Luteal concentrations of oxytocin decline during early pregnancy in the ewe

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Summary. The concentration of oxytocin in ovine corpora lutea dropped during pregnancy from 373 ng/g wet weight on Day 14 to <5 ng/g between Day 50 and term. The major decrease occurred before Day 20 after mating. Circulating concentrations of oxytocin also decreased between Days 10 and 15 after mating, and were low in late gestation; however, concentrations of oxytocin in plasma on Days 16 and 17 were raised. Mean luteal concentrations of oxytocin ranged from 730 to 2482 ng/g between Days 8 and 14 of the oestrous cycle in non-pregnant sheep. Secretion of oxytocin by the corpus luteum is thought to be one of the mechanisms leading to luteal regression; therefore loss of oxytocin from the corpora lutea in early gestation may contribute to their maintenance.

Introduction

Circulating concentrations of oxytocin parallel those of progesterone during the oestrous cycle in the ewe (Sheldrick & Flint, 1981; Webb, Mitchell, Falconer & Robinson, 1981; Schams, Lahlou-Kassi & Glatzel, 1982a) and the corpus luteum of the cyclic ewe contains oxytocin (Wathes & Swann, 1982; Flint & Sheldrick, 1982a). Active immunization against oxytocin prolongs the life of the corpus luteum (Sheldrick, Mitchell & Flint, 1980; Schams, Prokopp & Schmidt-Adamopoulou, 1982b) and it has been suggested that oxytocin secreted by the corpus luteum may be involved in the process of luteal regression (Flint & Sheldrick, 1982b, 1983).

The concentration of oxytocin in corpora lutea is reduced in hysterectomized ewes, in which luteal function is prolonged beyond the normal time of luteolysis (Wiltbank & Casida, 1956; Sheldrick & Flint, 1983). The purpose of the present investigation was to determine whether a reduction in the concentration of oxytocin in the corpus luteum is also associated with the maintenance of luteal function in early pregnancy.

Materials and Methods

Animals. Clun Forest ewes kept in paddocks with raddle-bearing intact or vasectomized rams were checked daily for signs of oestrous behaviour.

Jugular venous concentrations of progesterone and oxytocin were measured in 9 ewes sampled daily for one oestrous cycle. Progesterone and oxytocin were also measured in samples taken during the first 20 days of pregnancy from 8 of these ewes, and 5 of these animals were also sampled daily between 8 and 32 days *pre partum*. Blood samples were taken by venepuncture, usually between 14:00 and 16:00 h, into heparinized tubes. Plasma was stored at -20°C until assay.

Corpora lutea from 20 pregnant ewes and from 12 non-pregnant ewes between Days 8 and 14 of the oestrous cycle were extracted and processed for radioimmunoassay as previously described (Flint & Sheldrick, 1983).

Radioimmunoassays. Oxytocin and progesterone were measured in plasma and corpus luteum extracts by radioimmunoassay (Sheldrick *et al.*, 1980; Sheldrick & Flint, 1981; Flint & Sheldrick, 1983). For oxytocin assays, sensitivity, inter- and intra-assay coefficients of variation and extraction recovery from plasma were 2.6 pg/tube, 10.5%, 8.9% and 78.2%. For progesterone assays these values were 14 pg/tube, 11.4%, 5.7% and 89.2% respectively.

Results

Mean $\pm$ s.e.m. concentration of oxytocin in 10 corpora lutea from 8 non-pregnant ewes between 8 and 12 days after an infertile mating was 1970 ± 616 ng/g wet weight (Table 1). The level on Day

Text-fig. 1. Concentrations of oxytocin and progesterone measured in samples of jugular venous plasma obtained daily (between 14:00 and 16:00 h) from 9 sheep during one infertile oestrous cycle and in 8 of these sheep after mating to an intact ram. Values are means $\pm$ s.e.m. All pregnant ewes delivered live lambs at normal term (145 ± 3 days).

Table 1. Concentrations of oxytocin measured by radioimmunoassay in acid extracts of corpora lutea

Day after mating	No. of:		Wt of corpus luteum (mg)	Luteal oxytocin conc. (ng/g wet wt)
	Animals	Corpora lutea		
<i>Non-pregnant</i>				
8	6	6	598 ± 69	2482 ± 995
10	1	2	491; 512	823; 914
12	1	2	843; 708	1259; 1814
14	4	6	514 ± 64	730 ± 266
<i>Pregnant</i>				
14	4	6	694 ± 86	373 ± 256
15	1	2	780; 400	103; 208
17	3	4	830 ± 63	310 ± 52
20	1	1	730	17
21	1	2	726; 882	111; 123
30	1	2	571; 540	37; 31
53	1	2	640; 530	3; 4
60	1	3	503 ± 59	1 ± 0.3
70	2	4	817 ± 20	4 ± 0.4
76	1	2	400; 870	3; 5
121	1	2	590; 780	1; 2
133	1	2	550; 678	1; 2
135	1	2	450; 460	1; 1
137	1	1	910	2

14 (730 ± 266 ng/g) was below this mean value ($P < 0.005$, t test). In pregnant animals, luteal oxytocin levels decreased sharply after Day 14, and remained in the range 1–5 ng/g from Day 53 to late in gestation. It is not known whether these levels were maintained until parturition. The concentration on Day 14 of pregnancy was not significantly different from that on Day 14 of the cycle.

Circulating concentrations of oxytocin and progesterone increased with formation of the corpus luteum after non-fertile and fertile matings, as reported previously (Flint & Sheldrick, 1983; Text-fig. 1). However, peripheral plasma oxytocin levels were low on Days 14 and 15 of pregnancy, subsequently rising again on Days 16 and 17; the mean level on Days 14 and 15 was 5.7 ± 1.2 pg/ml ($n = 14$ samples); on Days 7–12 this value was 12.2 ± 1.3 pg/ml ($n = 46$; $P < 0.001$), and on Days 17–20, 13.9 ± 1.9 pg/ml ($n = 28$; $P < 0.001$). In 5 ewes sampled daily between 8 and 32 days *pre partum* the mean peripheral plasma oxytocin concentration was 6.2 ± 0.5 pg/ml ($n = 115$ samples). A transient rise in circulating progesterone concentration ($P < 0.005$) accompanied the reduced level of oxytocin on Days 14 and 15.

Discussion

The drop in the concentration of oxytocin in luteal tissue during pregnancy may be caused by a uterine factor or result from the process of luteal maintenance. The fact that hysterectomy leads to reduced luteal oxytocin levels does not help to differentiate between these possible mechanisms, since luteal function is also maintained after hysterectomy, as in pregnancy.

Evidence implicating luteal oxytocin secretion in the process of luteolysis includes the effect of active immunization against the peptide (Sheldrick *et al.*, 1980; Schams *et al.*, 1982b), the luteolytic effect of administered oxytocin which, although less marked than in the cow and goat (Armstrong & Hansel, 1959; Cooke & Knifton, 1981), has been reported in sheep (Milne, 1963), and the episodes of oxytocin secretion detected in sheep at luteal regression (Fairclough *et al.*, 1980; Flint & Sheldrick, 1983). In addition to these findings, which indicate that oxytocin may be involved in systemic mechanisms controlling luteal regression, there is evidence that gonadotrophin-stimulated steroid synthesis is inhibited by neurohypophysial hormones in isolated Leydig cells and

luteal cells in culture (Adashi & Hsueh, 1981, 1982; Tan, Tweedale & Biggs, 1982a, b). Therefore, in the context of local or systemic mechanisms, the loss of oxytocin from the corpus luteum in early pregnancy would be expected to lead to luteal maintenance. This is consistent with the decline in the antiluteolytic influence of the embryo between Days 16 and 21 of gestation, as indicated by failure of embryo extracts made after Day 15 to 16 to prevent luteal regression when administered into the uterus (Rowson & Moor, 1967; Martal, Lacroix, Loudes, Saunier & Wintenberger-Torrès, 1979). Since the time at which oxytocin disappears from the corpus luteum corresponds to the period over which embryonic antiluteolytic activity is lost, it is tempting to speculate that the loss of embryonic antiluteolysin is tolerated because of the disappearance of luteal oxytocin. The reduced susceptibility of the corpus luteum of pregnancy to administered prostaglandin (Nancarrow, Evison & Connell, 1982) may also be a reflection of this change in the mechanisms maintaining luteal function. A decrease in luteal oxytocin, besides reducing the likelihood of luteal regression, might also be expected to reduce uterine contractility, representing a further factor contributing to the successful establishment of pregnancy.

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