Repeatability of events during spontaneous and gonadotrophin-induced oestrus in bitches

G. C. W. England and W. E. Allen

Obstetrics Unit, Royal Veterinary College, University of London, Hawkshead Lane, North Mymms, Hatfield AL9 7TA, UK

Summary. Oestrus induction using equine chorionic gonadotrophin and human chorionic gonadotrophin was successful in five out of six bitches, although the first day of increased plasma progestagen concentration differed considerably between bitches. Induced oestrous periods differed from spontaneous cycles in the timing of vaginal epithelial cell cornification; plasma oestrogen concentrations were generally greater and progestogen concentrations were less in induced cycles. These results suggest that this schedule of oestrus induction would not be suitable for allowing mating on a predetermined day.

Keywords: oestrus induction; gonadotrophins; progestogen; oestrogen; oestrus; dog

Introduction

The interoestrous interval of bitches is 31 weeks, on average (Rowlands, 1950; Christie & Bell, 1971). Bitches with longer than average interoestrous intervals and those that are slow to reach puberty (Chakraborty *et al.*, 1982) have reduced chances of becoming pregnant. The development of a method to induce oestrus effectively and predictably in bitches would be advantageous, especially in a breeding establishment where a continuous supply of pups is required.

Equine and human chorionic gonadotrophins were first used for oestrus induction by Scorgie (1939); many other workers have used these preparations in different regimens and doses with varying success (Greenblatt & Pund, 1941; Bell & Christie, 1971; Wright, 1972; Jones *et al.*, 1973; Thun *et al.*, 1977; Archbald *et al.*, 1980; Wright, 1980; Allen, 1982; Barta *et al.*, 1982; Wright, 1982; Toniollo & Ferreira, 1984; Nakao *et al.*, 1985). Others have attempted the induction of oestrus using oestrogens alone or in combination with gonadotrophins (Papanicolaou & Blau, 1927; Hancock & Rowlands, 1949; Renton *et al.*, 1981; Chakraborty *et al.*, 1982; Ishihara *et al.*, 1982; Moses & Shille, 1988) and a variety of other compounds (Einer-Jensen, 1967; Auskova *et al.*, 1974; Carter, 1980; Vanderlip *et al.*, 1987; Cain *et al.*, 1989; Concannon, 1989; Shille *et al.*, 1989).

The success of induction differs between reports, even when similar regimens are used. There are problems in comparing results because most workers used few animals and definitions of anoestrus based upon behavioural and cytological information, and made no distinction between early and late anoestrus. Some workers used normal bitches, but others used bitches with prolonged anoestrus, and the criteria for success were different, e.g. ovulation, pregnancy or whelping.

The feasibility of mating on a fixed day following the initiation of an oestrus induction regimen was investigated and the findings were compared with spontaneous oestrus in the same bitches.

Materials and Methods

The method of Arnold *et al.* (1989) was used to induce oestrus because the reported plasma hormone concentrations resembled those of natural oestrus. Six clinically normal crossbred bitches were used for the study; each was 3–4.5 months from the end of the previous oestrus, and vaginal smears were typical of anoestrus.

Oestrus induction was attempted in each bitch with five daily subcutaneous injections of 20 iu equine chorionic gonadotrophin (Folligon: Intervet UK Ltd, Cambridgeshire, UK)/kg and 500 iu of human chorionic gonadotrophin (Chorulon: Intervet UK Ltd, Cambridgeshire, UK) given intramuscularly on the fifth day.

Heparinized blood samples were collected from the jugular vein for oestrogen and progestagen determination by radioimmunoassay. Total unconjugated oestrogens were measured by radioimmunoassay as described by Challis *et al.* (1971) using an antiserum that mainly cross-reacted with oestradiol-17- β (100%), 6- α hydroxyoestradiol (82%), oestradiol-17- α (5.3%), oestrone (4.8%) and oestriol (1.3%). The assay detection limit was 5 pg/ml and the interassay coefficient of variation at 25 pg/ml was 6.8%; at 250 pg/ml it was 19.3%. Progestagens were measured as progesterone by the radioimmunoassay method described by Cockrill & Allen (1978). The antiserum cross-reacted mainly with progesterone (100%), 11- α -hydroxyprogesterone (85%), 17- α -hydroxyprogesterone (12.5%), 5- β -pregnane-3,20-dione (12.5%), 5- α -pregnane-3,20-dione (3%), 5- β -pregnane-3- β -ol-20-one (1.7%), 11-deoxycorticosterone (1.1%) and 5- α -pregnane-3- β -ol-20-one (1%). The assay detection limit was 0.05 ng/ml and the interassay coefficient of variation at 0.63 ng/ml was 35% and at 1.79 ng/ml was 16.8%.

Vaginal smears were collected daily. The smears were stained with M&D Diff-Quik (American Hospital Suppliers, Didcot, Oxfordshire, UK) and cytology was examined. The percentage of epithelial cells assessed as cornified using this stain was calculated as the 'cornification index' (van der Holst & Best, 1976). When examined in this manner, the number of cornified cells increases in oestrus and, after a small decrease, increases again. This double cornification peak has been reported previously (van der Holst & Best, 1976; England & Allen, 1989).

The degree of vulval swelling was scored visually on a scale between 0 (anoestrus) and 5 (maximal vulval oedema). The behavioural response to teasing with a male was scored as 0, aggression; 1, lack of interest; 2, playing; 3, teasing behaviour by the bitch; 4, tail deviation; and 5, standing to be mounted.

Approximately 10 days after the onset of metoestrus (following induction), as assessed by vaginal cytology, the ovaries were removed at laparotomy. They were fixed in 10% buffered formal saline; histological sections were then cut and stained with haematoxylin and eosin.

All variables except metoestrous ovarian histology had been previously recorded for all the bitches during a spontaneous oestrous cycle. The timing of the events of the induced cycles (proestrus and cornification) were compared with the spontaneous cycles to examine their repeatability. Each event was timed from the onset of hormone therapy (Day 1) or the onset of spontaneous proestrus (Day 1) for comparison. The length of proestrus was defined as the period from the onset of vulval swelling to the onset of standing oestrus.

However, it has been shown previously (England *et al.*, 1989) that the clinical events of spontaneous cycles may not be repeatable in the same bitch, so that comparisons between spontaneous and induced cycles are difficult to make. To overcome this problem, cycles were also compared relative to the first day that plasma progestagen concentration increased above 0.9 ng/ml; this was designated Day 1.

Statistical analysis of the results was performed using the Student's *t* test; values were considered significant when $P < 0.05$. Values are given as means $\pm$ s.d.

Results

The behavioural manifestations of oestrus were successfully induced in five of the six bitches (Fig. 1). Histological examination of the ovaries of these five bitches demonstrated 3–6 corpora lutea within each ovary. These were characterized by thick, infolded luteal tissue with small, central, fluid-filled cavities. In the remaining bitch, behavioural oestrus was not induced and there was no significant change in plasma hormones or vaginal cytology; ovarian histology was not examined. This bitch had the shortest interval (3 months) from the previous oestrus. With the exception of the onset of the rise in plasma progestagen concentrations, the timing of events following the beginning of hormone therapy was consistent within 4 days (Table 1).

When spontaneous and induced oestrous periods were compared relative to the first day of increased plasma progestagen, the onset of the first peak of cornified epithelial cells occurred significantly earlier when oestrus was spontaneous (Table 2). The greatest difference between spontaneous and induced cycles was the range of each event when related to the first day of increased plasma progestagen. Variations were greater during induced cycles for all events, with the exception of the first day of standing oestrus.

The maximum percentage second peak cornification during spontaneous cycles was 93 ± 5.1 (s.d.) and for induced cycles was 86 ± 3.9 (s.d.); these values were significantly different ($P < 0.05$).

There was considerable variation in oestrogen and progestagen concentrations between spontaneous cycles (Fig. 1). In the induced cycles, oestrogen concentrations increased after the administration of equine chorionic gonadotrophin and were greater than those of the spontaneous cycles in three of the five bitches. Progestagen concentrations were always low when compared with the

Fig. 1. Plasma hormone concentrations during spontaneous (S) and induced (I) cycles in five bitches relative to the first day of increased plasma progestagen concentration. Bars represent periods of proestrus (p) and oestrus (o); oestrogen (—●—) and progesterone (·····) in S cycles; oestrogen (—) and progesterone (·····) in I cycles.

Table 1. Timing of events of induced oestrus measured from the onset of hormone therapy for five bitches

	Mean (days)	± s.d.	Range (days)
Onset of vulval swelling	6.0	1.7	4–8
Length of proestrus	7.4	1.5	5–9
Onset of standing oestrus	12.0	1.9	10–14
Length of standing oestrus	5.2	1.3	4–7
First cornification peak	12.2	1.6	10–14
Second cornification peak	14.5	1.9	12–16
First day of increased progestagen	12.0	3.5	9–18

spontaneous cycles in the same animals. Mean progestagen concentrations 5 days after the initial rise were 7.4 ± 4.6 and 3.2 ± 1.5 (s.d.) ng/ml for spontaneous and induced cycles, respectively; these values were not significantly different.

Table 2. Comparison of the timing of events of spontaneous (S) and induced (I) oestrus in five bitches. Values are the mean number of days before (–) or after (+) the first day of increased plasma progesterone concentration

		Mean (days)	± s.d.	Range (days)
Onset of vulval swelling	S	–9.0	1.4	–11 to –8
	I	–6.0	3.3	–11 to –2
Onset of standing oestrus	S	+1.8	3.2	–1 to +5
	I	+0.8	3.2	–1 to +3
1st cornification peak	S	–2.3*	0.9	–1 to –3
	I	+1.4*	3.6	–5 to +4
2nd cornification peak	S	+2.5	1.3	+1 to +4
	I	+3.3	3.6	–2 to +5

*Significantly different $P < 0.05$.

Discussion

Oestrus was induced in five of the six bitches as determined by behaviour and the presence of corpora lutea. The bitch that did not respond had the shortest interval from the previous oestrus. The time of occurrence of the onset of vulval swelling, the length of proestrus, the onset and length of standing oestrus and the occurrence of vaginal cytological cornification were similar between animals after hormone administration, but the onset of increased plasma progesterone concentrations (Days 9–18), presumably reflecting the timing of ovulation, was very variable. This would not allow for the mating day to be predetermined and confirms the differences that can occur between hormonal indices and clinical events (Wildt *et al.*, 1978).

The timing of the onset of proestrus, the onset of standing oestrus and the second peak of vaginal cell cornification was not statistically different between induced and spontaneous cycles when they were related to plasma progesterone concentrations. However, only a few animals were used and when the cycles were induced there was a considerable range for each event. These observations suggest that, when oestrus is induced in bitches, the timing of the associated events will not be either repeatable or similar to those of spontaneous cycles.

There was a significant difference between the induced and spontaneous cycles in the timing of the onset of the first cornification peak relative to the first day of increased plasma progesterone. The earlier onset of cornification in the induced cycles may be related to the abnormal oestrogen profiles. The regimen of Arnold *et al.* (1989) was used because these workers had found that longer periods of equine chorionic gonadotrophin treatment resulted in hyperoestrogenism. The results of the present study show that excessive oestrogen production may occur even with this regimen. This could have been due, in part, to differences in the potency of preparations of equine chorionic gonadotrophin, or related to the oestrogen assays.

The degree of vaginal cell cornification was significantly less in the induced than in the spontaneous cycles; this is difficult to explain because oestrogen concentrations were generally greater, but similar findings were reported by Leatham & Morrell (1939). Progesterone concentrations following induction were low, and mean values 5 days after the initial progesterone rise were lower (although not significantly) than for spontaneous cycles. This has been reported previously (Jones *et al.*, 1973), and may be related to the small size and short lifespan of corpora lutea formed after such treatment (Bell & Christie, 1971; Jones *et al.*, 1973; Barta *et al.*, 1982).

Fertility was not assessed in the present study, although in induced cycles high oestrogen concentrations might prevent implantation, and low progesterone concentrations might be insufficient to support pregnancy. This method of oestrus induction appears to be pharmacological and does

not produce repeatable physiological events. Despite these problems, Arnold *et al.* (1989), using the same regimen, achieved pregnancy in three of six bitches.

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