THE ROLE OF NAJIN
in the
BIORESCUE PROJECT
An ethical assessment

October 2021

BioRescue Ethical Team

Ethics Laboratory for Conservation,
Veterinary Medicine and Animal Welfare

INDEX

With the contribution of International Experts and leading Partners.

Quote as: "BIORESCUE, *The role of Najin in the Biorescue project: An ethical assessment.* Technical Report. October 2021"

INTRODUCTION

This Report details the decision making-process within the B*io*R*escue* consortium on the *Role of Najin in the Biorescue project*, which took place in January-September 2021. Najin is one of the two remaining northern white rhinos (*Ceratotherium simum cottoni*, Lydekker 1908, from here on NWR) on Earth at this time. The Report supplies the main factors – from a scientific, ethical, and decision-making standpoints – that were considered to reach an ethically-solid and scientific-sound conclusion about the topic.

A subspecies of the white rhino (*Ceratotherium simum*), the NWR, from once living in much of the savannah of Central Africa, was reduced, mainly by poaching, to a wild population of few individuals by the 1980s. Since 2007 there have not been any confirmed signs of its presence in the wild, and the taxon has been declared as "possibly extinct in the wild" (Emslie, 2020). Moreover, after the death of the last male Sudan in 2018, the small population living in captivity was reduced to two females – Najin and Fatu, both incapable of having a viable pregnancy due to health and age-related issues. For this reason, the NWR is presently considered "functionally extinct" (Emslie, 2020).

The only chance to revert this situation relies on pushing the boundaries of contemporary science (Saragusty et al., 2016; Hildebrandt et al., 2021a). More specifically, the strategy individuated by the participants of the B*io*R*escue* project – an International consortium led by the Leibniz Institute for Zoo and Wildlife Research in Berlin and coordinated by Zoo Dvůr Králové – combines advanced assisted reproductive technologies (aART) and stem-cell associated techniques (SCAT) (Hildebrandt et al., 2018). Both approaches involve the use of biomaterial from live as well as from deceased individuals, in the form of cryopreserved gametes (Hermes et al., 2018) and skin samples from fibroblast cultures. The ultimate goal of the project is to establish a self-sustaining and genetically healthy northern white rhino population to be reintroduced into the wild. The short term goals include instead collecting additional biomaterial from the two remaining living individuals, creating viable NWR embryos from IVF, and developing methods and protocols that can lead from successful embryo transfers to the birth of new individuals of the taxon.

Since both remaining females are unable of having a viable pregnancy, the participants in the project plan to employ females of southern white rhino (*Ceratotherium simum simum*, Bruchell 1817, from here on SWR) as recipient cows for the embryo transfer.

Najin and Fatu still play a central role in the project as:

- Donors of genetic material (mainly in the form of oocytes).
- Transmitters of species-specific behavioural patterns and social skills to the next expected generations of NWR.
- Ambassadors of their species.

However, reasons emerged to question Najin's role as a donor of genetic material. The female is 32 years old, has very weak hind legs due to bilateral alterations of the Achilles tendons, and has a large ovarian cystic formation on her left adnexus. It came to attention of the team, performing on a regular basis veterinarian screening and Ovum pick-up (OPU) procedures on Najin, that the ovarian cyst is immensely increasing in volume. Moreover, both general anesthesia and standing sedation can lead to increased risks of complications due to the age and health problems of the animal. Finally, Najin has been offering a limited number of oocytes during the OPU procedures, no oocytes at all in the last two interventions, and no viable embryos were ever produced via IVF of her eggs.

Attention to the welfare of the individual animals involved, as well as for any other ethical dimension involved in the project, is a central aspect of the B*io*R*escue* consortium. It is also one of the core values of Zoo Dvůr Králové, the formal owner of Najin. In 2009, the Management Committee was established in Ol Pejeta to oversee any activity that involves the northern white rhino transported from Zoo Dvůr Králové to Kenya, including Najin. In 2019, Zoo Dvůr Králové became one of the partners in the BioRescue project. For this reason, the Biorescue consortium and the management of Zoo Dvůr Králové requested a transparent discussion about the possible future role of Nájin to be held among all relevant stakeholders.

Three possible options were specifically discussed during the decision-making process, as detailed in this Report:

- **First**, to continue, more or less, to attempt on a regular basis (approximately every three months) the OPU procedure on Najin, while constantly monitoring her health conditions and reopening this decision-making process in the event of a further change in conditions.
- **Second**, to employ Najin one last time as a potential donor of genetic material by performing the surgical procedure of ovariectomy.
- **Third**, to stop employing Najin as a donor of genetic material and let her be just an ambassador of her species.

1. RELEVANT SCIENTIFIC INFORMATION

In this section the most relevant scientific information on the case are supplied. In particular, the section contains descriptions of:

1) Najin's background
2) OPU procedure
3) Ovariectomy procedure
4) Latest ultrasound examination on Najin
5) Issues relative to anesthesia

1.1. Najin's background

Najin was born in 1989 at ZOO Dvůr Králové. Her parents were Sudan and Nasima. Nasima, a female wild-born in Uganda in 1965 c.ca and transferred to Zoo Dvůr Králové in 1977, birthed three out of four NWR calves born in captivity, making her the most productive NWR in captivity. She died in 1992 at about age 27. Sudan, caught from the wild in what is now South Sudan in 1975 at about 2 years of age, also lived at Zoo Dvůr Králové until 2009, when he was moved to Kenya. He died in 2018, and he was the last known male of the subspecies. Najin, after mating with Saút, in

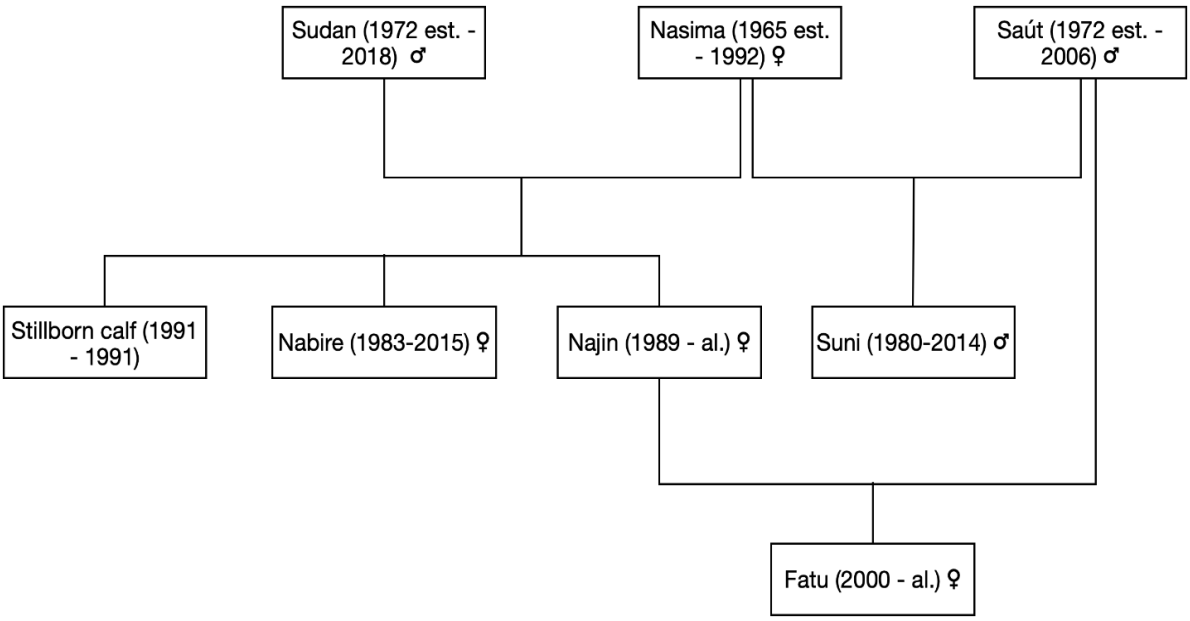


Figure 1: Najin and Fatu genealogical tree

In December 2009, the last four NWRs potentially able to reproduce, Najin, Fatu, Sudan, and Suni, arrived to Ol Pejeta Conservancy for a "Last Chance to Survive" breeding program. Unfortunately, the animals didn't reproduce and in 2014 an examination performed by the Leibniz Institute for Zoo and Wildlife Research in cooperation with Zoo Dvůr Králové, Ol Pejeta Conservancy and Kenya Wildlife Service revealed that none of the females was capable of natural reproduction anymore (IZW (Germany), KWS (Kenya) and Dvur Kralove Zoo (Czech Republic) 2014).

Moreover, male Suni died in 2014 of natural causes, while Sudan had a meager sperm count, showed degeneration in his testicular tissue, and died on the 19th of March 2018.

1.2. OPU procedure

OPU procedures in rhinoceros are a relatively new intervention (Hermes et al. 2009). In the context of the B*io*R*escue* project, they involve GnRH stimulation, full anesthesia, and transrectal ultrasound guided oocyte recovery (Hildebrandt et al. 2018).

Oocyte harvesting from Najin and her daughter Fatu began on the 22nd of August 2019 with a first intervention on both females. Since then, the OPU procedures have been repeated other four times, for a total number of procedures at the present time of n=6. **Table 1** recaps for each female the number of oocytes collected in each procedure, and the number of embryos created for each attempt.

Table 1: Results of OPU and IVF as of July 2021 – from Galli, unpublished data

	Najin		Fatu	
	Oocytes	Embryos	Oocytes	Embryos
1. (08/22/19)	5	0	5	2
2. (12/17/19)	3	0	6	1
3. (08/18/20)	2	0	9	0
4. (12/13/20)	0	–	14	2
5. (03/28/21)	–	–	21	4
6. (07/06/21)	–	–	17	3
Total:	10	0	72	12

Procedures have been planned with a minimum of 3 months pause between them in order to keep a tight schedule of collections while, at the same time, providing the animals with a safe interval to recover and ensure their welfare. Each intervention is preceded by the application of a specially designed ethical tool – the Ethical Assessment Tool (ETHAS) – in order to monitor the procedure (de Mori et al. 2021). Application of the tool ensures that any risks are considered, measures are taken to reduce their scope, and ethical concerns are discussed among the project partners.

Travel restrictions due to the Covid-19 pandemic led to the cancellation of the procedure planned for May 2020 (Hildebrandt et al. 2021b). In fact, one of the possible explanations for the failure to create embryos following the 18th August 2020 procedure is the prolonged interval between interventions due to the forced pause caused by the pandemic, as the females, in absence of cycling activity, had possibly retained follicles from the previous hormonal stimulation in December 2019, and the oocytes harvested in August 2020 were consequently aged (Hildebrandt et al., 2021b). As it can be seen from **Table 1**, this was the only time that an embryo has not been created from oocytes collected from Fatu.

In no case, instead, it has been possible to create an embryo from an oocyte collected from Najin. Moreover, with the exception of the first intervention, the number of oocytes retrieved form Najin has also been lower than the one retrieved from Fatu. During the fourth procedure (13th December 2020), no oocytes at all were collected from Najin. In the fifth procedure (28th March 2021), after preliminary ultrasound screening under standing sedation, it was decided not to continue with the attempt on Najin, due to the low estimated probabilities of obtaining viable oocytes. The same option was chosen for the July 2021 intervention.

Najin was born in 1989. Ovarian response to GnRH stimulation prior to OPU in white rhinoceros aged more than 30 years in the European OPU program has so far been very poor (n=5). This suggests that female rhinoceros past the age of 30 years enter a reproductive senescence in which GnRH stimulation of the ovary hardly induces any follicular growth. For this reason, these animals were removed from the European OPU program and retired.

1.3. Ovariectomy procedure

Abdominal surgery in rhinos is extremely challenging, and complete success of a ovariectomy procedure would be extremely uncertain. This intervention – to the best of our knowledge – has been attempted in rhinoceros thrice, twice in a SWR (Bronx Zoo and San Diego Safari Park) and once in a NWR (San Diego Safari Park). The surgical removal of ovarian tissue was done endoscopically. Only in one case the animal survived the intervention, and for a short period of time. No oocytes were collected in a known case of ovariectomy of a SWR 32 years old female (Pennington et al. 2019).

Concerning the potential expectations of ovariectomy it is also useful to look at the known cases of post mortem harvest of ovarian tissue. Ovarian tissue harvest post mortem in senescent females in the EAZA ex situ program has so far not yielded promising results in terms of oocyte retrieval or residual ovarian cortex (n=3). Fibrotic state of the ovaries from these older, senescent females resulted so far in zero oocytes and limited amounts of remaining germinative tissue harvested.

Yet, this small but very limited germinative tissue removed during this procedure may be useful once in vitro follicle culture will be developed and made operational for the NWR (Hildebrandt et al., 2021a), a technique that so far has been already successful in some species, for instance, in cats (Fassbender et al., 2007).

1.4. Latest ultrasound examination on Najin

The latest ultrasound examination of Najin to this date was conducted in Ol Pejeta, Kenya, on the 28th March 2021 by Robert Hermes, Frank Göritz and Thomas Hildebrandt (Hermes et al., 2021).

Summary of main sonographic findings:

Cervix:	Small leiomyoma 1.7 cm
Uterus:	Multiple, small, leiomyoma in both horns ≤ 1.5cm
	Uterine adenoma of 1.6 cm in the right horn
Ovary:	Right ovary, small and inactive with 2 follicles ≤ 1.5 cm
	Left ovary septed-cystic structure with diameter of 25 cm

To sum up

Pathologies found in Najin have been described and histologically validated in a previous publication (Hermes et al. 2006) containing images that match those of Najin in 2014 and 2021.

Main concerns on Najin's genital health in 2021 are:

- The extensive size of the ovarian cyst on the left ovary.
- Potential malignancy of the identified uterine adenoma.

Ad 1)

Ovarian cysts originate from the mesovarium or oviduct serosa. In the NWR these become so large that the affected ovary becomes atrophic. In 2014, the cysts in Najin's left ovary had a diameter of 10 cm, which converted into a volume of 0.5 l. The actual size (25 cm of diameter) converts into a volume of 8,2 l. This means an average increasing of size between 2014 and 2021 of 2 cm per year:

assuming this rate remain constant, this translates with a 3-4 l increase in volume in the next two years. GnRH treatment for ovarian stimulation bear the risk of further accelerating this growth, and causing the rupture of the cyst.

Ovarian atrophy on the left ovary and overall decrease of ovarian tissue leads to complete ovarian shut down and cease of follicular activity. Even when the ovary is stimulated, the other ovary shows none or very limited follicle response to the stimulation as observed during the last OPU.

A minimal invasive procedure aimed at aspiration of the fluid contained in the NWR ovarian cysts was attempted by the IZW team in Dvůr Králové in 2015. However, the fluid removal failed due to the extensive size and structure of the cysts.

Ad 2)

Active growing adenoma can potentially become malignant in white rhinoceros. In literature malignant uterine adenocarcinoma in white and Indian rhinoceros have been described multiple times (Wilson et al, 2010, Kim et al, 2021, unpubl. data.). Affected females were subject of euthanasia due to generalized metastasis. Further GnRH stimulations in Najin might promote uterine tumour growth of both leiomyoma and adenoma and induce malignancy of the adenoma to become an adenocarcinoma.

Figure 2: uterine leiomyom

Figure 3: left ovary 25 cm cyst

Figure 4: uterine adenom

1.5. Issues relative to anesthesia

The OPU procedures performed by the B*io*R*escue* team use a protocol specifically developed for this type of interventions.

The main advantages of this protocol are:

a) it is specifically tailored to the procedure; b) it is etorphine free, and, as such, prevents all the possible side-effects of this drug, which can have a high impact on the cardio-circulatory and respiratory systems; c) it uses a combination of 4 different drugs, reducing in this way their dosage, and hence their possible side-effects; d) each of these 4 drugs are reversible, as they have an antidote.

The protocol has been used for anesthesia of more than 500 rhinoceroses (both in zoos, and wild or semi-wild conditions), and it proved to have no consequences even for the animals that were anesthetized repeatedly (five or even more times).

In fact, through consecutive repetition of the protocol on the same animal it is possible to tailor it even better to the specificities of the individual.

Although Najin recovered well from full anesthesia using the optimized etorphine-free protocol in the past (n=5), during the fifth procedure (28th March 2021) she received (just) a standing sedation for dedicated transrectal ultrasound examination of her internal reproductive organs. In general, standing sedation is shorter in time and less detrimental to the cardiovascular and respiratory system of the animal. Recovery was fast and uneventful.

However, due to the weak and diseased hind legs (hyperextension of tendons) Najin started to have problems to bear her body weight and keep the balance towards the end of the procedure. Therefore, standing sedation longer than 10 min is not recommended.

2. RELEVANT ETHICAL ASPECTS

In this section, the most relevant ethical information on the case are supplied. In particular, the section contains a description of the:

1. Stakeholders involved
2. Ethical dimensions involved
3. Ethical desiderata

2.1. Stakeholders involved

The standpoint and interests of the stakeholders were analyzed and taken into account within the decision-making process. Stakeholders involved in the project and affected by the specific decision on Najin's future role were:

- Najin and Fatu. Although the subject of the decision is Najin, and her interest should therefore enjoy a central focus, both females were considered as stakeholders by virtue of their social bond. Their central interests are represented by their welfare: their health and functioning; the absence or minimization of negative affective states paired with the possibility of experiencing positive affective states; and the capacity to live species-specific natural lives. A decision that could lead to an eventual worsening of Najin's welfare could

likely have an indirect negative effect on Fatu as well by virtue of the social nature of these animals, who have lived together for decades.

- Biodiversity. There are several instrumental and non-instrumental reasons for conserving this rhino subspecies if we assume the standpoint of biodiversity conservation. The existence value of a taxon is already in itself an important reason for investing in its conservation. Furthermore, since great herbivores, like rhinos, are important ecosystem engineers, their disappearance can cause further ecological impoverishment, with a consequent loss of ecosystem services. As already mentioned, Najin's possible contribution in saving her species is twofold. On the one hand, as a donor of oocytes or other biomaterial that could be developed into gametes. On the other hand, through her crucial social competence, which could be hopefully transmitted to the next generation of NWR.

- Conservationists. From the standpoint of conservationists there are at least two important interests at stake beyond the obvious goal of saving the NWR. The project could help in establishing new methods and protocols applicable to the conservation of other threatened species, expanding in this way the intervention capacity of conservationists (Hildebrandt et al., 2021a). Moreover, the success of the project could have a strong positive impact on the biodiversity conservation narrative, attracting in this way new support, talents and resources to conservation. However, precisely for the latter reason (as well as for other ethical reasons), the ultimate goal of the conservation of NWR should not be pursued at all costs – that is, by putting aside any considerations relating to the welfare and health of the animals involved. Conservation obstinacy should be avoided. It is in this sense an immediate interest of conservationists to find a reasonable balance between the needs of respecting Najin's welfare and life, and put to good use its possible contributions to the project.

- People managing and protecting Najin and Fatu. This includes Zoo Dvůr Králové, which directly owns the two individuals and coordinates the NWR Rescue efforts, as well as all the keepers, veterinarians and other staff at the Ol Pejeta Conservancy, and all those, including members of the B*io*R*escue* Team and the NWR Management Committee, who have provided care and assistance on a daily or even on an occasional basis to Najin and Fatu. It is rather likely that these people have developed feelings of connection and affective bonds (of varying degrees) with the two animals. In this way, they care about their well-being in a more direct and substantial way, and would encounter stronger negative effects in case a decision could lead to the event of a threat to Najin's welfare and life.

- Kenya Wildlife Service. The final decision must conform to the frame of international regulations and best practices, and Kenyan laws on wildlife.

2.2. Ethical dimensions involved

Three main ethical dimensions were identified as involved in the decision:

The first relevant dimension is the need of respecting the welfare of Najin. Respect of animal welfare involves the pursuit of three complementary goals: a) assuring the physical health and functioning of the animal; b) minimize any eventual unpleasant affective states while allowing normal pleasures; c) allow the development and performance of natural lives according to the species.

The second relevant dimension is the need of respecting the life of Najin. This for both intrinsic and extrinsic reasons. Leaving aside the obvious intrinsic reasons for respecting life in general, we should consider the importance that Najin has for many different people. As an ambassador of her species

and iconic symbol for conservationists and public opinion, for instance, or as recipient of affection for the people who daily care and look after her.

The third relevant dimension is the need to preserve – and, whenever possible even restore – biodiversity, as it is a further source for many other relevant values. In this sense, the conservation of the NWR is especially important, for several reasons. It is at the same time a keystone species, an umbrella species, and, given its charisma, a potential flagship species. Moreover, the establishment of new protocols and procedures for its conservation could help in the future in the conservation of similarly numerically depleted mammal species.

These three value dimensions may conflict with each other. Important actions for the conservation of the taxon may require that some risks of animal welfare impairment – or even, in exceptional cases, of life-threatening events – should be taken. On the other hand, refusing to intervene means abdicating from the duty to conserve – for ourselves and the future generations – important elements of the Earth's biodiversity. The only viable solution, then, is to reach an acceptable equilibrium among the three different value dimensions.

2.3. Ethical Desiderata

From the previous analysis of the ethical dimensions involved, three main ethical desiderata relative to the decision were derived:

- Avoiding major accidents. Major accidents are defined as all those accidents that threaten the life of the animal. This desideratum follows from all the three previous ethical dimensions – as major accidents, beyond being life threatening, are also sources of welfare impairment and may compromise the chances of success of the conservation effort.
- Avoiding minor accidents. Minor accidents are defined as all those accidents that threaten the welfare of the animal but not her life. This desideratum follows mainly from the ethical dimension of respecting the welfare of the animal, even if good animal welfare is usually an important measure of success for conservation programs involving manipulation of animals for breeding.
- Obtain oocytes or other biomaterial useful for the conservation project. This desideratum follows from the ethical dimension of conserving biodiversity. It has to be stressed here that Najin is the mother of Fatu, and as such she share ~50% of the genetic information with her daughter, from whom it is still possible to harvest oocytes. The fact that it is only ~50% must not mislead, since, given the small number of individuals whose gametes survive or from which it will be possible to obtain them, it is still a great loss.

3. DISCUSSION

The decision to reconsider Najin's role within the project was made in consideration of her age, health status, and poor results obtained – in terms of oocytes collected and embryos produced. It was thus decided to perform, together with all the partners of the consortium, a decision-making process that would discuss, from an ethical standpoint, the present scenario and eventual alternatives to it.

After performing semi structured interviews within the consortium, three possible options were identified and a first draft report was drawn. This draft was then circulated among the members of

the consortium in a first round in which participants were able to advance their own observations and ideas. Participants were also requested to contribute populating a Pros and Cons table.

The document thus prepared was presented during a meeting of the members and partners of the consortium organized in Ol Pejeta in Kenya on July 8th 2021. Data were further collected after the meeting through an online survey distributed among members of the consortium. Participants were asked how likely was, in their opinion, the possibility to meet the previously identified ethical desiderata (avoiding major accidents, avoiding minor accidents, obtaining oocytes) according to a labelled six points Likert scale (option "I do not know" was also provided).

During the decision-making process some support tools were prepared and used: a Decision tree, a Bateson's cube, and a pros and cons table. The tools were built and discussed during the participatory process, helping in this way framing the analysis of the case.

3.1. Pros and cons table

A pros and cons table was populated to evaluate the impact of each option to the three value dimensions previously identified (Table 2).

Table 2: Pros and cons table

	Conservation	**Welfare**	**Life**
OPU	**Pro**: not giving up on this option means at least leaving an open door for opportunistic harvesting after preliminary ultrasound screening **Con**: the age, health conditions and history of the last two planned procedures do not give positive hope regarding the possibility of collecting new oocytes. Furthermore, no viable embryo from an oocyte collected by Najin has ever been obtained.	**Pro**: no clear pro **Con**: GnHR stimulation may worsen pre-existent health conditions. As prolonged standing sedation is not viable due to the hind leg status, preliminary screening for opportunistic OPU has to be done during a short time window.	**Pro**: no clear pro. **Con**: Cyst growth may lead to a scenario where the life of the animal is seriously threatened by the possibility of a rupture.
Ovariectomy	**Pro**: possibility of obtaining biomaterial from which to extract or produce gametes. **Con**: the extraction of biomaterial can be done also post mortem. Chances of obtaining oocytes depend on technology (in vitro follicle culture) not yet fully established for NWR.	**Pro**: ovariectomy would solve some of the health conditions relative to the genital apparatus. **Con**: invasive surgery is needed to perform the intervention.	**Pro**: no clear pro. **Con**: similar interventions in the past have shown that this is a life-threatening procedure.

Stop to OPU, no ovariectomy	**Pro**: Najin's role in the project could still be crucial, thanks to her social competence, which would be vital to transmit to the next generation of NWR. Post mortem collection of ovarian tissue would still make possible, with some chance, to obtain new oocytes in the future through in vitro follicle culture. Moreover, Najin could still donate during her life other tissues (subcutis or gingiva) in case it is needed for stem cell research. **Con**: no immediate chance of further oocyte.	**Pro**: the veterinary screening the animal has undergone in recent years can still continue. No specific welfare risks. **Con**: no clear con.	**Pro**: this is the least risky choice. **Con**: no clear con.

3.2. Decision tree

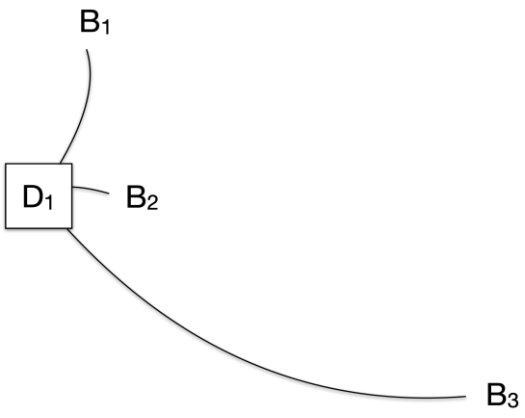
A decision tree was built in order to analyze all the various probable outcomes of the decision. The tree was built following six steps:

- Step one (S1): identification of the main choices.
- Step two (S2): identification of the possible chance nodes.
- Step three (S3): identification of the end nodes and ranking of outcomes.
- Step four (S4): identification of the probabilities for each course of events.
- Step five (S5): comparison between outcomes and probabilities.
- Step six (S6): discussion of strengths and limitations of this methodology.

S1) Identification of the main choices

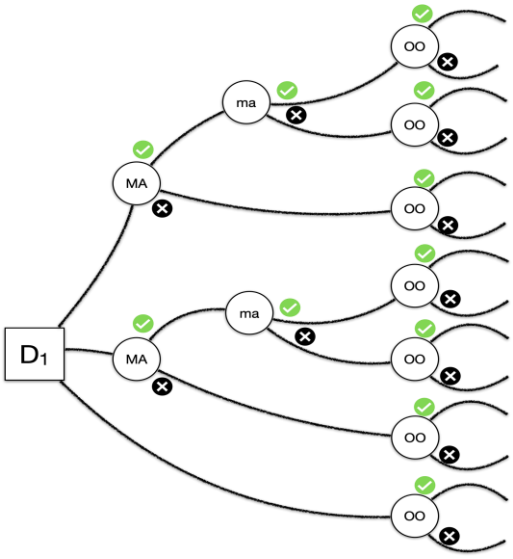
The first step in preparing the decision tree was to draw the main decision-node (square node) and to identify the branches, each representing one of the discussed options. More specifically (Figure 5):

- Branch B1 represents the choice to continue to perform OPU procedures on Najin.
- Branch B2 represents the option to stop OPU procedures and practice ovariectomy.
- Branch B3 represents the option to stop OPU procedures.



S2) Identification of the possible chance-nodes

The second step was the identification of the chance-nodes (circle node). Each chance-node represents a probabilistic event that exercises a relevant influence on a course of action, resulting in different possible outcomes. Three specific type chance-nodes where identified, each for the previously discussed ethical desiderata:



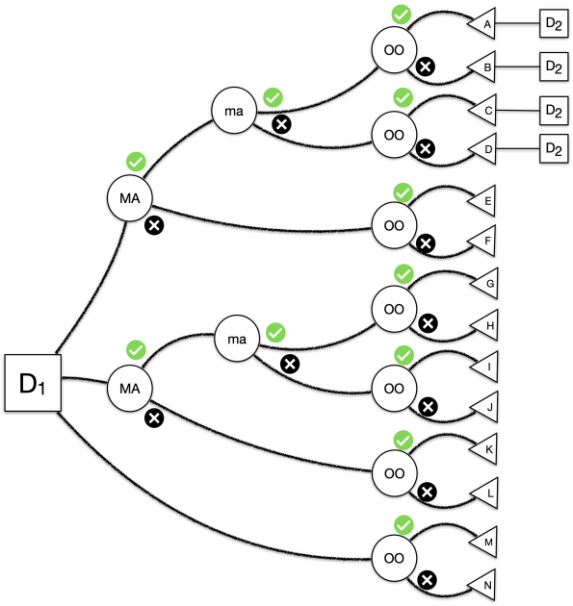
- Chance-nodes labeled as **MA** represents avoidance of major accidents. Major accidents are here defined as all those accidents that threaten the life of the anima. Major accidents can occur during any phase of the procedure.
- Chance-nodes labeled as **ma** represents avoidance of minor accidents. Minor welfare accidents are here defined as all those accidents that threaten the welfare of the animal but not her life – an harm of mild intensity and relatively short duration, which can be usually recovered over time without chronic or permanent effects.
- Chance-nodes labeled as **OO** represents obtaining viable oocytes – through direct harvesting or through in vitro follicle culture method (Hildebrandt et al., 2021a).

The tree does not take into account possible course of events where both a major and a minor accidents happen. In this case, a simplification was preferred, in order to reduce the number of possible outcomes, considering that a course of action in which a major accident occurs is already a really bad outcome per se.

Moreover, although very important, the tree does not directly discuss the possibility that Najin might contribute to the conservation of the taxon by transmitting her social competence to the next generation of NWR, since it would have required acceptable estimates of both the life expectancy of the animal, and of the time still required to see the birth of a NWR calf. Furthermore, this would have required the inclusion of an additional chance node at the end of several branches, multiplying in this way the number of outcomes.

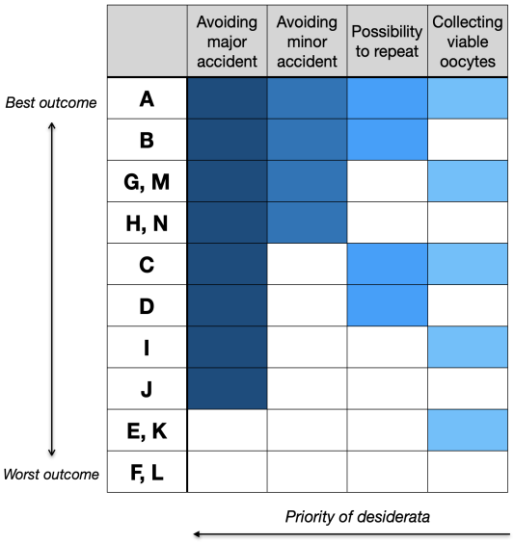
S3) Identification of end-nodes and ranking of outcomes

The third step was identification of end-nodes (triangle node). At the end of the branching process determined by the chance nodes, the tree culminates in fourteen end-nodes. Four of them lead to a new decision node similar to the one at the base of the tree: if the decision to carry out the OPU procedure on Najin gives rise to a course of action in which no major welfare accident occurs, then, the starting question comes up again, that is, whether to continue further, proceed with the ovariectomy, or not perform any more operations. In all other cases, instead, the decision-making process does not need to be repeated, and can be considered definitively concluded.



The end-nodes were then ranked on a scale representing their desirability as outcomes. Ranking was not made by assigning a numerical payoff to each end node, but on the basis of their capacity to satisfy four lexically ordered desiderata:

1. Avoiding major accident
2. Avoiding minor accident
3. Possibility to repeat the procedure
4. Collecting viable oocytes



The four desiderata are lexically ordered, meaning that satisfaction of a higher ordered desideratum trumps in importance satisfaction of all the other lower desiderata (i.e. avoiding major welfare accident trumps in importance satisfaction of all other three desiderata). The desideratum "Possibility to repeat the procedure" was added in order to reflect the option value provided in the decision to perform the OPU procedure compared to the other choices.

Lexical ordering of the desiderata was obtained on the basis of their capacity to respect the value dimensions described previously. Avoiding life threatening situation, for example, is important both from the standpoint of respecting the life of the animal per se, but also to avoid possible suffering, and to ensure the ultimate success of the conservation project. Similarly, ensuring compliance with a good level of welfare, in addition to being a commendable objective in itself, is also important from a conservationist standpoint.

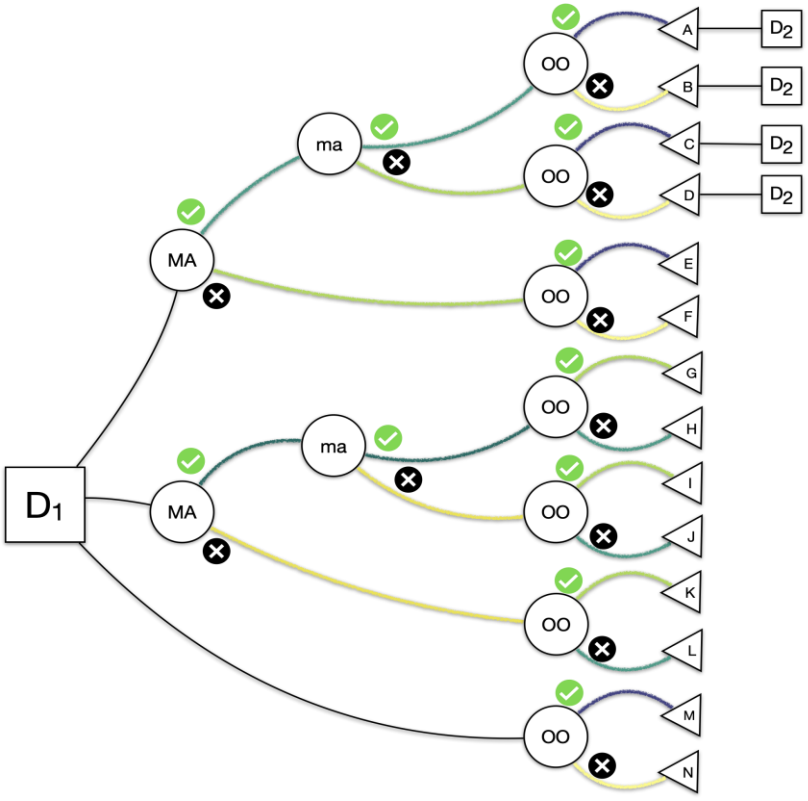
S4) Identification of the probabilities for each course of events

The fourth step was the identification of the possible probabilities for each event. For this purpose, a scale was used consisting of three progressive ranks of probability of occurrence of an event (unlikely, very unlikely, extremely unlikely) and their complementaries (likely, very likely, extremely likely). At each bifurcation following an event-node, the branches were classified according to this scale by the expert veterinarians in the project.

Concerning branch B_1 (continue OPU), chances of major accidents happening have been estimated likely. If the cyst growth is further stimulated by hormones, there is a mechanical risk that the thin cyst wall will rupture when Najin goes down under anesthesia – something that already happened in the past in another NWR, Nabire. The released content of the cyst would kill the animal in very short time. Chances of minor incident were similarly estimated likely, as growth of the cyst, even if not leading to a life-threatening situation is still a welfare impairment, as it is manipulation of the animal due to the status of the leg. Chances of obtaining viable oocytes through OPU were estimated as extremely unlikely, due to past results (see **table 1**), and data on performance of animal of the same age range of Najin.

Concerning branch B_2 (ovariectomy), possibilities of avoiding major accidents have been estimated very unlikely, due to the discouraging known precedents. Similarly, chances of avoiding minor accidents have been estimated as very unlikely, due to the invasiveness of the intervention and the need for prolonged recovery. Chances of obtaining oocytes have been instead estimated as likely, since the techniques required for obtaining oocytes from the germinative tissue eventually harvested from the ovaries, while not yet developed, has already been shown as possible in other mammal species.

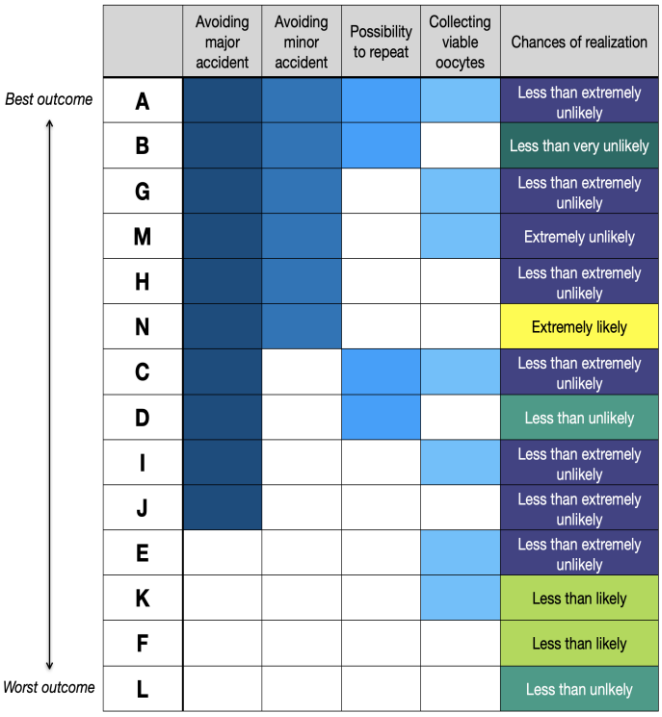
Concerning branch B_3 (do not perform OPU or ovariectomy) chances of obtaining oocytes have still been assessed as possible yet extremely unlikely due to the possibility of post mortem harvesting of ovarian tissue for future in vitro follicle production.



Probability	Scale	Color
Extremeley unlikely	0,001	
Very unlikely	0,01	
Unlikely	0,1	
Likely	0,9	
Very likely	0,99	
Extremely likely	0,999	

S5) Confronting outcomes and probabilities

In this last step it becomes possible to weight the effective value of an outcome, based on its ranking and on the estimated chances of its realization. In this way it should be possible to have a better comparison between the perspectives offered by each option on the table.



	Avoiding major accident	Avoiding minor accident	Possibility to repeat	Collecting viable oocytes	Chances of realization
A					Less than extremely unlikely
B					Less than very unlikely
G					Less than extremely unlikely
M					Extremely unlikely
H					Less than extremely unlikely
N					Extremely likely
C					Less than extremely unlikely
D					Less than unlikely
I					Less than extremely unlikely
J					Less than extremely unlikely
E					Less than extremely unlikely
K					Less than likely
F					Less than likely
L					Less than unlkely

What emerges is that the expected realization of the best outcomes for branch B_1 (continue with OPU) and B_2 (perform ovariectomy) are rather low. In particular, the best outcome for B_1 has the lowest chance of realization, while the best outcome for B_2 has the second lowest chance of realization. For both branches, the end-nodes with the highest chances of realization fail to satisfy most of the desiderata. For B_1 the most probable outcome is the worst scenario of a major accidents paired with no oocytes. For B_2 this worst scenario has the second highest estimated chance of happening, while the most probable outcome is that of a major accident.

OPU end-nodes analysis

End-node	Expected realization	Drawbacks
F	Less than likely	Major accident, no oocytes. Worst scenario for B_1, highest estimated chance to happen if procedure is repeated
D	Less than unlikely	Minor accident, no oocytes. Possibility to repeat the procedure is nearly worthless as chances of obtaining oocytes remain low
B	Less than very unlikely	No oocytes. Possibility to repeat the procedure is nearly worthless as chances of obtaining oocytes remain low
E	Less than extremely unlikely	Major accident
C	Less than extremely unlikely	Minor accident
A	Less than extremely unlikely	No accidents, oocyte retrieved. Best scenario for B_1, lowest estimated chance to happen

Ovariectomy end-nodes analysis

End-node	Expected realization	Drawbacks
K	Less than likely	Major accident.
L	Less than unlikely	Major accident, no oocytes. Worst scenario for B_2, second highest estimated chance to happen.
I	Less than extremely unlikely	Minor accident
J	Less than extremely unlikely	Minor accident, no oocytes
G	Less than extremely unlikely	No accidents, oocyte retrieved. Best scenario for B_2, second lowest estimated chance to happen
H	Less than extremely unlikely	No oocytes

3.3. Bateson cube and self-administered questionnaire

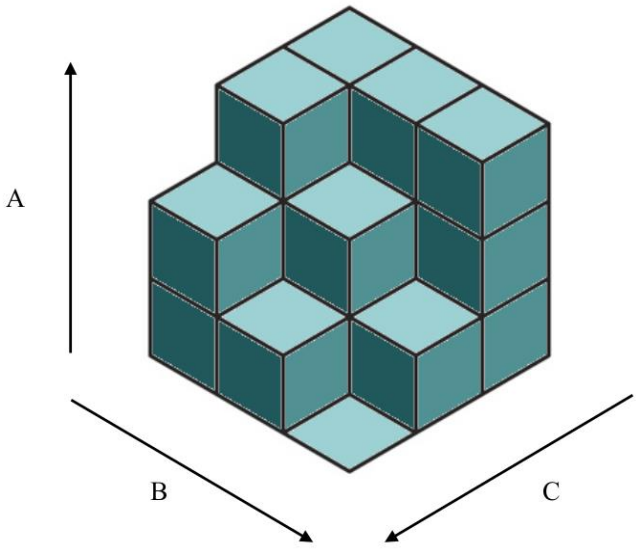
The Bateson's cube is a model for decision making that summarizes and displays the possible combinations between scores attributed along three dimensions. It was originally developed and introduced by Patrick Bateson to assess the ethically acceptability of scientific research involving animals (Bateson 1986; Driscoll and Bateson 1988). Its main structure, however, can be adapted to assess other scenarios involving three ethically relevant dimensions.

In this specific case, the three dimensions of the Bateson cube were made to correspond to each of the previously identified ethical desiderata:

- Dimension **A** (height): Obtaining oocytes or other biomaterial useful for the project.
- Dimension **B** (width): Avoiding minor incidents.
- Dimension **C** (depth): Avoiding major accidents.

In a Bateson cube each dimension needs to be assessed through a 1-3 range score. High score represents high possibilities of obtaining the desideratum (avoiding major/minor accidents, obtaining oocytes), while a low score represents low possibilities. Acceptable scenarios are represented by the clear space in the cube, while unacceptable scenarios are instead represented by solid space. It is important to note that on one of the axis (in this case the one representing the "avoiding major accidents" desideratum) at least a medium score is necessary (but not sufficient) in order for the result to be acceptable.

In this way, a Bateson cube can track the acceptability or non acceptability of 27 possible variants of a general scenario.



In order to attribute a score along the three dimensions of the Bateson's cube for each option discussed data was collected through an online survey distributed among members of the consortium (n = 20). The survey was based on an anonymous, computer-assisted self-completion questionnaire (CASI) conducted with Google Forms, a user-friendly web-based tool. While preparing the survey, recommendation about questionnaire design based on common wisdom and scientific literature were followed. To identify confusing items in questions, respondent problems, mistakes, and potential biases, different researchers reviewed the questionnaire. A tailored invitation email was used to

distribute the link to access the survey. Participants then completed the self-administered questionnaire individually from July 19th 2021 to 23rd.

Participants were asked to consider how likely was, in their opinion, the occurrence of three desiderata (avoiding major accidents, avoiding minor accidents, obtaining oocytes) for each of the three options at stake. Response options consisted in a labelled six points Likert scale, from "extremely unlikely" (1 point) to extremely likely (6 points). Answer option "I do not know" was also provided. Sixteen participants completed the survey, with a response rate of 80%. Descriptive statistics was performed on the results, and for each scenario measures of central tendency were used to identify which scenario variants are considered the most likely by the respondents (**Figures 5-7**).

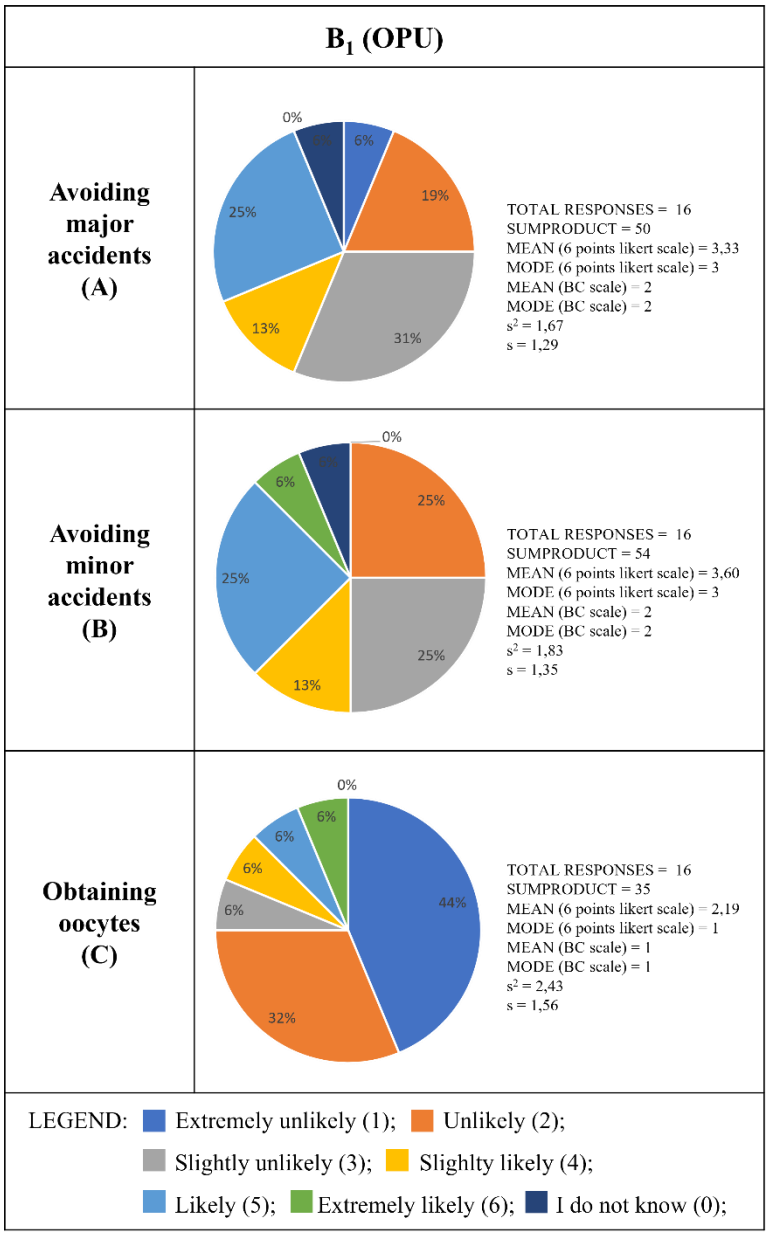


Figure 5: Respondents'answers about the occurrence of the three desiderata (avoiding major accidents, avoiding minor accidents, obtaining oocytes) for the B_1 (OPU) option.

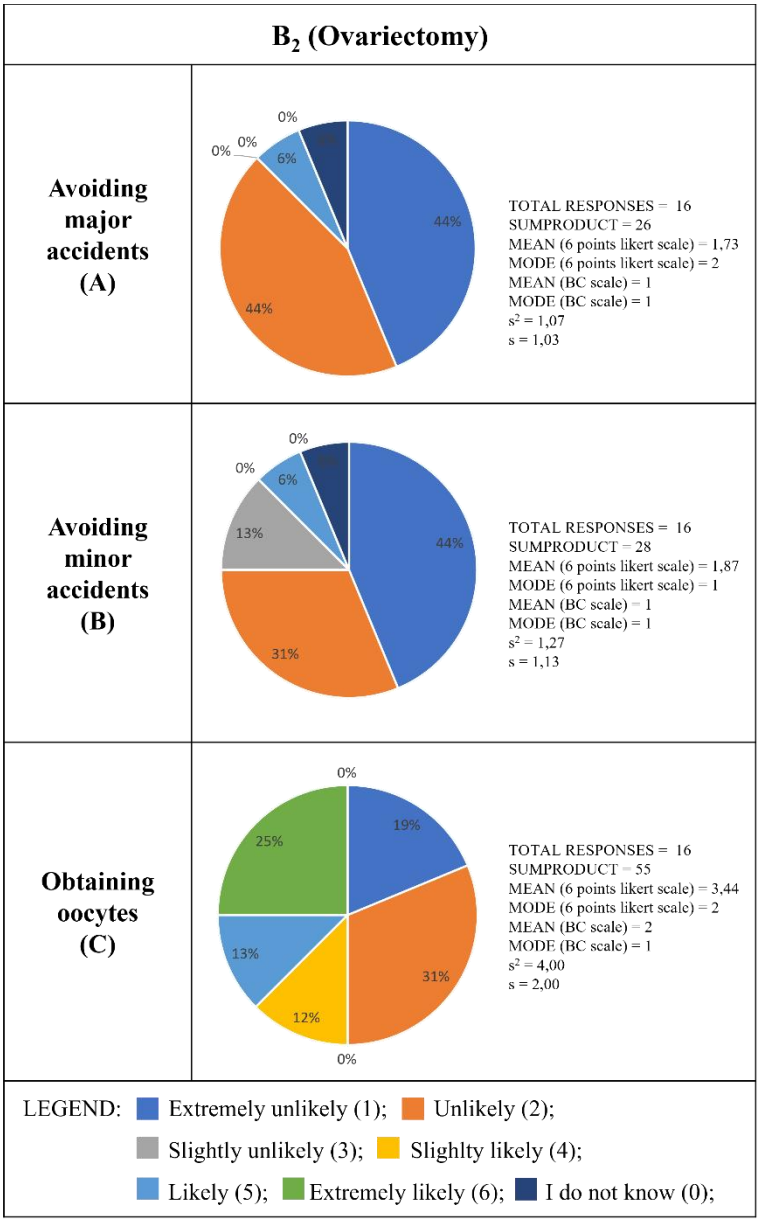


Figure 6: Respondents'answers about the occurrence of the three desiderata (avoiding major accidents, avoiding minor accidents, obtaining oocytes) for the B_2 (Ovariectomy) option.

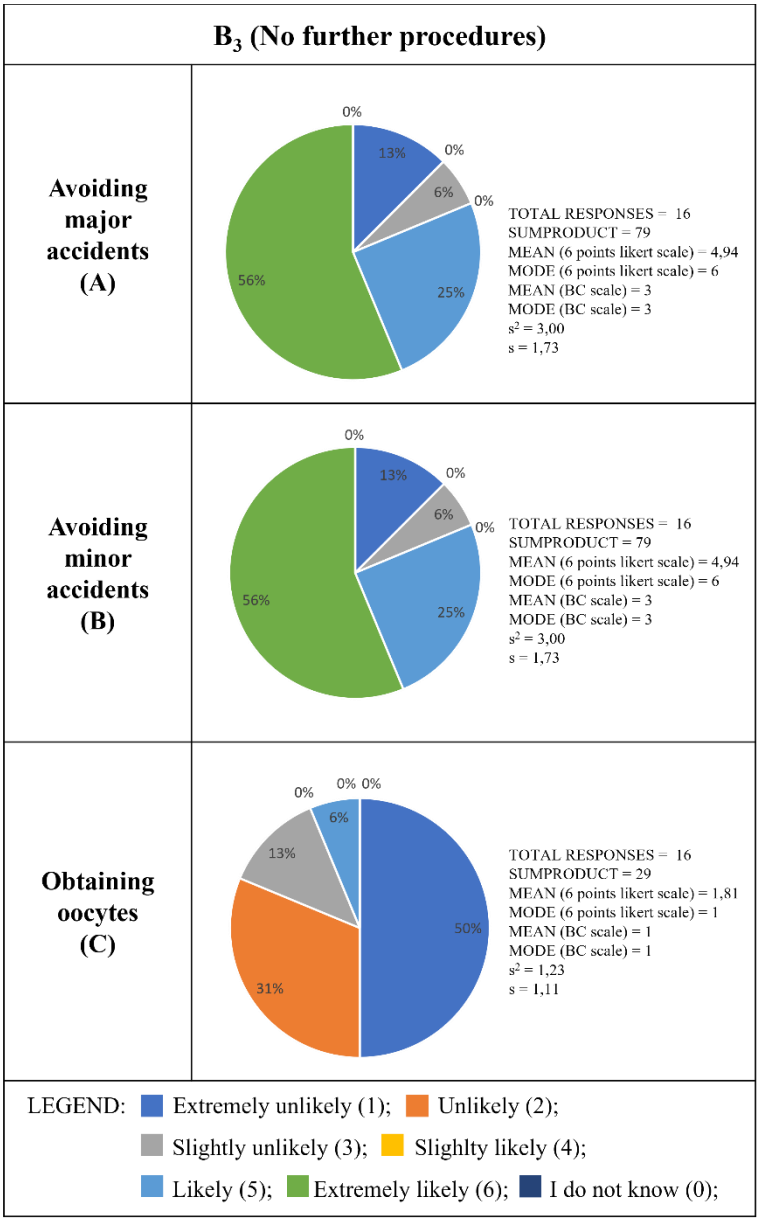


Figure 7: Respondents'answers about the occurrence of the three desiderata (avoiding major accidents, avoiding minor accidents, obtaining oocytes) for the B_3 (No further procedures) option.

The six point results were then converted in a 3 point scale and used for evaluating the acceptability of each option through the Bateson's cube. Mode and mean scores where both used to define the most likely scenario variants. **Tables 2** recap the 27 possible scores of a cube, dividing them in acceptable and not acceptable and ordering each category from the worser (higher rows) to the better (lower rows) result. Colored rows represent the scores obtained through mode by the three options discussed. Azure is option B_1 (continuing OPU); red is option B_2 (ovariectomy); green is option B_3 (ceasing intervention). Both mode and mean scores considered, the options of continuing to attempt on a regular basis the OPU procedure and the one performing ovariaectomy were deemed not ethically acceptable (with a worse result for ovariectomy). Ceasing to use Najin as a living donor of genetic material was instead considered acceptable.

Table 3 – Acceptable and non acceptable scores on the BC

Acceptable scores			Non acceptable scores		
A. Avoiding major accidents	B. Avoiding minor accidents	C. Obtaining oocytes	A. Avoiding major accidents	B. Avoiding minor accidents	C. Obtaining oocytes
2	2	3	1	1	1
2	3	2	1	1	2
2	3	3	1	1	3
3	1	3	1	2	1
3	2	2	1	2	2
3	2	3	1	2	3
3	3	1	1	3	1
3	3	2	1	3	2
3	3	3	1	3	3
			2	1	1
			2	1	2
			2	1	3
			2	2	1
			2	2	2
			2	3	1
			3	1	1
			3	1	2
			3	2	1

4. CONCLUSION

Given the scientific data collected and the ethical analysis performed, the decision making process allowed to reach a shared conclusion. What emerged at the end of the participatory process was the following:

> The most responsible course of action is to cease any further intervention on Najin and to stop using her as a living donor of genetic biomaterial.

This decision was strengthened by the results provided by the tools built during the decision-making process. According to the decision tree built for the occasion, the decision to stop intervention had the best expected outcomes given the ethical desiderata of avoiding accidents that may harm the animal or even threaten its life. The results provided by the Bateson's cube, following the analysis of data provided by the survey, were that proceeding with OPU or performing ovariectomy are not ethically acceptable choice, and the only commendable decision in this sense is to stop interventions.

It is worth noting that, at least concerning OPU, this analysis does not support a general condemnation of these kinds of intervention. It is because of the unique circumstances surrounding Najin's health and age that the best option is to stop all procedures.

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