Ovarian hyperstimulation syndrome in a bitch caused by recombinant human chorionic gonadotropin treatment of suspected luteal insufficiency – a case report

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Abstract

Two bitches with a history of hypoluteoidism were treated by recombinant human chorionic gonadotropin (r-hCG) in repeated doses during early dioestrus. The level of progesterone increased, but the therapy led to hyperstimulation of the ovaries which resulted in ovarian hyperstimulation syndrome (OHSS), with subsequent development of oestrogen toxicity. This is the first case documenting OHSS in a canine after administration of r-hCG. Although manifested during dioestrus in our case report, the occurrence of OHSS is associated with similar administration of r-hCG to women. The described use of r-hCG is not appropriate for luteal insufficiency treatment, but seems to have a place in assisted reproduction technology because of its surprising effects. Further research of luteotrophic factors in bitches is warranted because of these unpredictable results. Lower doses and frequency of administration need to be considered for a better outcome.

Dog, cystic ovarian disease, hypoluteoidism, hCG, ultrasonography

Progesterone is key hormone for pregnancy maintenance in canines. Insufficient secretion of progesterone by corpora lutea (CL) during pregnancy, so-called hypoluteoidism or luteal insufficiency, is one of the possible causes of pregnancy loss or infertility (Root Kustritz 2001; Zedda et al. 2017). A progesterone peak is observed during the first half of a pregnancy, with a minimum concentration of 47.7 nmol/l, according to various authors (Hadley 1975; Fernandes et al. 1987; Concannon 2009). The only source of progesterone in bitches are the CL (Kiso and Yamauchi 1984; Concannon 2009).

Luteinizing hormone (LH) plays a very important role in luteal function as one of the luteotrophic factors, and its failure causes canine hypoluteoidism (Concannon 1980; Fernandes et al. 1987). Human chorionic gonadotropin (hCG) can substitute for LH in assisted reproduction protocols. No serious side effects of hCG have been reported in animals (Tilley and Smith 2007; Plumb 2008). Because of its purity, recombinant hCG (r-hCG) shows fewer local side effects and provides more consistent results (The European Recombinant Human Chorionic Gonadotrophin Study Group 2000; Ludwig et al. 2003). However, the administration of both hCG and r-hCG can cause ovarian hyperstimulation syndrome (OHSS) in women (The European Recombinant Human Chorionic Gonadotrophin Study Group 2000; Ludwig et al. 2003). In dogs, OHSS after r-hCG administration has not yet been described. Herein, we present this condition in two bitches as an iatrogenic complication of r-hCG treatment of early luteal insufficiency.

Case description

Two bitches with suspected hypoluteoidism were treated by r-hCG during early dioestrus. The first case was well documented; information regarding the second case is incomplete.

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Case One

A five-year-old, nulliparous Shar Pei, weighing 20 kg, had a history of early embryonic resorption with luteal insufficiency. She expressed insufficient progesterone concentrations during her previous oestrus cycles and short interoestrus intervals (4–5 months) independently on previous mating. She was inseminated twice during two different oestrus cycles and the second artificial insemination (AI) was followed by the r-hCG treatment. Nine months before the r-hCG treatment, the first AI was performed by intrauterine laparotomic technique with frozen-thawed sperm on day LH 6 (LH 0 determined by progesterone measurement) despite the fact that the progesterone was 48.7 nmol/l (Immulite® 1000 Immunoassay System, Siemens Healthcare Diagnostics Inc., NJ, USA). Low rise of progesterone concentration after ovulation occurred regularly in this bitch during all three oestrous cycles when progesterone was measured. We confirmed an embryonic resorption thirty-five days after surgical insemination. Vaginal cytology demonstrated a typical dioestral smear; haematological and biochemical indices, including thyrotropin and thyroxine, were without significant changes. The bitch was without signs of any disease, no discharge was present. There was no suspicion in her history that the cause of the embryonic resorption was an infectious disease, therefore, other tests were not performed. The progesterone concentration of 16.6 nmol/l was considered to be the cause of the pregnancy loss.

Case Two

A three-year-old Tosa, weighing 72 kg, was inseminated intravaginally six months before the r-hCG treatment, during her previous cycle (timing with progesterone, missing data). The pregnancy was excluded by ultrasonography 30 days after that without signs of embryonic resorption. Thyroxine and progesterone concentrations as determined by a private veterinarian were 32 nmol/l and 31.8 nmol/l, respectively.

Procedures

All examinations and therapeutic procedures were performed at the explicit request and with the informed consent of the dog owners.

Both bitches underwent AI by an endoscopic technique described by Hollinshead and Hanlon (2017) at our workplace. It was performed with frozen-thawed sperm on progesterone at a concentration of 59.1 nmol/l in the Shar Pei (her second AI), and with fresh semen on progesterone at a concentration of 77.9 nmol/l in the Tosa. Progesterone evaluation during the second week after AI revealed low concentrations in both bitches. Progesterone concentrations during the oestrus cycle and after AI are shown in Fig. 1.

Treatment using r-hCG at subcutaneous doses of 50 IU/kg, every 48 h (Ovitrelle 250 micrograms/0.5 ml, Merck Serono S.p.A., Italy) was initiated from day 9 and 11 after AI in the Tosa and the Shar Pei, respectively. Five doses were administered in total.

The first ultrasonography during treatment revealed normal-sized ovaries (Plate VII, Figs 2A,B) in both bitches, an intact uterus in the Tosa, and an early pregnancy with embryonic resorption in the Shar Pei. Progesterone concentrations increased above 63.6 nmol/l in both bitches. The same side effects were reported in both bitches after the fourth and fifth doses (Tables 1, 2).

Nonregenerative anaemia and thrombocytopaenia developed within 13 to 19 days from the start of the r-hCG treatment. Concentrations of both oestradiol and progesterone were increased, and enlarged polycystic ovaries were recorded during this period (Plate VIII, Figs 2C,D). Both bitches were treated by supportive and symptomatic therapy. No further information on the Tosa is available. We recorded basal oestradiol concentrations and regression of clinical signs in the Shar Pei 10 days after the termination of the r-hCG treatment. Initial leukocytosis was replaced by leucopaenia and all clinical signs disappeared

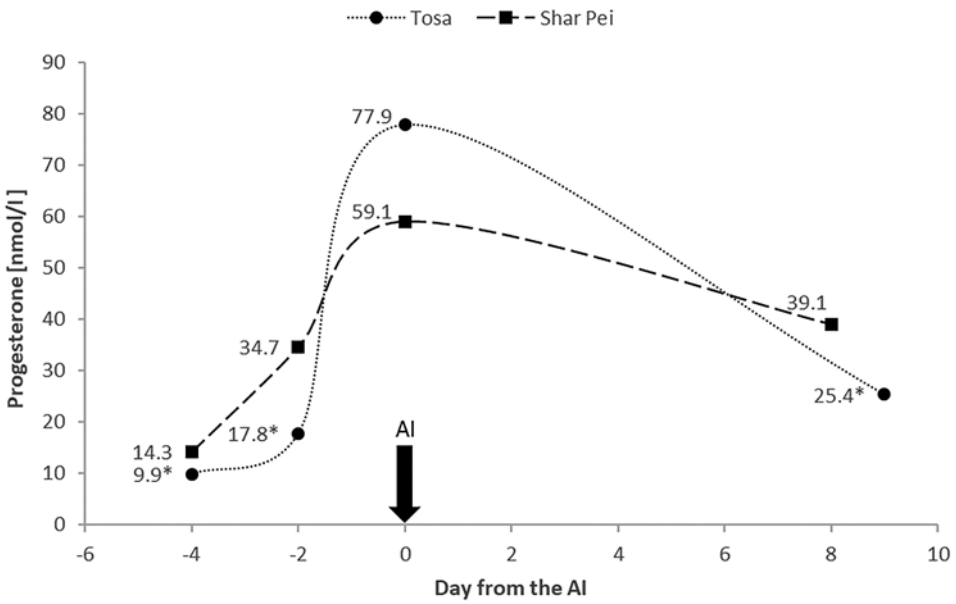

Fig. 1. Progesterone before, during and after artificial insemination (AI) in both bitches. Progesterone measured by Immulite® 1000 Immunoassay System, Siemens Healthcare Diagnostics Inc., NJ, USA. Values marked with * were measured in private practice.

20 days after the end of the r-hCG treatment. All haematological indices were within the reference range 3 months after the last dose of r-hCG (this examination performed by a private veterinarian). Detailed descriptions of both cases are shown in Tables 1 and 2.

Discussion

Luteal insufficiency is generally observed from the 4th week of pregnancy and later (Root Kustritz 2001; Tibold and Thuróczy 2009). We recorded low progesterone concentrations during the 2nd week, although progesterone concentrations reach their peak between days 5 and 25 of dioestrus (Hadley 1975; Fernandes et al. 1987; Concannon 2009). Low progesterone concentrations were considered as hypoluteoidism, although a luteinization defect may have occurred. Common therapy by progestins was excluded to avoid teratogenicity in pups (Curtis and Grant 1964; Root Kustritz 2005; Zedda et al. 2017); therefore, we suggested the r-hCG treatment.

The views of various authors on the luteotrophic effect of LH differ. While results reported by Okkens et al. (1986) indicate that CL are independent of the LH production until day 24 to 28, Concannon (2009) reported that LH is luteotrophic already from day 14 following the LH surge, if not earlier. The response of both bitches to r-hCG treatment supports the claim made by Concannon.

Recombinant human chorionic gonadotropin increased the secretion of endogenous progesterone, which was observed even 20 days after administration (Tables 1, 2). This establishes the LH effect of r-hCG, which was expected because of hormone homology (Chandrasekher et al. 1994; The European Recombinant Human Chorionic Gonadotrophin Study Group 2000). The unexpected oestradiol increase shows that r-hCG probably has effects of both LH and follicle-stimulating hormone in bitches.

Table 1. Description of Case One (Shar Pei). Recombinant human chorionic gonadotropin (r-hCG) was administered on days 1, 3, 5, 7 and 9 of r-hCG treatment at a 48-h interval.

Day after insemination	11	18	19	23	29	39
Day of r-hCG treatment	1	8	9	13	19	29
Number of r-hCG doses administered	1	4	5	5	5	5
Progesterone (nmol/l)	Missing data	64.9	Missing data	126.6	> 127	67.4
Oestradiol (pmol/l)	Missing data	Missing data	Missing data	1083	< 36.7	Missing data
Clinical signs	No signs, good health	No signs, good appetite	Lethargy, inappetence, polyuria/polydipsia, serosanguinous vulvar discharge, abdominal enlargement, pain	Lethargy, inappetence, polyuria/polydipsia, serosanguinous vulvar discharge, no pain	Mild polydipsia, without vulvar discharge	Usual drinking and appetite
Haematology, biochemistry	Missing data	Missing data	Missing data	Leukocytosis with a left shift (neutrophilia, monocytosis), slight thrombocytopaenia, other indices within the reference range	Leukocytosis with a left shift (neutrophilia), anaemia, severe thrombocytopaenia	Anaemia, leukopaenia (neutropaenia), severe thrombocytopaenia
Ultrasonography (maximum measured size) Uterus	Missing data	Two spherical 8 mm gestational sacs without embryos, several places with embryonic resorption	Missing data	Several places with resorption, 14 mm collapsed sacs with hypoechoic content	7–8 mm in diameter with several irregular sacs without embryos	Small amount of anechoic content, cystic endometrial hyperplasia
Description of ovaries		Rounded thick-walled structures about 6 mm in diameter		Multiple anechoic cystic structures up to 9 mm in diameter	Fewer 6–7 mm cystic structures	Fewer anechoic rounded structures up to 5 mm
Left ovary (cm)		3.0 × 1.5		3.8 × 2.6	3.2 × 2.4	3.1 × 1.4
Right ovary (cm)		3.2 × 1.5		4.2 × 3.4	4.2 × 2.4	2.9 × 1.8

Table 2. Description of Case Two (Tosa). Examination performed by private practitioner. Recombinant human chorionic gonadotropin (r-hCG) was administered on days 1, 3, 5, 7 and 9 of r-hCG treatment at a 48-h interval.

Day after insemination	9	16	19	24
Day of r-hCG treatment	1	8	11	16
Number of r-hCG doses administered	1	4	5	5
Progesterone (nmol/l)	25.4	Missing data	> 63.6	> 63.6
Oestradiol (pmol/l)	Missing data	Missing data	Missing data	955
Clinical signs	No signs, good health	Lethargy, inappetence, polyuria/polydipsia	Serosanguinous vulvar discharge, abdominal enlargement, pain	Missing data
Haematology, biochemistry	Missing data	Missing data	Indices within the reference range	Anaemia, thrombocytopaenia
Ultrasonography	Missing data	Missing data	Non-pregnant, typical dioestral	Polycystic enlarged ovaries similar to kidneys in size

Excessive hormonal stimulation led to a similar ovarian hyperstimulation syndrome known in women with similar clinical signs. Enlarged ovaries with multiple cystic structures were detected by ultrasonography. Enlarged ovaries caused abdominal pain and enlargement, and presumably nausea-induced inappetence. Abdominal discomfort could lead to lethargy. Ascites described in women (Beerendonk et al. 1998) was not present in either bitch. Polyuria/polydipsia is not present in humans, while oliguria is common (Manau et al. 2002). Cystic endometrial hyperplasia (CEH) which was found on day 29 of the r-hCG treatment in the Shar Pei could be caused by overproduction of oestradiol and progesterone which are responsible for CEH development in bitches (Chen et al. 2001; Marinković et al. 2018). This potential side effect can then negatively affect the reproductive potential of the bitch.

OHSS resulted in oestrogen toxicity in both bitches. High oestrogen concentrations can cause myelotoxicity leading to thrombocytopaenia, anaemia, and leukopaenia, as we describe in both bitches. Oestrogen-induced myelotoxicity has been observed after repeated administration of exogenous oestrogens (Tsutsui et al. 2006) and in cases of testicular and ovarian tumours with endogenous production (McCandlish et al. 1979; Suess et al. 1992; Sanpera et al. 2002). The observed initial leukocytosis with a left shift followed by leukopaenia in the Shar Pei is consistent with reported symptoms (Crafts 1948; Gaunt and Pierce 1986).

Conclusion

The treatment by r-hCG caused an increase in progesterone concentration, however, it did not lead to pregnancy preservation and even induced OHSS with subsequent oestrogen toxicity. However, it is impossible to draw any clear conclusions based on these surprising findings, as only two animals were treated. It seems that

r-hCG might be used in assisted reproductive techniques, however, further research is warranted. The authors recommend a reduction of the dose and frequency of administration, together with numerous check-ups to prevent unwanted side effects.

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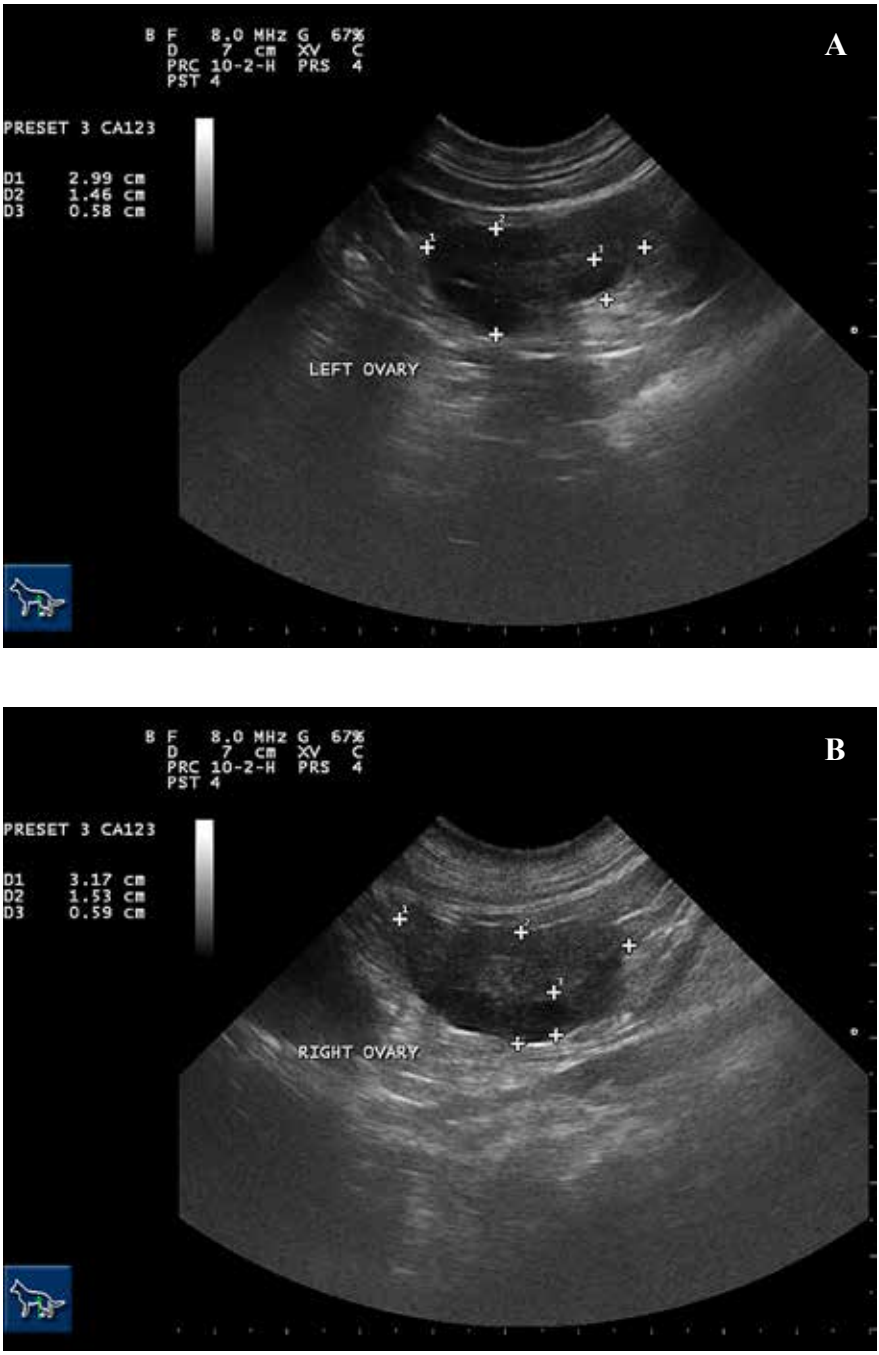

Fig. 2A,B. Ultrasonography of the Shar Pei's ovaries during recombinant human chorionic gonadotropin treatment. Sagittal view of both ovaries, performed by MyLab™ 40 VET, Esaote S.p.A., Genova, Italy. A and B show ovaries on day 18 after insemination

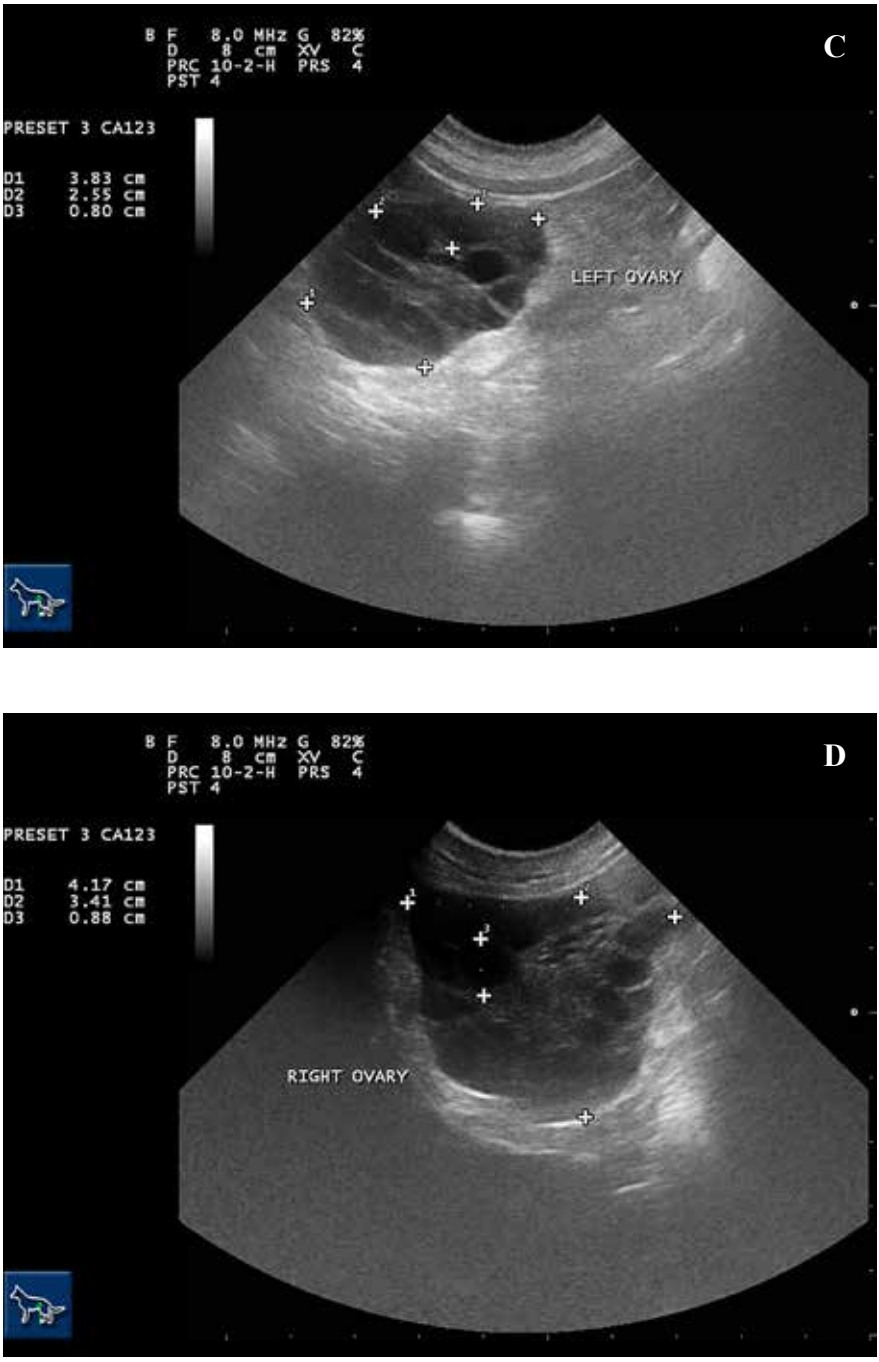

Fig. 2C,D. Ultrasonography of the Shar Pei's ovaries during recombinant human chorionic gonadotropin treatment. Sagittal view of both ovaries, performed by MyLab™ 40 VET, Esaote S.p.A., Genova, Italy. C and D show ovaries on day 23 after insemination.