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Ovarian follicular fluid concentrations of prostaglandins E_2, $F_{2\alpha}$ and I_2 during the pre-ovulatory period in pigs

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Summary. The concentrations of prostaglandin (PG) E_2, $PGF_{2\alpha}$ and PGI_2 (measured as 6-oxo-$PGF_{1\alpha}$) in follicular fluid collected from the ovaries of mature, cycling pigs during the immediate pre-ovulatory period have been estimated by radioimmunoassay. The concentrations of all three prostaglandins were low 24-32 hrs before the expected time of ovulation, with the ratio of the mean concentrations of PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ being 0.7:1.1:1.0. The concentration of PGE_2 showed a small increase during the next 22 hrs but, in the 2 hr period before the expected time of ovulation, the mean concentrations of PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ increased 119-, 11- and 5-fold, respectively, which produced a ratio of these mean concentrations of 14.8:2.2:1.0. One sample of follicular fluid contained the highest concentration of 6-oxo-$PGF_{1\alpha}$ (54.4 ng/ml) yet reported for any tissue from any species, but this concentration was still lower than those of PGE_2 (529 ng/ml) and $PGF_{2\alpha}$ (94.6 ng/ml). These results show that prostaglandin concentrations increase in the follicular fluid of mature, cycling pigs in the immediate pre-ovulatory period, with PGE_2 being the predominant prostaglandin produced. In « pre-ovulatory » follicles which failed to ovulate, prostaglandin concentrations were at baseline values. The findings in this study are consistent with follicular prostaglandins being essential for ovulation in the pig.

Introduction.

The pre-ovulatory surge of gonadotrophic hormones from the anterior pituitary gland is usually considered as the overriding endocrine event influencing the mature follicle. A consequent series of changes is found in the follicular tissues, culminating in the release of a secondary oocyte at ovulation. There are changes in the concentration in the follicular fluid of steroid hormones (*e.g.* Short, 1961 ; Younglai, 1972 ; Moor *et al.*, 1973 ; Baird and Fraser, 1975 ; McNatty *et al.*, 1975 ; Channing and Coudert, 1976), gonadotrophic hormones (McNatty *et al.*, 1975 ; Henderson, McNeilly and Swanston, 1982), prolactin (McNatty, Sawers and McNeilly, 1974) and prostaglandins (Tsafriri *et al.*, 1972 ; LeMaire *et al.*, 1973, 1975 ; Brown and Poyser, 1984), in addition to enzymatic

and structural modifications to the follicle wall (reviewed by Lipner, 1973 ; Espey, 1974, 1980 ; Weir and Rowlands, 1977 ; Thibault and Levasseur, 1979) in diverse mammalian species. There are several reports of steroid hormone and of gonadotrophin concentrations in the Graafian follicles of pigs (*e.g.* Chang *et al.,* 1976 ; Hunter, Cook and Baker, 1976 ; Cook, Hunter and Kelly, 1977 ; Eiler and Nalbandov, 1977 ; Daguet, 1979), but original papers recording follicular fluid concentrations of prostaglandins in pigs are few and limited to gonadotrophin-treated, prepubertal animals (Ainsworth, Baker and Armstrong, 1975 ; Tsang *et al.,* 1979). Since the interval between the gonadotrophin surge and ovulation of approximately 40 hours in the pig (Dziuk, Polge and Rowson, 1964 ; Liptrap and Raeside, 1966 ; Hunter, 1967) is one of the longest reported for domestic species and is closely similar to the interval in women (Edwards, 1980), there is a special interest in examining the concentration of prostaglandins in follicular fluid during the pre-ovulatory period in mature, cycling pigs. Ovarian prostaglandins may have a direct bearing on modifications to the follicle wall at ovulation and on the oocyte so released (see Szöllösi *et al.,* 1978), as well as on changes in oviduct function and on the physiology of spermatozoa stored in the oviduct isthmus (Hunter, Cook and Poyser, 1983). Consequently, the concentration of prostaglandin (PG) E_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ (the chemically hydrated product of PGI_2) have been measured in pig follicular fluid just prior to ovulation.

Materials and Methods.

Experimental animals. — Thirty-six sexually mature Large White × Landrace gilts from the herd at the School of Agriculture, Edinburgh, were used in this study ; they were aged $6\frac{1}{2}$ - $9\frac{1}{2}$ months and weighed 91-138 kg. They were penned as groups of 5-8, maintained under natural lighting, fed a proprietary diet twice daily, and checked for standing oestrus with a teaser boar at 09.00 and 16.00 hrs. All animals included in this study had shown at least one oestrous cycle of 20-22 days. During late pro-oestrus of a subsequent cycle, animals were checked individually every 3-4 hrs for the onset of standing oestrus and thus the approximate time of the pre-ovulatory surge of gonadotrophic hormones (see Liptrap and Raeside, 1966 ; Niswender, Reichert and Zimmerman, 1970). Eleven gilts in late pro-oestrus (*i.e.* having a red and swollen vulva together with excitable mounting behaviour) were given a single intramuscular injection of 500 i.u. HCG (human chorionic gonadotrophin : Chorulon, Intervet) to predetermine the time of ovulation : this is known to occur 40-42 hrs later (du Mesnil du Buisson, 1954 ; Dziuk *et al.,* 1964 ; Hunter, 1967).

Collection of follicular fluid. — Sampling of follicular fluid occurred at known intervals after the onset of standing oestrus or after the pro-oestrous injection of HCG. Samples were taken immediately after ovariectomy during a mid-ventral laparotomy under general anaesthesia (4 animals) or within 20 min of *post mortem* evisceration of the reproductive tract and ovaries at the City of Edinburgh abattoir (32 animals). In the latter instances, tissues were returned to the laboratory in a vacuum flask on ice.

Actual collection of fluid involved needle puncture at the base of each pre-ovulatory follicle (26 gauge × 1/2" intradermal needle) and aspiration of the contents into a chilled 1.0 ml plastic syringe (Gillette Scimitar). Any sample contaminated with blood — a rare event — was discarded. Fluids from the pre-ovulatory follicles in each animal were pooled in chilled glass vials, coded so that their origin was unknown until the results were obtained, and deep frozen at − 20 °C until assay.

Follicles considered pre-ovulatory in this study measured 7-10 mm in diameter, and nearly always represented a clearly distinguishable population. Follicles of 5-6 mm in diameter, probably destined for atresia, were not aspirated. But in three animals in which ovulation was several hours overdue, perhaps because of surgical adhesions between the ovarian surface and oviduct, follicular fluid was collected from 9-11 mm diameter follicles.

Measurement of prostaglandin concentrations. — Each sample of follicular fluid was diluted with 9 ml water and, after lowering the pH to 4.0 with N-HCl, was shaken twice with 20 ml ethyl acetate. The two ethyl acetate fractions were combined and evaporated to dryness on a rotary evaporator at 45 °C. The dried extract was redissolved in 2 ml ethyl acetate and was stored at − 20 °C until assayed. The recovery of PGs by this method is greater than 80 % (Poyser and Scott, 1980 ; Swan and Poyser, 1983). The results have not been corrected for procedural losses.

PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ were measured using antibodies raised in rabbits and tested in the Department of Pharmacology, Edinburgh (Dighe *et al.,* 1975 ; Poyser, 1980 ; Poyser and Scott, 1980 ; Lytton and Poyser, 1982). The only significant cross-reactivities were PGE_1 (66 %), PGA_2 (25.5 %) and PGB_2 (11.8 %) with the PGE_2 antiserum, and $PGF_{1\alpha}$ (100 %) with the $PGF_{2\alpha}$ antiserum. An enzyme converting PGE_2 into PGA_2 (and hence into PGB_2) has not been reported for any tissue, and prostaglandins of the 1-series are only minor products in most tissues. Consequently, it is likely that the PGE_2 and $PGF_{2\alpha}$ antibodies were measuring predominantly the prostaglandin to which they had been raised. The intra-assay coefficients of variation, calculated from the variation between the duplicate results obtained for each sample, were 10.6 % (PGE_2), 9.5 % ($PGF_{2\alpha}$) and 10.8 % (6-oxo-$PGF_{1\alpha}$). The inter-assay coefficients of variation, calculated from the results of adding a known amount of the appropriate prostaglandin to each assay were 9.2 % (PGE_2), 8.5 % ($PGF_{2\alpha}$) and 12.3 % (6-oxo-$PGF_{1\alpha}$). The limits of detection were 40 pg (PGE_2), 30 pg ($PGF_{2\alpha}$) and 40 pg (6-oxo-$PGF_{1\alpha}$).

Statistical analysis. — Differences between mean concentrations of individual prostaglandins were assessed by a Chi-squared test.

Results.

The results are summarised in table 1. In the 8-12 hr group of animals, the mean concentrations of PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ were all low and were in the ratio of 0.7:1.1:1.0. In the 20-30 hr and 31-39 hr groups, the mean

concentration of PGE_2 showed a small but gradual increase while the concentrations of $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ did not change. The ratio of the mean concentrations of PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ in the 20-30 hr and 31-39 hr groups were 2.9:1.1:1.0 and 3.4:1.0:1.0, respectively. In the 40-42 hr group, in which follicles were judged to be on the verge of ovulation but in which ovulation had not actually commenced, there was a highly significant ($P < 0.001$) increase in the concentration of each prostaglandin compared with the 3 earlier groups. The mean concentrations of PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ increased 119-, 11- and 5-fold, respectively, from the 8-12 hr group to the 40-42 hr group. The highest concentrations of PGE_2 (529 ng/ml), $PGF_{2\alpha}$ (94.6 ng/ml) and 6-oxo-$PGF_{1\alpha}$ (54.4 ng/ml) were all recorded in the same sample of pooled follicular fluid. The ratio of mean concentrations of PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ in the 40-42 hr group was 14.8:2.2:1.0.

The concentrations of prostaglandins in follicles in ovaries already showing some very recent ovulations were at baseline values. Likewise, prostaglandin concentrations in follicles from ovaries whose time of ovulation was considered overdue were also very low and had shown no increase above baseline levels.

There was no detectable influence of the pro-oestrous HCG injection on prostaglandin concentrations in pooled follicular fluid. Eight of the injected animals were distributed among the 8-12 hr, 20-30 hr and 31-39 hr groups. The remaining 3 injected animals were in 40-42 hr group, but were not represented in the highest concentration of each prostaglandin recorded in this study. There was a suggestion of higher concentrations of prostaglandins in fluid from pre-ovulatory follicles of 9-10 mm diameter when compared to those of 7-8 mm diameter, but the distribution of samples did not enable demonstration of a significant trend.

TABLE 1

The mean concentration of three prostaglandins in follicular fluid aspirated at laparotomy or autopsy from six different groups of pigs according to the time interval elapsing from the onset of standing oestrus or a pro-oestrous injection of HCG.

Interval from onset of oestrus or HCG (hours)	No of animals sampled	Concentration in ng/ml					
		PGE_2		$PGF_{2\alpha}$		6-oxo-$PGF_{1\alpha}$	
		Mean	(Range)	Mean	(Range)	Mean	(Range)
8-12	4	1.4	(0.7- 1.9)	2.3	(1.6- 2.8)	2.1	(0.9- 3.1)
20-30	8	4.0	(1.1- 9.5)	1.6	(0.3- 3.9)	1.4	(0.3- 3.5)
31-39	10	6.2	(2.6- 16.7)	1.8	(0.3- 4.9)	1.8	(0.3- 5.1)
40-42	8	166.1	(8.9-529.2)	24.6	(2.3-94.6)	11.2	(0.8-54.4)
Ovulation commenced	3	1.1	(0.5- 1.9)	0.8	(0.6- 1.1)	1.5	(1.0- 1.8)
Ovulation overdue	3	2.0	(1.4- 2.8)	0.2	(0.1- 0.2)	0.2	(0.1- 0.4)

Discussion.

There is much evidence that ovulation in several mammalian species is dependent upon a pre-ovulatory rise in the concentration of prostaglandins in the Graafian follicles (reviewed by Behrman, 1979 ; Poyser, 1981). The pre-ovulatory LH surge is the stimulus for this increase. Concerning the pig, it has been reported that the concentrations of PGE_2 and $PGF_{2\alpha}$ increase in the follicular fluid of pre-pubertal animals, treated with pregnant mare's serum gonadotrophin (PMSG) and human chorionic gonadotrophin (HCG), up to the expected time of ovulation (Ainsworth *et al.*, 1975 ; Tsang *et al.*, 1979). We have extended the studies on prostaglandin involvement in ovulation in this species by showing that there is an increase in the follicular fluid concentration of PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ (which reflects PGI_2 production) following the gonadotrophin surge in mature, cycling pigs, with a particularly large increase in the follicular fluid prostaglandin concentrations in the 2 hr period immediately preceding the expected time of ovulation. Tsang *et al.* (1979) reported that the follicular fluid concentration of PGE exceeds that of PGF except during the 10 hr period preceding ovulation where the PGF concentration is similar to or even exceeds the PGE concentration. In contrast, we found that the PGE_2 concentration was slightly lower than the $PGF_{2\alpha}$ concentration 24-32 hrs before ovulation, but then the PGE_2 concentration increased to a much greater extent than the $PGF_{2\alpha}$ concentration, indicating a preferential synthesis of PGE_2. A similar reversal of the $PGF_{2\alpha}$ to PGE_2 ratio in follicular fluid in the immediate pre-ovulatory period has been found in the rat (LeMaire, Leidner and Marsh, 1975). In the present study, there was also an increase in the follicular fluid concentration of 6-oxo-$PGF_{1\alpha}$, although the increase was not as great as those for PGE_2 and $PGF_{2\alpha}$. Nevertheless, the highest concentration (54.4 ng/ml) of 6-oxo-$PGF_{1\alpha}$ found is the largest value yet recorded for any tissue in any species. An increase in the concentration of 6-oxo-$PGF_{1\alpha}$ at the time of ovulation in the rat ovary has been reported previously (Brown and Poyser, 1984). Overall, the present findings are consistent with ovarian prostaglandins having an essential rôle in ovulation in the pig. Furthermore, follicles which failed to ovulate had low concentrations of prostaglandins. Studies on rabbit ovaries have shown that prostaglandin concentrations increase only in those follicles which actually ovulate (Yang, Marsh and LeMaire, 1974).

The granulosa cells and the thecal cells from pig follicles have the ability to synthesize PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$, with PG synthesis by both cell types increasing significantly in the immediate pre-ovulatory period (Veldhuis, Klase and Demers, 1982 ; Evans *et al.*, 1983 ; Ainsworth *et al.*, 1984). PGE_2 tends to be the major prostaglandin produced by each cell type, and the thecal cells tend to produce more prostaglandins than the granulosa cells (Evans *et al.*, 1983 ; Ainsworth *et al.*, 1984). These studies, together with our findings, suggest that PGE_2 produced by the follicular cells is the major prostaglandin involved in ovulation in the pig. However, in pre-pubertal pigs treated with PMSG and HCG in which ovulation has been prevented by indomethacin treatment, $PGF_{2\alpha}$ and not

PGE_2 is effective in overcoming this blockade of ovulation. Indeed, PGE_2 may even antagonize the effect of $PGF_{2\alpha}$ (Downey and Ainsworth, 1980). This finding suggests that $PGF_{2\alpha}$ is the major prostaglandin involved in ovulation in pigs, although this experiment needs repeating in mature, cycling pigs. The precise rôle of prostaglandins in ovulation remains unkown.

Macroscopically detectable changes in pig Graafian follicles in the last hour before ovulation include a softening of the tissues with the wall becoming flaccid and pendulous, a marked increase in the capillary network with a spread of petechial haemorrhages, and an increased coagulability in the fluid itself (reviewed by Hunter, 1984a). Other workers have recorded changes in the concentration of proteolytic enzymes such as collagenase (Espey, 1974), perhaps acting to destabilise the follicular wall, and in plasminogen activator (reviewed by Thibault and Levasseur, 1979 ; Poyser, 1981), perhaps underlying changes in the physical characteristics of the follicular fluid. Even so, the precise involvement of prostaglandins with each of these steps, with possible muscular contractions in the thecal layers (Guttmacher and Guttmacher, 1921), and with changes in the oocyte and its granulosa cell investment (see Thibault, 1977) merit investigation.

Whilst there is no specific evidence so far for an extra-ovarian rôle of the follicular prostaglandins in reproduction, their involvement in oviduct physiology and especially in sperm activation and release from reservoirs in the oviduct isthmus of domestic animals is certainly worth considering (Hunter, 1984b). If the high titres of prostaglandins found shortly before ovulation were able to leave the follicular tissues in a manner similar to that for steroid hormones by passing into the vascular bed — which at this stage has crossed the basement membrane and colonized the granulosa cells (Corner, 1919) — then they may reach the neighbouring oviduct tissues via a counter-current transfer from the ovarian vein into the oviduct branch of the ovarian artery (Hunter *et al.,* 1983). Such a local transfer of prostaglandins could act to alter the patency and contractility of the oviduct, permitting hyperactivation and progression of spermatozoa from the caudal isthmus to the site of fertilization (Hunter and Nichol, 1983). At present, the complete route of such a transfer is hypothetical and its proof would require the monitoring of the passage of radio-labelled prostaglandin from the pre-ovulatory ovary to the oviduct wall. An alternative, albeit post-ovulatory, route for transfer of high concentrations of prostaglandins to the oviduct is immediately upon follicular collapse at ovulation, when the fluid largely enters the oviduct ampulla. This is not to infer passage of follicular fluid down the oviducts — in fact luminal fluid flow is in the other direction shortly after ovulation — but follicular fluid prostaglandins could nonetheless have a transient and dramatic effect on oviduct physiology (Hunter, 1977).

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Résumé. *Concentration des prostaglandines (E_2, $F_{2\alpha}$ et I_2) dans le liquide folliculaire des ovaires de truies durant la période pré-ovulatoire.*

Les concentrations des prostaglandines PGE_2, $PGF_{2\alpha}$ et PGI_2 (cette dernière estimée en tant que 6-oxo-$PGF_{1\alpha}$) ont été mesurées par radioimmunoessai dans le liquide folliculaire d'ovaires de truies cycliques, adultes, prélevé pendant la période préovulatoire. Dans les 24-32 h qui précèdent l'ovulation, ces concentrations sont faibles. Leurs proportions relatives sont 0,7:1,1:1,0. La concentration de PGE_2 s'élève légèrement pendant les 24 h suivantes, mais dans les 2 heures qui précèdent le moment de l'ovulation, les concentrations moyennes de PGE_2, $PGF_{2\alpha}$ et 6-oxo-$PGF_{1\alpha}$ sont multipliées respectivement par 119, 11 et 5, ce qui aboutit à des proportions relatives de 14,8:2,2:1,0. Un échantillon de liquide folliculaire contient 54,5 ng/ml de 6-oxo-$PGF_{1\alpha}$, la concentration la plus élevée rapportée jusqu'ici, quels que soient le tissu et l'espèce examinés. Mais les concentrations de PGE_2 (529 ng/ml) et de $PGF_{2\alpha}$ (94,6 ng/ml) sont beaucoup plus élevées.

Ces résultats montrent que la période préovulatoire immédiate se caractérise chez la truie cyclique adulte par une augmentation générale du taux des prostaglandines dans le liquide folliculaire, avec une prédominance de la PGE_2. Lorsque l'ovulation ne se produit pas, les concentrations des prostaglandines restent au niveau de base.

Ces résultats montrent le rôle essentiel des prostaglandines dans l'ovulation chez le porc.

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