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Artificial fertilization for amphibian conservation: Current knowledge and future considerations

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Abstract

Amphibian populations in the wild are experiencing massive die-offs that have led to the extinction of an estimated 168 species in the last several decades. To address these declines, zoological institutions are playing an important role in establishing captive assurance colonies to protect species in imminent danger of extinction. Many of the threatened species recently placed into captivity are failing to reproduce before they expire, and maintaining founder populations is becoming a formidable challenge. Assisted reproductive technologies, such as hormone synchronization, gamete storage and artificial fertilization, are valuable tools for addressing reproductive failure of amphibians in captive facilities. Artificial fertilization has been commonly employed for over 60 years in several keystone laboratory species for basic studies in developmental biology and embryology. However, there are few instances of applied studies for the conservation of threatened or endangered amphibian species. In this review, we summarize valuable technological achievements in amphibian artificial fertilization, identify specific processes that need to be considered when developing artificial fertilization techniques for species conservation, and address future concerns that should be priorities for the next decade.

Keywords: Amphibian; Artificial fertilization; Egg; Hormones; Sperm

1. Introduction

Compared to mammals, amphibians display a wide range of reproductive strategies. Indeed, their reproductive strategies are so diverse that one would be challenged to make a general statement that reflects the entire taxa. Amphibians have evolved reproductive mechanisms often involving both an aquatic and terrestrial life stage that are successful based on their specific habitats. Most of the current literature on anurans (frogs and toads) is for temperate *Bufo* and *Rana* species; however, the great diversity of reproductive patterns in tropic anurans remains relatively unstudied. Caecilian reproductive mechanisms are even less well understood than those of tropical anurans. Fortunately, there is more known about the diversity of urodele (salamanders and newts) reproductive patterns because of their high density in the southeastern USA, where they are easily studied. The three living orders of Amphibia use both external and internal fertilization mechanisms reflecting a wide range of oviparous, ovoviviparous and viviparous strategies [1]. Typically, anurans are oviparous, salamanders and newts are ovoviviparous and caecilians are viviparous, although

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there are some exceptions to these categorizations, especially in anurans. Extensive reviews on amphibian reproduction can be found in Duellman and Trueb [1], Salthe and Mecham [2], and Whitaker [3].

The reproductive patterns of *Xenopus*, *Bufonids* and *Ranids* have been described in great detail for nearly a century. All three families of anurans share common traits that make them ideal model organisms, especially in the fields of developmental biology and embryology. These anurans have external fertilization, large eggs that are easily manipulated, developmental rates that proceed at a highly advanced pace compared to mammals, have no parental involvement, and have fecundity rates that can reach as high as 80,000 eggs per reproductive event [1]. It is therefore not surprising that 10% of all Nobel Prize recipients in physiology and medicine used frogs as a model (www.nobelprize.org), or that the first animal ever cloned was a frog [4].

Many of these early developmental biology studies required researchers to develop artificial fertilization (AF) techniques for greater control over their study designs. This dearth of knowledge on AF for several key laboratory species is now being applied to the conservation of endangered species within these same families (e.g. *Bufonidae* and *Ranidae*). In one context, this accumulation of knowledge on fertilization mechanisms, coupled with the ease of external fertilization in the lab, places assisted reproductive technologies (ARTs) for amphibians at a much more advanced stage than for any other companion animal or non-domestic species. A case in point is the release of over 2000 endangered Wyoming toad tadpoles produced by AF into the wild [5]. No mammalian conservation program can boast such numbers of released animals produced by ART. It is noteworthy that the terms AF and *in vitro* fertilization (IVF) are often used interchangeably by amphibian reproductive biologists to denote the artificial insemination (AI) of eggs in a Petri dish. However, AF is probably a more appropriate term for anurans that demonstrate primarily external fertilization compared to salamanders, newts and caecilians that exhibit internal fertilization. Whereas the majority of topics within this special issue of *Theriogenology* will focus on AI for companion animal and non-domestic mammalian species, a comparative paper on amphibian AF is warranted, considering their global extinction threat.

The aim of this review paper is to first introduce the amphibian extinction crisis and then the global efforts to stem their loss. As a result of securing so many relatively undescribed species in biosecure facilities, a captive breeding crisis is now growing that will require rapid development of ART until more is known about how to induce natural breeding. The remainder of this paper will discuss the current state of knowledge regarding AF for amphibians and some of the related technologies or unique reproductive adaptations that impact gamete interactions during fertilization.

2. Amphibian extinction crisis

The global loss of amphibian biodiversity is a stark example of how increasing anthropogenic actions impact our global ecosystems. Currently, amphibian extinctions are 200 times higher than the mean extinction rate for all species over the last 350 million years [6], leading many paleontologists to describe our current global biodiversity deficit as parallel to the loss of the dinosaurs. One of the most comprehensive surveys for an entire class of vertebrates, the global amphibian assessment (http://globalamphibians.org), indicates that approximately 32% of the nearly 6000 amphibian species known to science are in imminent danger of extinction. This level of extinction debt is much greater than for mammals (22%) or birds (12%) [7]. In general, the public is more likely to identify with, and financially support, charismatic flagship species such as elephants, lions or giant pandas [8] than they are to espouse frog conservation. It is estimated that approximately 168 amphibian species have likely gone extinct since the early 1980s; even more alarming is that 43% of the total number of remaining species are continuing to decline [9]. Although habitat loss is the primary threat to amphibians in the wild [10], other factors such as disease, climate change and pollution are affecting amphibian species worldwide. The rapid spread of a global epizootic fungal disease known as chytridiomycosis [11] has decimated populations in protected areas with pristine habitat. Hence, finding the way forward for conserving amphibian biodiversity is much more challenging than for mammals, because threats to mammalian biodiversity are well-known and conservation efforts to alleviate these threats primarily address habitat loss, poaching and genetic bottlenecks. Stressors such as climate change and pollution are believed to be interacting with the spread of chytridiomycosis [12], which poses the question of how to confront these population collapses, especially in remote or protected areas where many of the declines are occurring.

In 2005, the IUCN species survival commission hosted an international summit in Washington DC to address the catastrophic loss of so many amphibians. Out of this summit, an amphibian conservation action