize and to differentiate several types of interferons (Hayes *et al.*, 1979). Table 3 shows that the antiviral activity present in a pooled sample of uterine flushings from Large White gilts differs from that of two species of pig IFN- α , the natural leucocyte IFN, and the recombinant subtype rIFN- α 1, by 2 main criteria. First, the relative activity on human cells was very low (0.26% of activity on MDBK cells) compared to PoIFN-Le (6.4%) and to rPoIFN- α 1 (3.5%). Secondly, a difference appeared when cytotoxic effect on pig kidney cells was tested. It has been reported that cultures of primary or low-passage key kidney cells are extremely sensitive to a cytotoxic effect induced by various IFN- α and virus-induced leucocyte IFNs (Laude & La Bonnardière, 1984). Conceptus IFN was toxic to such a pig primary kidney cell strain (RPA cells) at a titre (reverse of limit dilution) equivalent to 110% of the antiviral activity on MDBK cells (Table 3). Corresponding data for PoIFN-Le and rPoIFN- α 1 were 0.4% and 0.9%, respectively.

Table 3. Cross-species antiviral activities ($\log_{10}$ IU/ml) in uterine flushings from Large White gilts

IFNs	Cell lines (species)					
	MDBK (cattle)	MM10 (sheep)	WISH (human)	L929 (mouse)	RPa (pig)	
					AVE*	CCE†
Uterine flushings	3.6	3.7 (110)‡	1.0 (0.26)	<1.6 (0.8)	<3.95 (190)	3.7 (110)
porIFN-le (natural)	3	3.2 (170)	1.8 (6.4)	2.3 (19)	2.5 (33)	0.6 (0.4)
porIFN- α 1 (recombinant)	4.1	4.5 (280)	1.95 (7.2)	2.6 (3.5)	4.2 (125)	2.0 (0.9)

*Antiviral effect.

†Cytocidal effect. Data are presented as log (units/ml), as for antiviral effect. They represent the reverse of the highest IFN dilution inducing a toxic effect on RPa cells. The usual calibration with international standard IFN was done.

‡Figures in parentheses are % of IFN titre on MDBK cells.

The antiviral activity from Large White flushings was sensitive to pH 2.0 (after a 24-h dialysis), slightly less than pure recombinant pig IFN- γ . PoIFN- α 1 was completely resistant to this treatment (Table 4).

Table 4. Acid pH stability of uterine antiviral activity from Large White gilts

IFN	Activity after treatment (U/ml)		
	pH 7.4	pH 2	Residual activity (%)*
rPoIFN- α 1	27 000	27 000	100
rPoIFN- γ	5 200	960	18
Flushing	5 200	1 260	24

*U/ml at pH 2: U/ml at pH 7.4 $\times$ 100.

Antigenicity is another important criterion to characterize an interferon. Antiviral activities in individual flushings and in conceptus culture medium from Day-16 samples were measured in neutralization tests using antisera to IFN- α and IFN- γ (see Materials & Methods). Table 5 shows the results obtained with Large White flushings. Surprisingly, both antisera were individually able to neutralize to some extent the antiviral activity. Moreover, a mixture of the two antibodies, each at the same concentration, brought about nearly complete neutralization of the bioactivity, suggesting that conceptus-derived IFN is a mixture of IFN- α (like) and IFN- γ (like) proteins. Also, these data suggest that the proportion of IFN- γ is relatively higher in conceptus cultures than in uterine flushings. The same results, not presented here, were obtained with flushings from Meishan gilts.

Finally, the same neutralization assay using rabbit antiserum to ovine trophoblastin was totally negative (data not shown).

Discussion

High antiviral activities were detected in uterine flushings from all gilts between Days 14 and 20 of gestation and correspondingly elevated in conceptus conditioned culture medium from the same

Table 5. Seroneutralization of antiviral activity in uterine flushings and conceptus conditioned medium from Large White gilts

Fluid	Antibodies to:		
	IFN- α	PoIFN- γ	Both
Flushing ($n = 5$)	1.01 $\pm$ 0.32*	1.04 $\pm$ 0.24	2.74 $\pm$ 0.48
Medium ($n = 5$)	0.44 $\pm$ 0.25	1.14 $\pm$ 0.29	2.46 $\pm$ 0.40

*Antiviral titre difference ($\log_{10}$) between control and seroneutralized samples (mean $\pm$ s.e.m. of 5 individual seroneutralizations). All samples were collected at Day 16 of gestation.

(or corresponding) pregnant gilts. The levels of activity in the present study were higher than reported by Cross & Roberts (1989). We cannot explain the nearly 20-fold difference between their results and the present results, at least in Exp. II, in which samples were assayed 1–2 weeks after collection. The difference is even more pronounced for the IFN titres in conceptus culture medium. One possible reason might be that all culture media in the present work contained the protease inhibitor aprotinin, which was shown by Beers *et al.* (1990) to protect antiviral activity from degradation. This partly invalidates the statement that conceptuses in culture produce far less activity (0.1–0.01%) than *in vivo* (Cross & Roberts, 1989). However, Day-16 sheep conceptuses do secrete 50–100-fold more interferon than do Day-16 pig conceptuses on a per g conceptus wet weight basis (Pontzer *et al.*, 1988). Nevertheless, considering the low titres obtained in Exp. I (Fig. 1), we confirm previous reports of the lability of pig conceptus interferons.

In the present experiments, maximum antiviral activity in uterine flushings was obtained at Day 16, although titres from Days 14 and 16 differed significantly only for Large White^H gilts. The similarity in timing of IFN activities in flushings and culture medium suggests that the Day-16 peak titre corresponds to the day of maximum potential for synthesis and not to an accumulation of IFN in the uterine lumen. As judged by quite low IFN titres on Days 22 and 25 in Meishan conceptus cultures, the major period of IFN synthesis was between Days 14 and 20. If present results are compared with those for ovine trophoblastin (Roberts, 1989), pig interferon activity seems to appear and to peak on about the same days. In sheep, oTP-1 synthesis followed by quantifying specific mRNA from conceptuses in culture (Farin *et al.*, 1989) was found to peak at Day 13, but one cannot exclude that a time shift occurs in the kinetics of activity due to artificial conditions of conceptus cultures.

The only significant difference between Meishan *versus* Large White and Large White^H gilts was higher activity in Meishan flushings on Day 14. Ideally, not only total activities per uterine horn, but those per g conceptus should be analysed for more accurate comparison between breeds, but the flushing procedure did not allow counting or weighing of the conceptuses. This difference was not true at Day 16. Therefore, Meishan uterine fluid can be considered to be more active around Day 14 which is the time of attachment (Geisert *et al.*, 1982b). At Day 14, conceptuses from all Large White and Meishan gilts are elongated (Bazer *et al.*, 1988). Therefore, regarding a possible effect of interferon upon conceptus elongation, differences in initial amounts of activity produced at Days 11 or 12 should be considered. In this respect, data from Exp. I, because they are not amenable to rigorous statistical analysis, do not permit a conclusion of earlier secretion by gilts of the Meishan breed. IFN activities in conceptus culture medium were not affected by breed, regardless of day of gestation, although maintenance of conceptuses in culture medium is in some ways an artefactual situation, as stated by Cross & Roberts (1989).

The nature of components with antiviral activity present in uterine flushings and conceptus culture medium is complex. In the present work, two different IFNs were identified on the basis of bioactivity and antigenicity. As it was not possible to isolate and/or purify each component, our

arguments in favour of the existence of two IFN species are indirect, but they are 3-fold. (i) We found a partial neutralization by each of two antisera, one towards IFN- α , and the other anti-PoIFN- γ . Moreover, the level of neutralization was much increased when both antibodies were mixed (Table 5). (ii) The uterine antiviral activity was clearly different from IFN- α by its spectrum of bioactivity on different cells, and in particular it exerted a much more pronounced toxic effect on primary pig kidney cells than did either IFN- α (Table 5). In fact, we found that uterine flushings were intermediate in toxicity between IFN- α and IFN- γ (not shown). (iii) Antiviral activity in uterine flushings was very sensitive to pH 2.0, much like recombinant IFN- γ (Table 4). The lability of gamma or immune IFNs is well documented (for a review, see De Maeyer & De Maeyer-Guignard, 1988).

Cross & Roberts (1989) have shown that the antiviral activity found in flushings from pregnant gilts was unstable upon storage and labile at pH 4.5. On the other hand, they found that, after electrophoresis of uterine proteins, at least one band with an M_r of 22 000 was immunodetected by antiserum to HuIFN- α . This protein was absent in uterine flushings from cyclic gilts. Taken together, their results might be accounted for by the properties of a mixture of IFNs of the alpha and gamma types.

More detailed characterization of each of these antiviral activities is needed. All interferon species found and characterized so far in early pregnancy in ruminants are related, but not identical, to IFN- α s of class II (Imakawa *et al.*, 1989). One paradoxical property of pig conceptus antiviral activity is that it is partly neutralized by immunoglobulins to human leucocyte IFN (Table 5) but not at all by an antiserum to oTP-1 (not shown). By analogy with the results and hypothesis of Adolf (1987), one cannot exclude the existence of a third sub-class of alpha-related genes, expressed also in induced leucocytes, and to which the pig alpha-like conceptus-derived IFN could belong. On the basis of seroneutralization data, the relative amounts of the two IFN species seem to be different *in vivo* and *in vitro*, which suggests either that conceptus synthesis is in some way regulated by the maternal environment, or that the activity of IFN- α -like species could be less stable than that of IFN- γ . Further characterization of the activity of IFN- γ and identification of cells secreting it are needed, but the possibility that IFN- γ is secreted by the pig conceptus early in gestation raises several questions. As far as is currently known, IFN- γ is a lymphokine produced by T lymphocytes (Stobo *et al.*, 1974). At the stages of conceptus development studied here, no lymphoid cells in conceptuses have ever been reported. One must, therefore, suppose that embryonic or trophoblastic cells are the source of this activity; this will have to be established.

Previous publications have stressed the difference between the pig and ruminants as to the role(s) that IFN(s) could play during gestation (Bazer *et al.*, 1989), as *in-vivo* infusion experiments seem to rule out an effect of conceptus proteins on corpus luteum lifespan (Harney & Bazer, 1989). The discovery of uterine IFN- γ might suggest an additional role(s) to those previously found or supposed (for a review, see De Maeyer & De Maeyer-Guignard, 1988); among them are the stimulation of antigens of the major histocompatibility complex at critical steps of the immune recognition by the mother (Feinman *et al.*, 1987), or an effect on the differentiation of embryonic or other cells (Honda & Rostami, 1989). IFN- γ could also exert a protective effect on the conceptus against maternal immune lysis (Renkonen *et al.*, 1988). Determination of the target tissue(s) of the two IFNs (α and γ) found during pregnancy in the pig will be one of the conditions needed before one can understand their role, individually and/or in synergy. However, in view of the well defined period of secretion reported here, one cannot rule out that interferons, in particular IFN- γ , play a role in a crucial step in differentiation of the pig conceptus.

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