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IOD treatment in practice



Iron Overload Disorder

Browsing Rhinoceros

Ex situ:

- Non-natural food
- Too much iron

The question remains: is IOD a primary disease
or is IOD a part of bigger metabolic disorder



Sophisticated iron intake... there is most likely no stop in the amount of iron intake.

The most
iron intake:
in the small
intestine



Imbalance!

Iron absorption



Iron "excretion"

- New Red blood cells
- Fetal growth



Imbalance Between Iron Absorption and Iron use ore excretion

After absorption, the iron is transported throughout the body. Transferrin is an iron-transport protein, Transferrin is the “mini cooper” of iron.



CONSEQUENCES

The effect of too much iron ...examples

- Cell damage
- Multiple organ/system failure
- More vulnerable to infections

SYSTEM FAILURE

Skin diseases, liver damage, hemolytic anemia
- Early death! -

Average lifespan black Rhino in captivity in Europe
+/- 22 years

IOD and Zoo's

- **Some never heard of IOD**
- **Underestimate IOD, animals aren't sick**
- **Ignore the problem**
- **Don't know where to begin**
- **Don't have the facilities / funding**





**Test all rhino food on the amount of iron! every new batch!
and put low iron batches aside for the black rhino's**

Food products - iron levels - Rotterdam Zoo

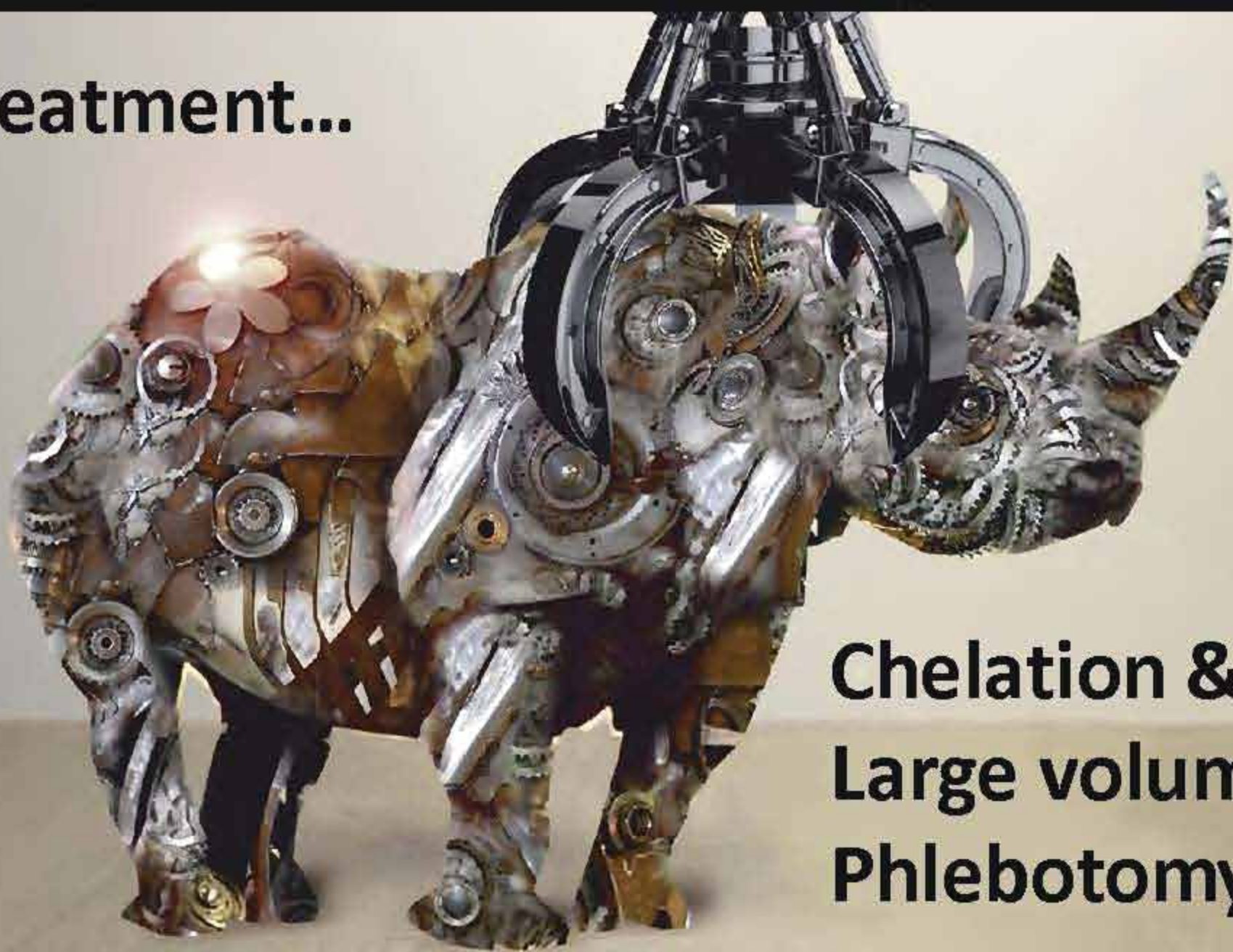


Date	Product - Research number	Iron mg/kg DM	Crude ash	DM gr/kg	Copper mg/kg DM
	Roughage				
11-12-2013	Lucerne 300706	137	99	885	
29-4-2014	Lucerne 301475	147	116	874	
31-10-2014	Lucerne 304993 /19-11-2014 test 2 305249	147	82	976	
26-11-2014	Lucerne 305281	147	97	838	
8-1-2014	Lucerne soilage 300832	147	123	723	
2-2-2016	Lucerne soilage nr.6 250185	140		655	
2-2-2016	Lucerne (loods) grote balen	148		829	
6-7-2016	luzerne soilage 1e snee 060716-3 252259	110		500	8,6
7-7-2016	lucerne 2e snee 070716-4 252275	127		240	7,8
12-11-2016	luzerne 4e snee frankrijk 258503	83	46		7,3
15-5-2015	Hay (dry grass) 739785	147		896	
2-4-2015	Hay (dry grass) "neushoornhooi 31-3-'15" 306136	147		876	
2-4-2015	Hay (dry grass) "gr.voorraad hooi 31-3-'15" 306137	149		871	
2-3-2014	Hay (dry grass) 736442	147	85	880	6,1
1-6-2014	Hay (dry grass) 735432	147	85	822	10,9
11-12-2013	Hay (dry grass) 300707	117	81	901	
31-10-2014	Hay (dry grass) 304934	107	61	974	
15-5-2015	Hay (grass) soilage 739784	144		727	
4-6-2015	Hay (fresh grass) before soilage 306847	151		337	
15-6-2015	Hay (fresh grass) "Hokke" soilage 306969	148		703	
21-8-2015	Hay (fresh grass) "hokke 2e snee 21082015-1" 308390	147		285	10,2
21-8-2015	Hay (fresh grass) "bakker 2e snee 21082015-2" 308391	147		494	7,3
24-8-2015	Hay "kokke dik 24082015-1" 308393	289			

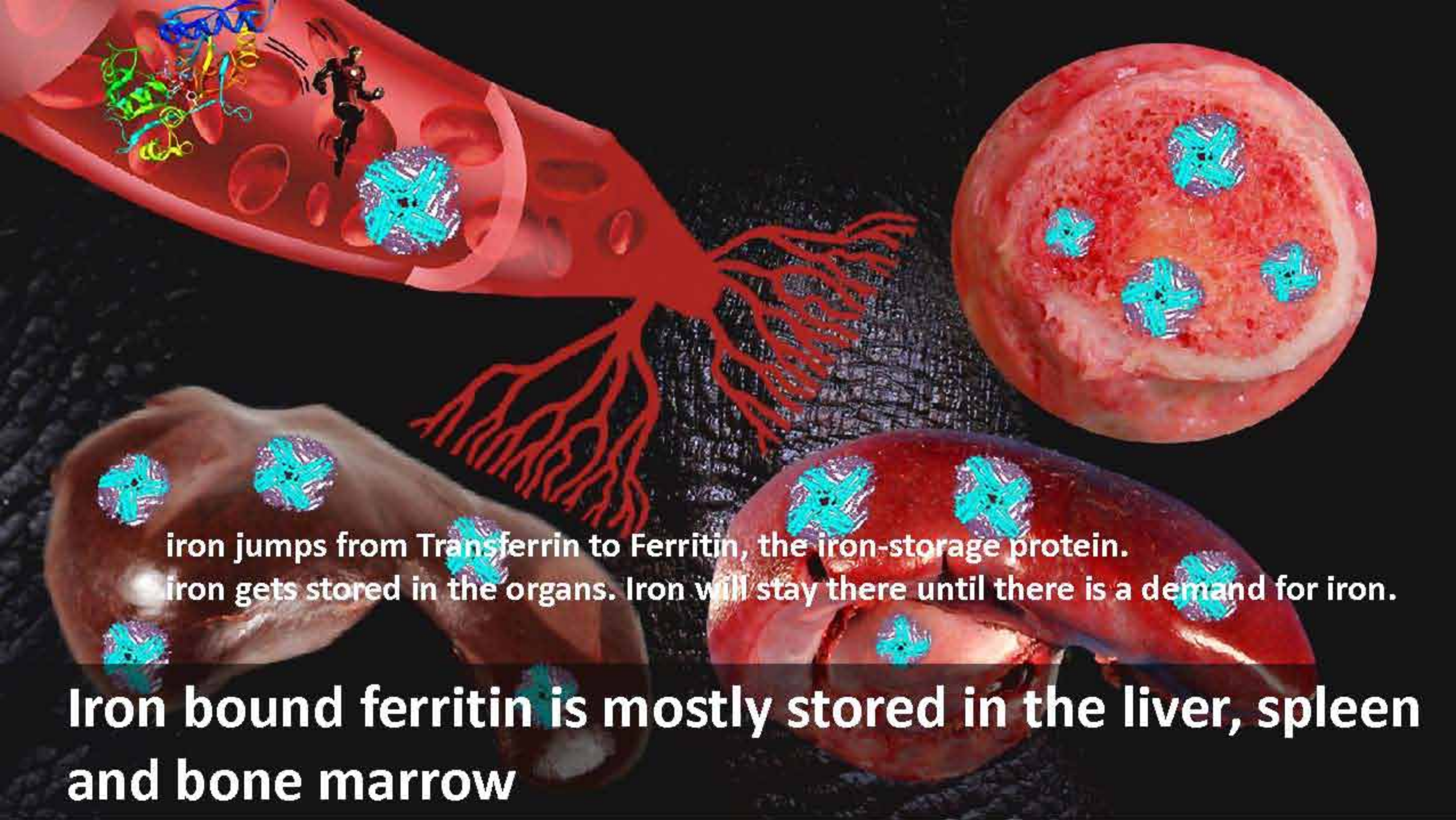
High iron blood levels



Treatment...



**Chelation &
Large volume
Phlebotomy**

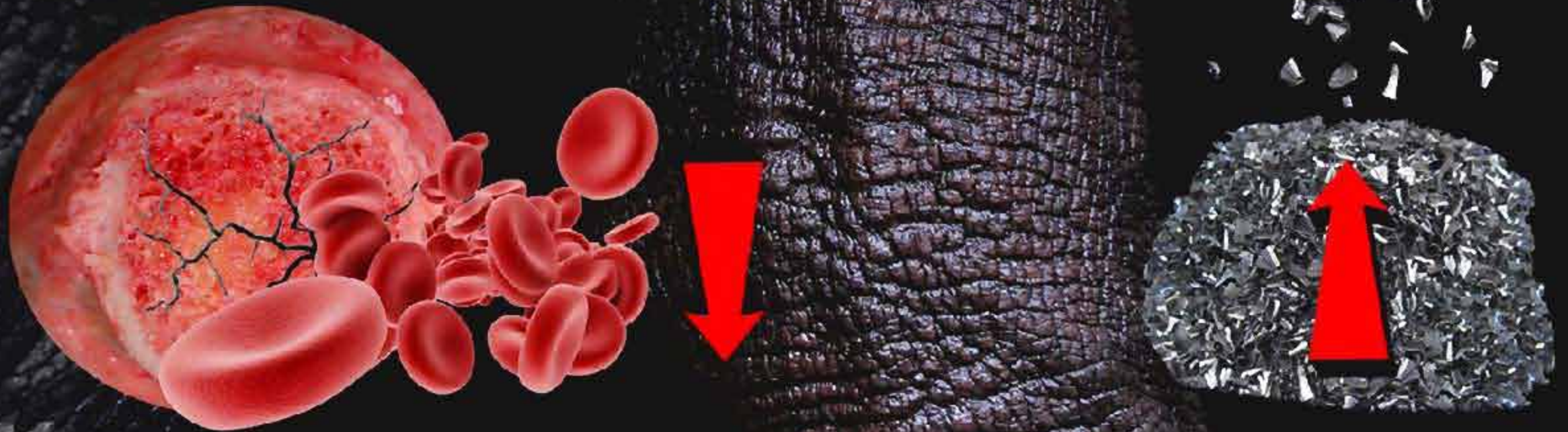


iron jumps from Transferrin to Ferritin, the iron-storage protein.

iron gets stored in the organs. Iron will stay there until there is a demand for iron.

Iron bound ferritin is mostly stored in the liver, spleen and bone marrow

**When the bone marrow is damaged...
Less red blood cells are made...
Less iron will be extracted from the "iron pool"...
The iron levels will rise**



Bone marrow produces red blood cells ... Red blood cells contains a lot of iron



Bound iron is harmless



DANGER
POISON



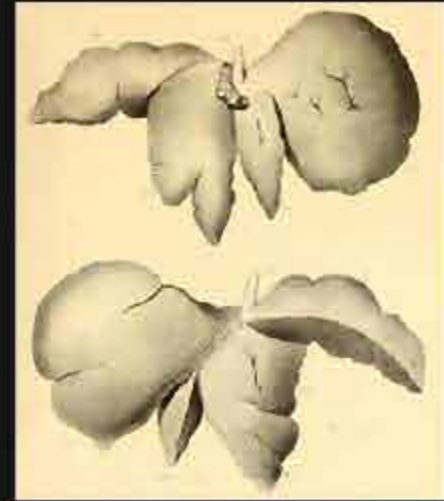
Free iron is dangerous!

When the liver is damaged...

Less transferrin  will be made

Less iron will be bound to the transferrin

The "free-iron" levels in the blood will rise



Normal transferrin: low free-iron



(Low) transferrin: a lot of free-iron



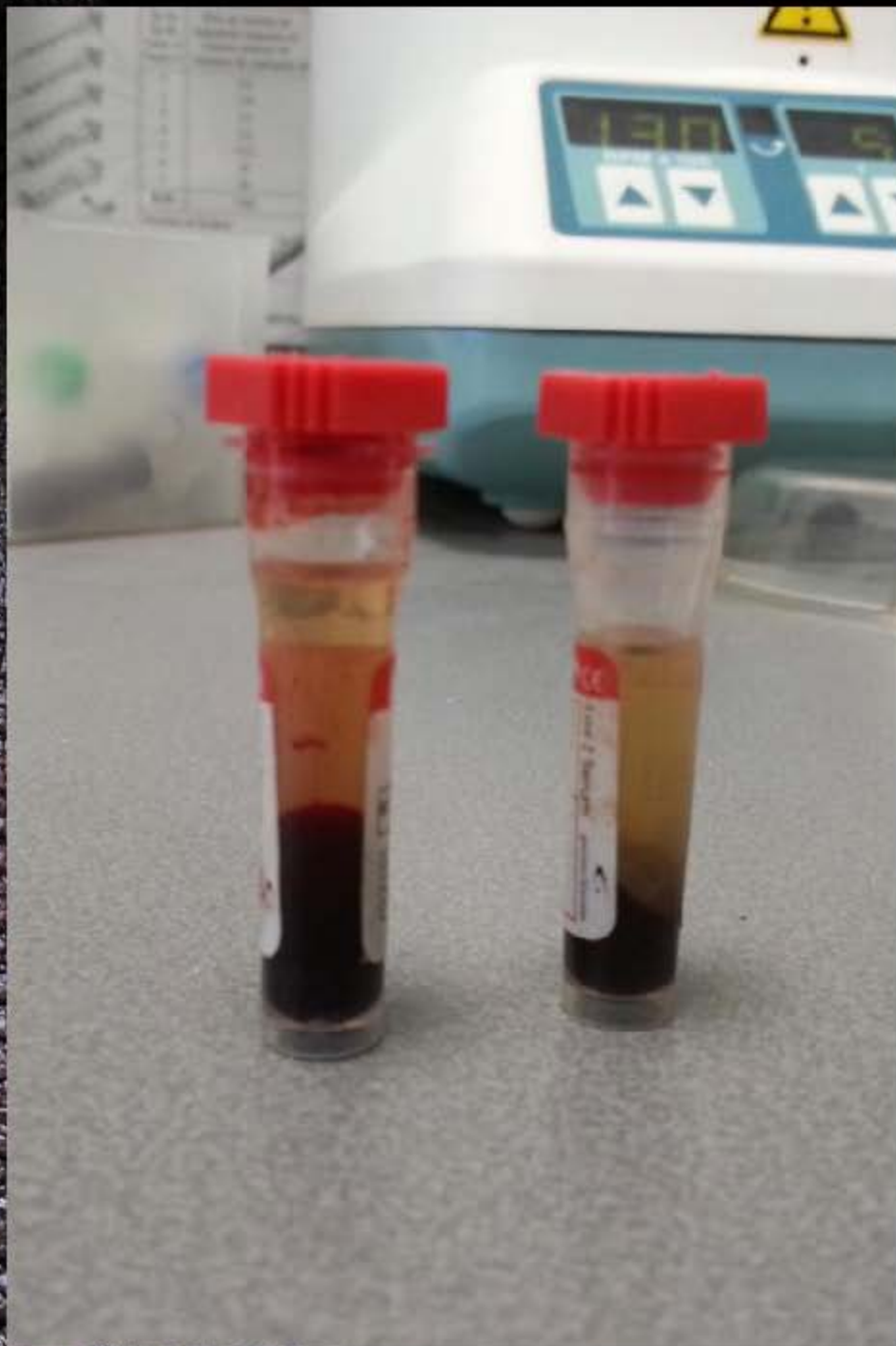
Free iron causes a lot of damage in the body

**When you don't test the blood of your rhino's,
you don't know if your rhino's are healthy.**

To begin....

**When you have black rhino's
in your collection:**

**You have to be able to test
all rhinos on iron values in
the blood.... Blood drawing!**



Small blood collection:

- **Butterfly needle 0,6 mm / 23 gauge**
- **Stop after 2-3 attempts**
- **Avoid sounds / movements**
- **Cold environment... use warm water**
- **2 people**
- **Take your time**



Europe: we test TIBC (Transferrin) and saturation

Monthly monitoring iron values, iron saturation and total iron binding capacity

Iron values:

Iron: amount of iron in the blood

Total iron binding capacity (TIBC): capacity of the blood to bind iron with transferrin

Iron saturation: % of protein (transferrin) which is bound to iron, how many buffer capacity is left in the blood

Ferritin: storage capacity of iron in the body (mainly liver and bone marrow; small amount present in blood), this is not available in Europe at this moment

Relatiecode : 726
Aanvrager : Diergaarde Blijdorp
Ordernummer : 1160728017
Afnamedatum : 26-07-2016
Uw referentie :
Kopie naar :
Vetware ordernr :

Eigenaar : Blijdorp Diergaarde
Adres : Postbus 532
Woonplaats : 3000 AM ROTTERDAM
Diernaam : Vungu Z13343 Zwarte Neushoorn
Soort / Ras : Zoogdier / neushoorns
Geboortedatum :
Geslacht : MAN
PIN :

Bepaling	Resultaat	Ref. waarden	Eenheid
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KLINISCHE CHEMIE

IJzer	67.2		µmol/L
TIJBC	78.6		µmol/L
IJzerverzadiging	85.5		%

Iron

Saturation

Saturation is the most important number! Should be around 40-50% ... above 70% LVP is needed. LVP is a good way to get rid of the excessive iron. This together with low iron (tannin rich) food



Dilation: we use warm water to raise the veins

We installed a thermostat crane between the boiler and the hoose, This way the water is always the same temperature.





Dilation of the vein with warm water... Usually 1 minute is enough.



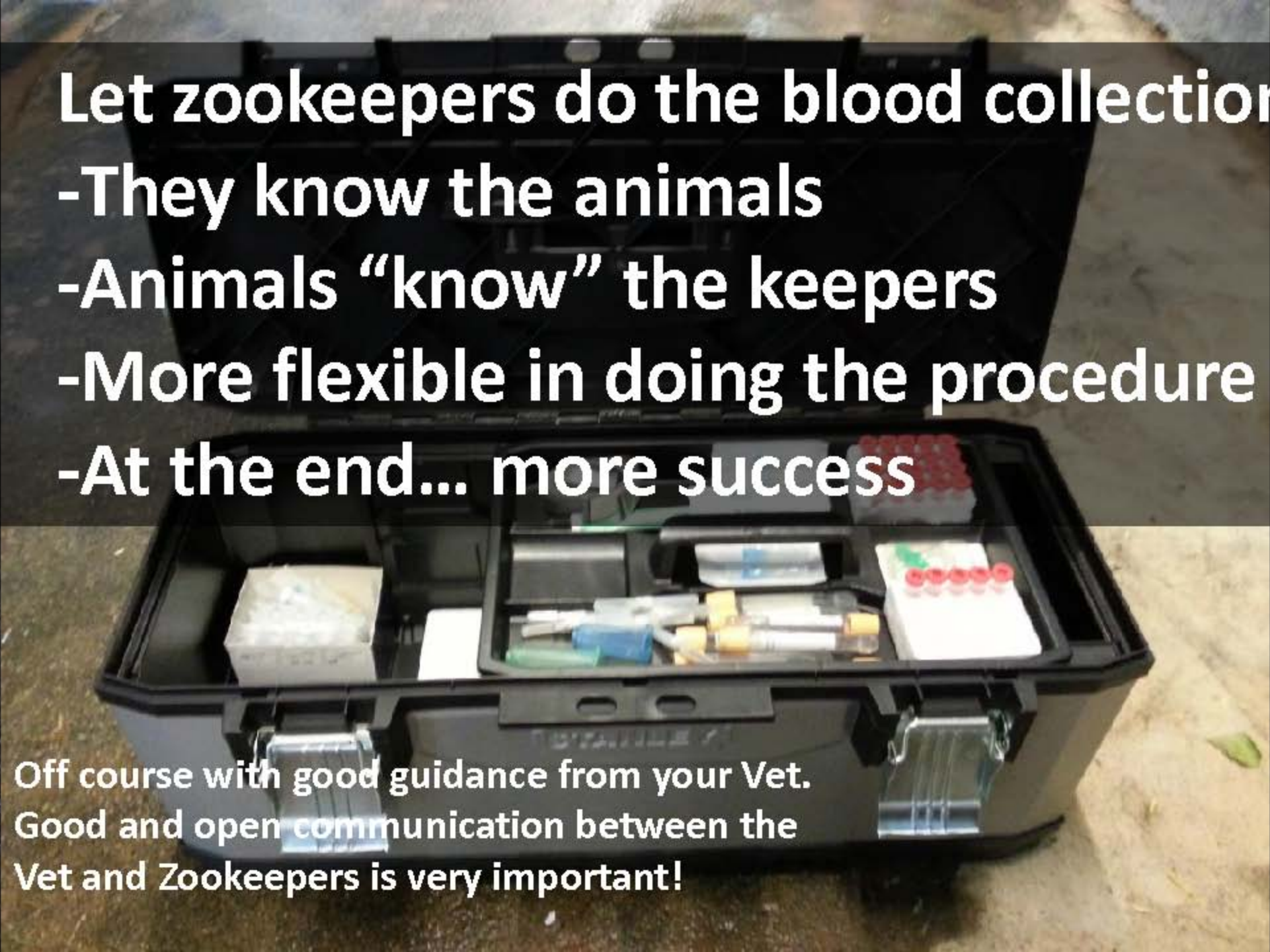
**Front leg blood
collection, inside of
the leg... (farrest
leg)**

When the veins don't dilate or when the Rhino doesn't cooperate we go to Plan B

The nearest front leg vein

- The leg closest to you, very easy to reach.
- Between inner and middle toe.
- It's hard to see the vein but you can feel it really good.
- Feel the vein before inserting the needle!
- Insert the needle on a 90 degree angle.



- 
- Let zookeepers do the blood collection**
 - They know the animals**
 - Animals “know” the keepers**
 - More flexible in doing the procedure**
 - At the end... more success**

Off course with good guidance from your Vet.
Good and open communication between the
Vet and Zookeepers is very important!

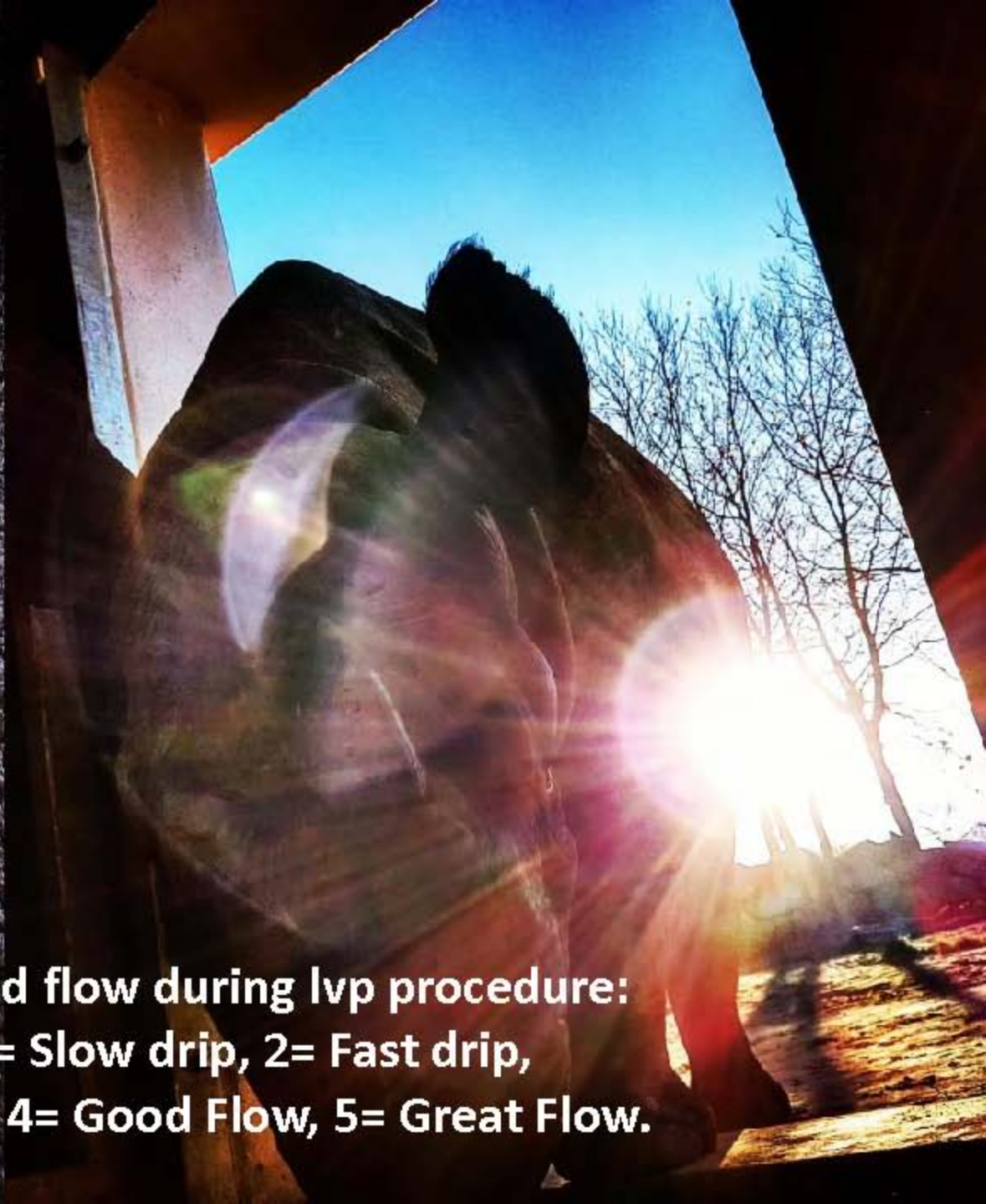
Large Volume Phlebotomy

- Take a lot of blood from the rhino (2-7 liter blood)
- **Body reacts on a created anemia** *Body gets a signal ---- short of red blood cells.*
- **Excessive iron** (stored iron) **will be used for new red blood cell production**
- **Iron saturation will drop**

LVP

- Needs a warm environment to dilate the veins
- Turn the heating on $\geq 23^{\circ}\text{C}$ (73°F)
- At the end of the day the veins are larger (due to heat or movement)
- 3 people needed
- Good communication is essential
- Enough Food 😊
- Medical equipment

Codes for the blood flow during lvp procedure:
0= No flow, 1= Slow drip, 2= Fast drip,
3= Slow flow, 4= Good Flow, 5= Great Flow.



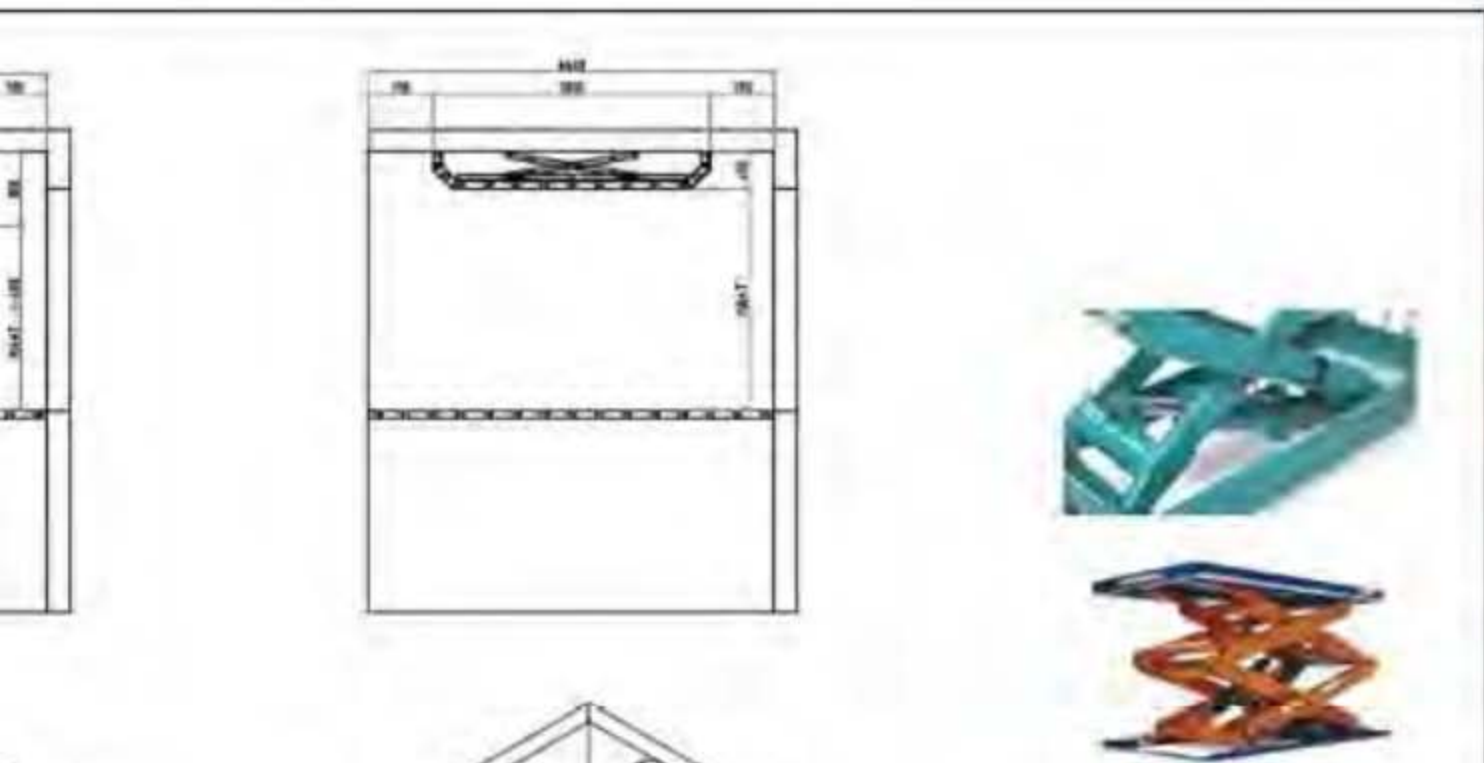
Safety...

....The chute

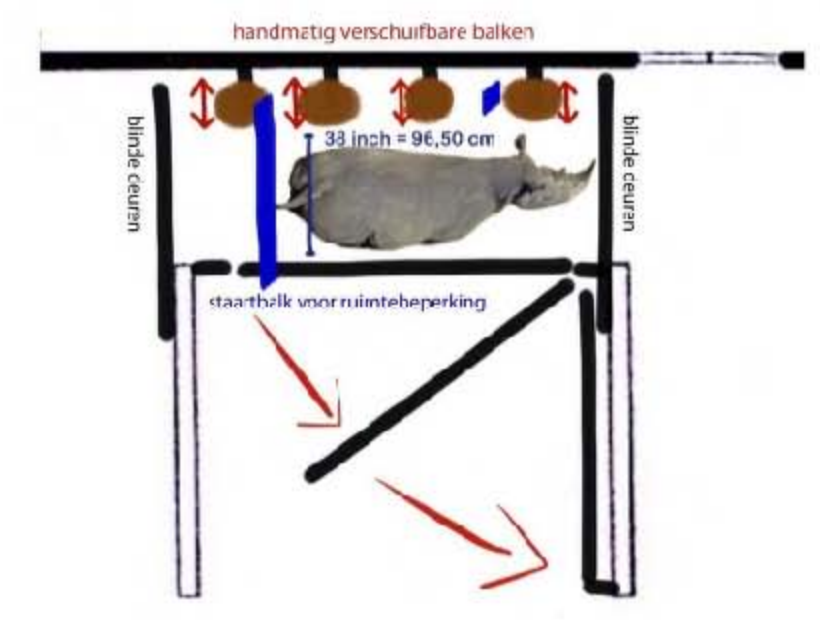




First we didn't have a chute... that wasn't safe



We designed and constructed a chute...





Guidance rail for LVP line (Tube/hoose)



**At the end ...The result is impressive!
Procedures are much safer with the chute.
We never lock them in the chute... when
panicking they can walk out backwards if they
want.**

**We measured the distance to the veins (skin
thickness) and diameter of the veins on the leg.
To search for the best spot to insert the needle
for LVP.**

+ 0.32cm
x 0.72cm

The bottle is normally used for vacuum wound (Redon) drainage on humans after surgery.

Complete set +/- 4 dollar.

First we used 1,1mm 19 Gauge- Flow wasn't good enough.

Switched to 1.27mm 18 gauge-Huge improvement, we had to have an extra flow code... number 5

(after opening the vacuum bottle by moving the white clip, squeeze the green rubber tube to really open the bottle properly)

The LVP setup:

18 gauge, 1,2 mm,

1 inch needle for

front leg placement





Needle placement: we don't use the hind legs...We want to, however our Rhino won't let us. You want to have the farrest leg, good veins are on the inner leg. Farrest front leg slightly behind other front leg.... This way no stretching of the vein. Not on carpal joint (front knee)! Follow the direction of the vein. When the needle placement is higher.... The vein often rolls away.



**When you don't want this behaviour
Also have a lot of positive experiences in the chute
With only negative experiences...You will lose cooperation.**



Also train in chute without using a needle

Black rhino's can freak-out from everything
Let them get used to the equipment and people involved

- for blood collection
- being comfortable around people
- stand still for long periods of time


I think you can't do LVP on every Rhino. Some Rhinos just won't allow it.



Emergency LVP procedure on Black Rhino male Vungu

- Vungu was very slow, didn't eat or move anymore
- His Iron-Saturation was 99%
- We didn't do voluntary LVP yet
- We took 8,5 liter blood, his iron levels dropped
- Vungu probably would have died if we didn't intervene





**We took a maximum of 6,9 liter blood without sedation.
Now we stop after 4 liter to prevent massive drop heamatocrit/hemoglobin
Keep monitoring heamatology!
For an succesfull LVP you should take 4-7 liter of blood per session (For example: 2x2 liter in a short time is also effective)
We had to do the procedure every two months, now +/- 2 in one year (monitor saturation to see when new LVP is neccessary)**

A close-up photograph of an elephant's face behind vertical metal bars of a cage. A large, red, distressed-style stamp is overlaid diagonally across the image, containing the text "NOT THE SOLUTION" in a bold, red, sans-serif font. The stamp has a thick red border and a black background for the text.

NOT THE SOLUTION



Prevention is the best treatment

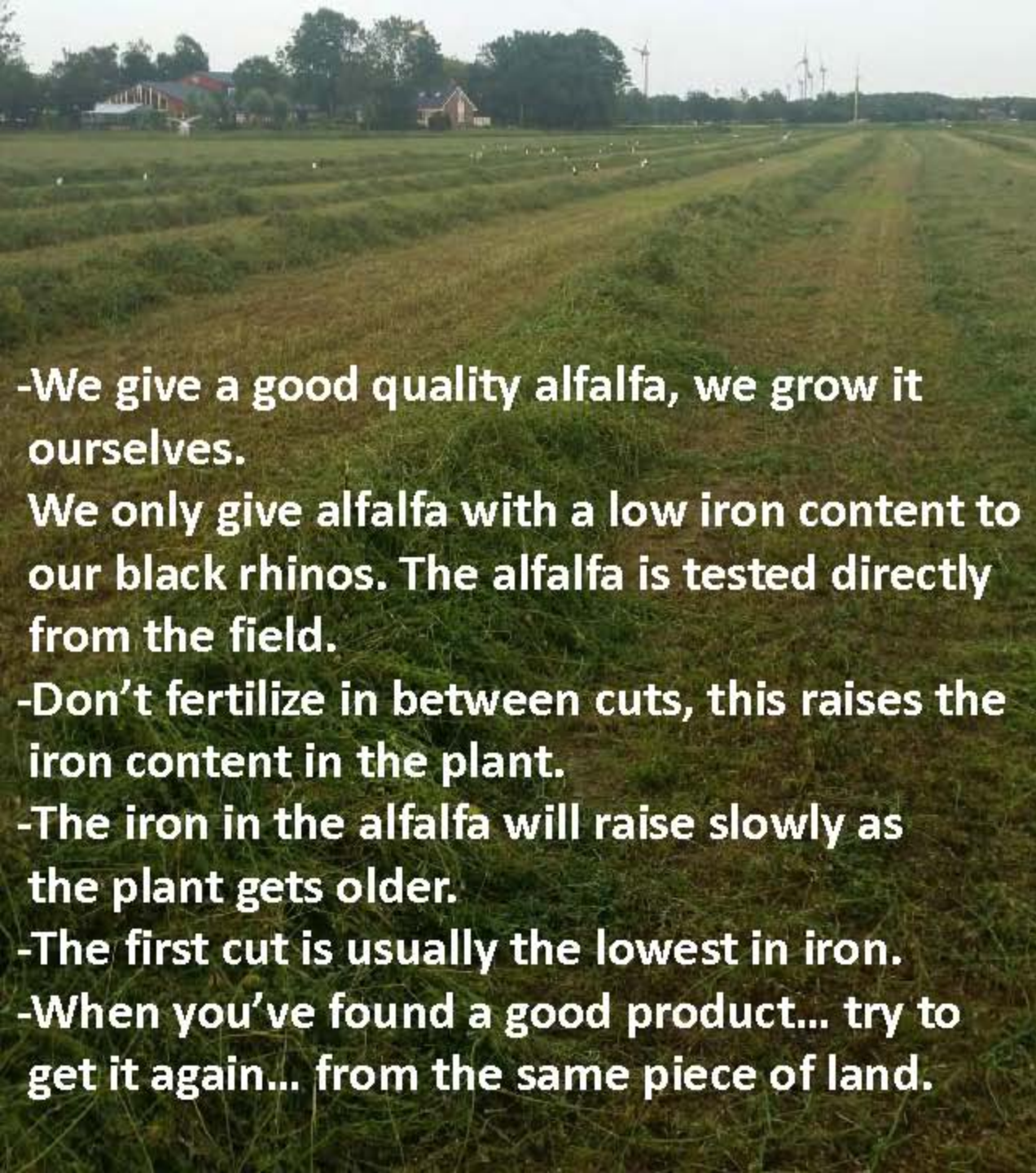


Natural chelation due to tannins in browse

Give a lot of browse...it's *natural food!*

Browse contains tannins.

Tannins are “natural” chelators *and will bind with iron, when iron is bound to tannins, it can't be uptaken in the body.*

- 
- We give a good quality alfalfa, we grow it ourselves.
 - We only give alfalfa with a low iron content to our black rhinos. The alfalfa is tested directly from the field.
 - Don't fertilize in between cuts, this raises the iron content in the plant.
 - The iron in the alfalfa will raise slowly as the plant gets older.
 - The first cut is usually the lowest in iron.
 - When you've found a good product... try to get it again... from the same piece of land.





Test every batch of roughage!
Use pellets with low amount of iron
Vit C enhances the uptake of iron! No produce!
Maximum iron for a black rhino <math><4000\text{ mg/day}</math> and maybe even below 3000mg
Give a lot of tannins! Tannins=Browse!





THE ORIGINAL TEA PLANT

ACACIA BUSH FIBRE
Reg. No.: V12333 Wet/Act 36/1947
Holder: Wes Enterprises
Reg. No.: 1970 / 004382 / 07



MASS 20 Kg MASSA
Produced by **WES** FFEUS
Geproduseer deur VOERE

A division of WES Enterprises (Pty) Ltd.
Wambati's Road, Thabazimbi
P.O. Box 340, Thabazimbi, 0350 FSA
Tel: (014) 777-1330
Fax: (014) 777-1334

LOT No:

**Investing in tannins:
Find out which available browse has
high tannin levels and add it to the
diet of your browsing rhino's.**



**You can't do IOD prevention alone...
it's a Team effort!**

- **Management: *time, funding***
- **Veterinarian(s): *support, supplies, knowledge***
- **Zookeepers: *support, commitment, knowledge***
- **Nutritionist(s): *Support, commitment, knowledge***
- **Animal nutrition department: *supplies, commitment, knowledge***
- **Researchers.... *We can always learn more***



DIERGAARDE
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Keep your Rhino's healthy!

Large Volume Phlebotomy in Black Rhino

Requirements

- Training of animals
 - for blood collection
 - for being comfortable around people
 - to stand still for long periods of time
 - keep it positive (also have training sessions without the use of a needle)
- 3 People needed for the procedure
 - 1 for handling the animal, 1 for needle control, 1 for flow control
 - Good communication during the procedure is essential
 - For example; Codes 0-5 for the blood flow*
0= No flow, 1= Slow drip, 2= Fast drip,
3= Slow flow, 4= Good Flow, 5= Great Flow.
- A chute/ crush to work safely on the animal
- Heat will help dilate veins
 - Warm environment ($\geq 23^{\circ}\text{C}$ or 73°F)
 - Warm water ($30^{\circ}\text{-}35^{\circ}\text{C}$ or $86^{\circ}\text{-}95^{\circ}\text{F}$)



Large Volume Phlebotomy in Black Rhino

Requirements

- Redon vacuum drainage system
 - Privac 600 ml, OK-model, hose 125 cm, Large Lock Connector
 - Vacuum 0,9-1 bar
- Hoose-needle-connector
- Male luer with lock ring x 1/8" hose barb, Nylon, 25/pk
- Needle
 - 1" 18G (Luer Lock)
 - $\geq 1,5$ " does NOT work
- Coolspray (chloor-ethyl)



Large Volume Phlebotomy in Black Rhino

Our advice

- Test animals regularly (every 3 months minimum)
 - Fe (2+), Fe saturation and TIBC (serum)
 - Ht and Hb (EDTA whole blood)
 - GGT, ureum, creatinin (heparin plasma, serum)
- Minimize iron intake!
 - Test every batch of roughage
 - Use pellets with low iron/ low amount of pellets
 - <6000mg daily iron uptake [Clauss] our experience <4000mg!



Iron Overload Disorder

Browsing Rhinoceros



The most iron intake is the small intestine

Erst! Non-natural food, too much iron



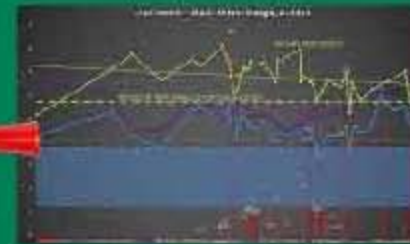
-Urine
-Skin
-New Red blood cells
-Macrophages



Imbalance



Transferrin: iron transport



Ferritin: iron binding

Iron bound ferritin is mostly stored in the liver, spleen and bone marrow

High iron levels in the body



When the bone marrow is damaged... Less red blood cells are made... less iron will be extracted from the 'iron pond'!



-Test all the rhino-food on iron levels. Create a diet that is low on iron.

-Provide a lot of browse. Browse contains tannins. Tannins are natural chelators, they are assumed to bind iron and make it less available for absorption.

-Train your rhino's for blood collection (Leg/ears). Test the blood samples regularly on iron levels.

-Start a Large Volume Phlebotomy program! It's the only proven solution for IOI.

Also train young rhino's for this procedure. The iron-levels will be critical within a few years.

-Chelation therapy can be an option in the future. More research is needed. Reduce stress!



-Cell damage
-Liver damage
-Hemolytic anemia
-Multiple organ/system failure
-More vulnerable to infections
-Skin diseases

Early death!

