Journal of the American Association for Laboratory Animal Science
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Vol 46, No 3
May 2007
Pages 64–67

Case Reports

Ovarian Hyperstimulation Syndrome in Gonadotropin-treated Laboratory South African Clawed Frogs (*Xenopus laevis*)

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Ovarian hyperstimulation syndrome (OHS) is a rare but sometimes fatal iatrogenic complication of ovarian stimulation associated with the administration of exogenous gonadotropins to women undergoing treatment for infertility. Laboratory *Xenopus* spp are commonly treated with human chorionic gonadotropin (hCG) to stimulate ovulation and optimize the number of oocytes harvested for use in biomedical research. Here we report cases of OHS in 2 gonadotropin-treated laboratory *Xenopus laevis*. After receiving hCG, the frogs developed severe subcutaneous accumulation of fluid, coelomic distention, and whole-body edema and were unable to dive, although they continued to eat and swim. At postmortem examination, extensive subcutaneous edema was present; ascites and massive numbers of free-floating eggs were found in the coelomic cavity and in aberrant locations: around the heart-sac and adhered to the liver capsule. Whole-body edema, gross enlargement of the ovaries, ascites, and abdominal distention are findings comparable to those observed in women with OHS. The pathophysiology of OHS is thought to be related to hormonally induced disturbances of vasoactive mediators, one of which may be vascular endothelial growth factor secreted by theca and granulosa cells. We know of no other report describing OHS-like symptoms in gonadotropin-treated frogs, and the cases described here are 2 of the 3 we have observed at our respective institutions over the last 6 y. According to these results, OHS appears to be rare in gonadotropin-treated laboratory *Xenopus*. However, the condition should be included in the differential diagnosis for the bloated frog.

Abbreviations: hCG, human chorionic gonadotropin; OHS, ovarian hyperstimulation syndrome; PMSG, pregnant mare serum gonadotropin

Under appropriate conditions, gametogenesis can occur year-round in the South African clawed frog (*Xenopus laevis*), a species that typically produces thousands of eggs each cycle. In laboratory settings, eggs and immature oocytes can be repeatedly collected from the same frog by treating the animal with gonadotrophic hormones, usually urinary human chorionic gonadotropin (hCG). The dose for hCG in frogs is not standardized, varies widely among research laboratories, and is generally between 50 and 800 IU per animal (injections are given in the dorsal lymph sac), either alone or in combination with a 'priming' dose of pregnant mare serum gonadotropin (PMSG; dose ranges from 100 to 800 IU/frog).[16,23,24] With this approach, laboratory *Xenopus* can be stimulated to provide a continuous and readily available supply of material for both basic and biomedical research in vertebrate embryology, cellular biology, physiology, genetics, and biochemistry.

Most laboratories can collect sufficient quantities of oocytes or mature eggs from the same healthy frog for several years without complications, by allowing the animal to rest 1 to 3 mo between collections.[16,24] However, the effect of hormone treatments on the animal's health and quality of eggs or oocytes is unknown. Here we report 2 suspected cases of ovarian hyperstimulation syndrome (OHS) in laboratory frogs, an apparently rare but fatal complication associated with induction of ovulation by exogenous administration of gonadotropic hormones for the purpose of harvesting eggs and oocytes for biomedical research.

Received: 17 Nov 2006. Revision requested: 29 Dec 2006. Accepted: 29 Dec 2006.
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Case Reports

Case 1. In Spring 2002, an adult, approximately 90 g, female South African clawed frog (*X. laevis*; purchased from NASCO, Madison, WI) housed in the *Xenopus* collection at University of California-Berkeley was primed with an injection of PMSG (100 IU subcutaneously; Sigma-Aldrich, St Louis, MO), and ovulation was induced with an injection of hCG (500 IU subcutaneously; Sigma-Aldrich) 1 wk later. After priming, the frog was placed in a 19-l plastic tank containing 15-l of fresh, filtered dechlorinated water and allowed to lay eggs overnight. The eggs were collected the following morning, and the frog returned to a 190-l pond-style static, self-flushing (100% water exchange) tank with 5 other postovulatory frogs. The water is filtered, dechlorinated, and maintained at a temperature range between 19 to 21 °C. The light cycle in the room is an alternating 12:12-h light:dark cycle. The frogs were fed *Xenopus* brittle (NASCO) 2 to 3 times weekly, 3 h prior to a complete water change. These procedures were covered under protocols approved by the University of California–Berkeley Institutional Animal Care and Use Committee. The animal was identifiable via a personal identification tag located subcutaneously.

Twelve days after gonadotropin injection, the frog presented with generalized distention of the subcutaneous space that extended over the whole body (Figure 1 A) and a superficial

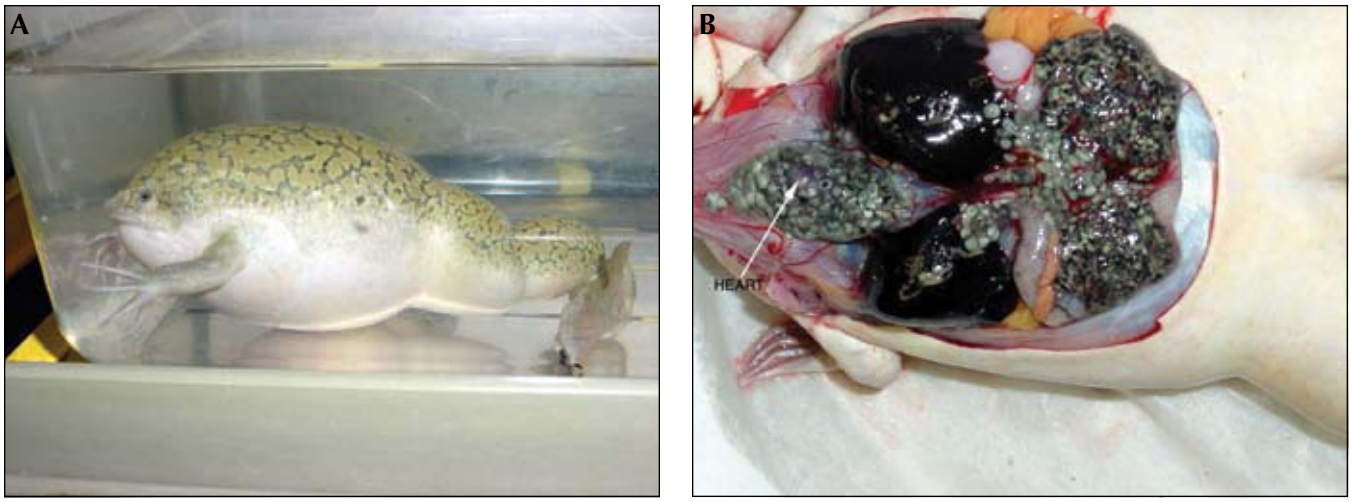

Figure 1. (A) Case 1: A 2- to 3-y-old female South african clawed Frog (*Xenopus laevis*) with ovarian hyperstimulation syndrome. Note bloating, whole-body edema, separation of the skin from the subcutaneous space and the blunted appearance to the snout. (B) At gross necropsy, numerous degenerative oocytes were free in the coelomic cavity and surrounded the heart.

abrasion (a presumed bite wound) on the right cranial limb. Copious amounts of clear, nonviscous, acellular fluid were aspirated from the dorsal lymph sac and other subcutaneous compartments. The specific gravity of the fluid was less than 1.003 according to a handheld refractometer. Examination of Wright-stained slides of the aspirate under a microscope did not reveal the presence of microorganisms. The frog was isolated and monitored daily for 1 wk, during which time the frog ate normally and the limb lesion began to heal, but the generalized edema remained unchanged. The frog was euthanized by immersion in buffered 1.0% benzocaine (Sigma) for 10 min, and a gross necropsy was performed. Numerous degenerative oocytes spilled from the coelomic cavity. Oocytes were free-floating as well as adherent to visceral organs, especially to the heart and liver (Figure 1 B). Neither microbial cultures of the coelomic fluid and organs nor additional diagnostics (histopathology, special stains for mycobacterium) were submitted for this case.

Case 2. In September 2004, a 2-y-old female laboratory South African clawed frog (*Xenopus laevis*; snout-to-vent length, 110 mm; body weight, 86 g; purchased from NASCO) was primed with PMSG (100 IU injected into the dorsal lymph sac). Four days later, ovulation was induced with hCG (800 IU injected into the dorsal lymph sac). The frog was placed in a bucket containing 7-l fresh egg-laying solution (high-salt modified Bath solution: 88 mM NaCl; 1 mM KCl; 0.7 mM $CaCl_2$; 1 mM $MgSO_4$; 2.5 mM $NaHCO_3$; 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.8), and eggs were laid spontaneously approximately 7 h later. This procedure was performed with approval of the Stanford University's Animal Care and Use Committee. The laid eggs were collected, and the frog was returned to rest in a compartmentalized section of regular pond housing (300-l, dark-green, opaque, bathtub-style self-flushing aquarium, 150 to 200 frogs/pond; as described for Case 1, the water is filtered, dechlorinated, and maintained at a temperature range between 19 to 21 °C). The room was on a 12:12-h light:dark cycle. All frogs in the animal facility, including the affected frog, are fed *Xenopus* brittle (NASCO) 3 times weekly, 3 h before a complete water change.

The frog was observed daily; the animal caretaker noted the frog was in good health, eating well, and able to dive. One week after egg laying, the frog was found bloated, unable to dive, and swimming at the water's surface. The initial differential diagnoses included renal failure and bacterial infection resulting in septicemia; however, neither petechial nor ecchymotic hemorrhage, signs that are consistent with bacterial sepsis, was present. No other frogs in the tank were affected.

The dose and route of administration of the PMSG and hCG were reviewed with the investigators and found to be appropriate. No environmental changes were identified, and routine water-quality testing (pH, temperature, conductivity, ammonia, nitrate, nitrite, free and total chlorine, monochloramine, copper, and dissolved oxygen) confirmed that all parameters were within normal limits. Because of the severity of the whole-body edema, the frog was euthanized by intracoelomic administration of a buffered 5% tricaine solution. A complete necropsy was performed. Tissue samples from all major viscera, as well as sections of skeletal muscle and skin, were collected and immersion-fixed in formalin, processed in paraffin, sectioned, and stained with Giemsa or hematoxylin and eosin for histopathologic evaluation.

Necropsy revealed that subcutaneous spaces over the rear legs, dorsal body, and head were expanded greatly by colorless, transparent fluid. The coelomic cavity was distended moderately with transparent fluid and filled with oocytes that were either floating freely or adherent to the hepatic ligaments and Glisson capsule, around the heart, and within the pericardial sac and expanding the parietal serosa of the dorsal coelomic cavity (Figure 2). The ovaries were mildly hemorrhagic. Dermal vessels over the dorsal and ventral body surfaces were moderately congested. There were no gross or histologic lesions in the kidneys or other organs. Microbiologic culture of coelomic fluid and heart blood collected at necropsy was negative for common *Xenopus* pathogens including, but not limited to, *Mycobacterium*, *Flavobacterium*, and *Aeromonas* spp. Acid-fast staining of tissues for mycobacteria was negative.

Discussion

The findings observed in these hCG-treated frogs are strikingly similar to those reported in women with OHS. This condition is an iatrogenic complication of ovarian stimulation and is characterized by excessive enlargement of the ovaries, ascites, abdominal distention, whole-body edema and, occasionally, death associated with the administration of exogenous gonadotropins.[11] In infertile women, the aim of supraphysiologic ovarian stimulation is to optimize the number of oocytes that can be harvested for use in assisted reproductive technologies. Fortunately, OHS in women is an uncommon complication, with a prevalence of 0.5% to 5%.[4,9,12]

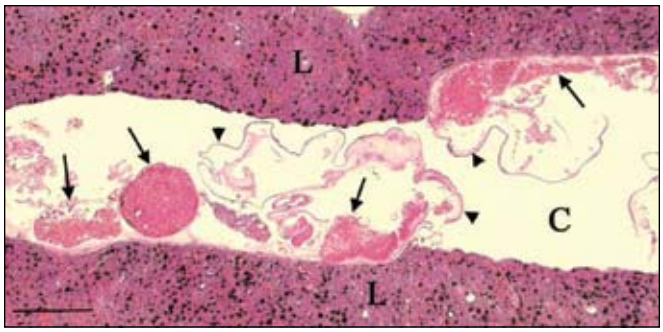

Figure 2. Case 2: Photomicrograph of oocytes located free within the coelomic cavity and adherent to the hepatic capsule. L, liver; C, coelomic cavity; arrowheads, ruptured zona pellucida; arrows, yolk material from degenerate ova. Hematoxylin and eosin stain. Bar, 1 mm.

Women younger than 30 y with good ovarian reserve, those who have developed OHS before, and those who have a low body weight or polycystic ovarian syndrome are at increased risk for the condition.[12,21,22] The diagnosis of OHS is based on clinical history and signs. Here we describe OHS-like symptoms in 2 frogs treated with gonadotropins.

We could find no report in the literature of OHS in gonadotropin-treated laboratory frogs. A retrospective review of Stanford's animal medical records (from 2000 to 2006) revealed that only 2 of the 49 bloated frogs submitted for necropsy had the appropriate history and displayed ascites and whole-body edema in conjunction with eggs adherent to the liver or pericardium or both (1 of the 2 frogs is described here; photographs and histology were not available on the other). From 2000 to 2006, the daily census in the *Xenopus* colony at Stanford averaged approximately 2000 animals, and the colony is composed almost exclusively of adult females used for egg and oocyte harvest. Given that only 2 frogs with OHS have ever been observed at Stanford University and only 1 at UC-Berkeley (where the *Xenopus laevis* colony is of comparable size, if not slightly larger) in the last 6 y, the condition appears to be rare in gonadotropin-treated laboratory frogs, as it is in women.

The main differential diagnoses for OHS in laboratory *Xenopus* include renal failure, primary bacterial sepsis such as that caused by *Chrysoebacterium* (*Flavobacterium*) *meningosepticum*,[14] and secondary or opportunistic bacterial septicemias (for example, *Aeromonas hydrophilia* and *Mycobacterium* spp) that may be precipitated by environmental stressors such as poor water quality and abrupt temperature changes or after surgical harvest of oocytes. In the frogs in this report, bacterial sepsis was excluded primarily in light of the absence of petechial and ecchymotic hemorrhages and negative bacterial cultures of blood and coelomic fluid or the absence of organisms in lymphatic aspirates.

Similar to women with OHS, the frogs in this report were young (less than 3 y old) and approaching their reproductive peak (with good ovarian reserve). The clinical signs of OHS in these frogs were not apparent until 1 wk (Case 2) or almost 2 wk (Case 1) after administration of hCG. As seen in women, exogenous hCG may last as long as 6 d,[6] and signs of OHS typically appear between 1 and 2 wks after hCG administration.[8,18,19]

In OHS, the increase in size of the ovaries is accompanied by abdominal distension and ascites.[11] One or more follicles may enlarge to several times its normal size and may rupture, leading to complications related to blood loss and accumulation in the abdominal cavity. We did not observe complications related to blood loss in the frogs, but the anuran follicular wall is notably avascular compared with those of mammalian species.

In OHS, extravascular protein-rich exudate may accumulate in the peritoneum (or as in the frog cases described here, in the coelomic cavity), and sometimes in the pleura and pericardial sac. This massive shift of compartmental fluids to the third space results in cardiopulmonary complications, hemodynamic and electrolyte changes, hemoconcentration, hypovolemia, hypoalbuminemia, and oliguria. Whole-body edema may result. In severe forms of OHS, liver dysfunction and thromboembolic phenomena are sometimes fatal complications. We did not attempt to treat these frogs for OHS, but in women, treatment for OHS is supportive and includes administration of intravenous fluids, electrolytes, albumin, and activated protein C.[2,11,15]

The pathogenesis of OHS is not clearly understood. As reviewed by several authors,[5,10,13,18] data from women and mammalian animal models show that edema formation involves several inflammatory mediators, probably secreted by the ovaries in response to hCG. In general, OHS appears to develop as a result of the disturbance of the normal inflammatory-like ovulation process. Vascular endothelial growth factor, secreted by theca and granulosa cells, has been implicated as one of the vasoactive mediators that may play a predominate role in OHS.[1,3,7,17,20] Secretion of vascular endothelial growth factor and other inflammation-mediated cytokines leads to capillary leakage of proteins and transmission of these factors to other compartments. We believe a similar pathogenesis occurred in these frogs. The clinical appearance of whole-body edema, often referred to as the nonspecific condition 'amphibian hydrops' in the older literature, likely is related to capillary leakage and disruption of the lymphatic system and accumulation of extravasated body fluids in the subcutaneous space between muscle and the loosely attached amphibian skin.

The finding of multiple eggs adherent to aberrant locations (for example, the pericardium) is uncommon in women but occurred in both frogs reported here and is related to the absence of a diaphragm in this semiaquatic anuran species. In addition, women tend to release 1 or perhaps several eggs at ovulation; in contrast, anuran amphibians release hundreds or more. Multiple mature follicles burst, and numerous eggs are released into the peritoneal cavity before they reach the oviduct.

Although the exact number of female *Xenopus laevis* used annually in laboratory research around the world is unavailable, a conservative estimate would approach hundreds of thousands of frogs. Laboratory animal veterinarians responsible for the care of these animals may encounter sporadic cases of OHS in female *Xenopus* treated with gonadotropins. Bloating and whole-body edema are nonspecific signs in laboratory *Xenopus* and most often occur in frogs that succumb to infectious disease, particularly bacterial septicemia. Distinguishing features of septicemia may include petechia and ecchymotic hemorrhages, cloudy coelomic fluid, and, of course, positive bacterial cultures obtained from tissue and body fluids. History of treatment with gonadotropins and ascites and large numbers of eggs in the coelomic cavity and in aberrant locations (around the heart sac, adhered to the liver capsule) were present in these frogs with OHS. Therefore OHS should be included as a differential diagnosis for the 'bloated' frog.

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