TARGET ORTHO

BANNED DRUGS AND ATHLETE IN SPORTS -2

FOP/MO (KHELO INDIA SCHOOL GAMES 2017)
TOURNAMNET I/C 5th Elite Women Boxing Championship
SCIENTIFIC COMMITTEE AT ISSEM
SENIOR RESIDENT DOCOTR at SPORTS INJURY CENTRE, VMMC & SJH , DELHI
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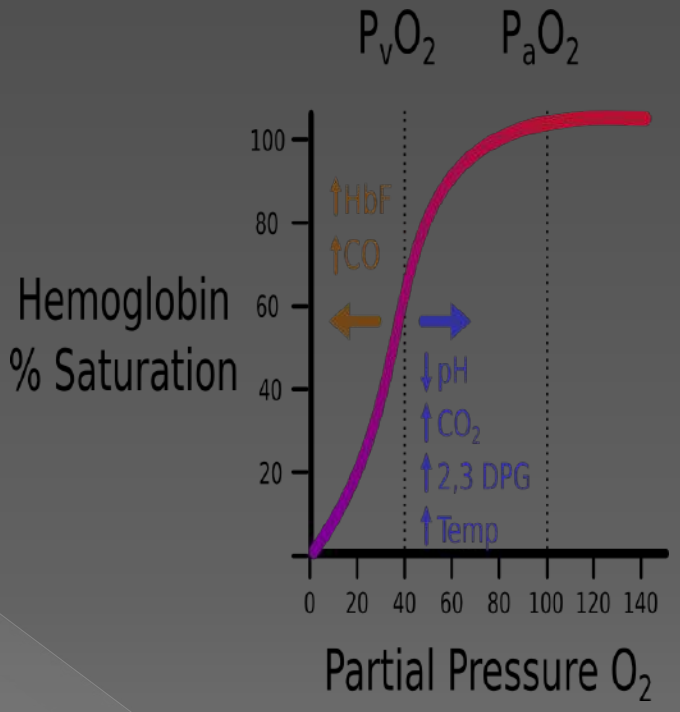
ISSEM
INDIAN SOCIETY OF SPORTS AND EXERCISE MEDICINE

MANIPULATION OF BLOOD AND BLOOD COMPONENTS- M1

- All prohibited methods in this class are non-*Specified* except methods in M2.2. which are *Specified Methods*.
- ALL TIMES
- Prohibited METHODS

BOHRS EFFECT

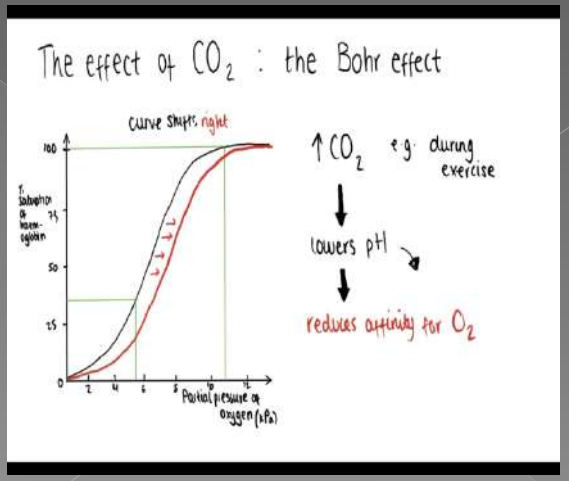
- The sigmoidal shape of the oxygen dissociation curve illustrates ----Hemoglobin's propensity for positive coo perativity, as hemoglobin undergoes conformational changes to increase its affinity for oxygen as molecules progressively bind to each of its four available binding sites.



BOHRS EFFECT

The Bohr effect describes hemoglobin's lower affinity for oxygen secondary to increases in the partial pressure of carbon dioxide and/or decreased blood pH.

This lower affinity, in turn, enhances the unloading of oxygen into tissues to meet the oxygen demand of the tissue



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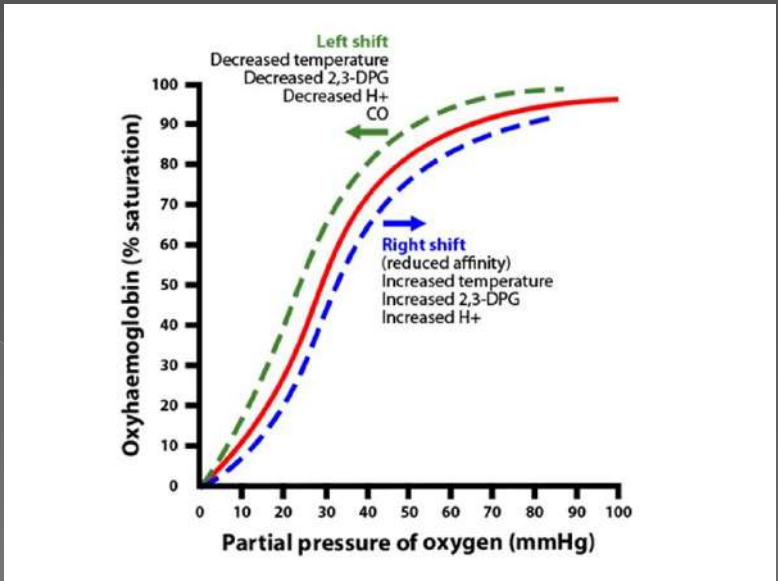
HB DISASSOCIATION CURVE

- SIGMOID SHAPE



The following are prohibited:

TARGET ORTHO

- 1. The Administration or reintroduction of any quantity of autologous, allogenic (homologous) or heterologous blood, or red blood cell products of any origin into the circulatory system.

- 2. Artificially enhancing the uptake, transport or delivery of oxygen. **Including, but not limited to**:
- **PERFLUOROCHEMICALS; EFAPROXIRAL (RSR13)** which modifies hemoglobin's affinity for oxygen and allows for oxygen to be unloaded at low tension **AND MODIFIED HAEMOGLOBIN PRODUCTS**, e.g. haemoglobin-based blood substitutes and microencapsulated haemoglobin products, excluding supplemental oxygen by inhalation.

- 3. Any form of intravascular manipulation of the blood or blood components by physical or chemical means.

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Hemoglobin mass and physical performance

- In AEROBIC sport–long-distance running, cycling or cross country skiing – the main factors determining performance are a high delivery of O2 to the exercising skeletal muscles and its utilization.
- The rate of maximal O2 uptake (O2 max) is dependent on a high cardiac output (Q) and a wide difference for arterial-venous O2 (a-vO2), i.e. the Fick equation:
- VO2 max = Qmax × a-vO2 max

- Predictive of O2 max --Its not the [Hb] , but the total mass of Hb (Hbmass).
- Volume loading, i.e. plasma volume expansion in itself does not lead to an improved exercise performance.
- **Plasma volume expander is administrated simultaneously with increments in Hbmass**, then performance will be likely to increase.

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The influence of Epo on exercise performance

- Subcutaneous (s.c.) administration of rhEpo at doses of 60-350 units (U) per kg-1 body weight (b.w.) and for 4-6 wks increases VO2 max and the time to exhaustion substantially

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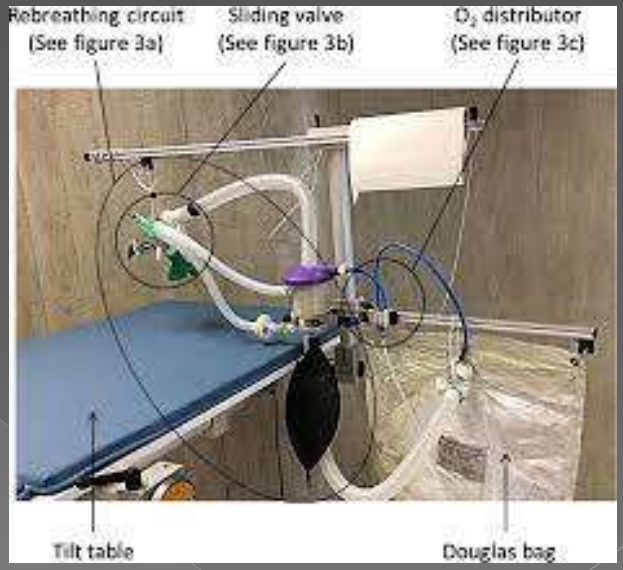
Direct detection of blood doping

- 1. RBC transfusion

- DIRECT- FLOW CYTOMETRY

- INDIRECT- CO re-breathing technique for detecting non-physiological increases in Hbmass.



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CO Rebreathing

- The CO rebreathing method is based on the **DILUTION PRINCIPLE**.
- Inhaled CO serves as a marker to tag circulating hemoglobin molecules, and the dilution of the tagged molecules in the "untagged" hemoglobin molecules is used to calculate the total number of circulating hemoglobin molecules and hence Hbmass.
- CO is used as marker due to its excellent lung diffusion capabilities and its strong affinity for hemoglobin.

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DILUTION PRINCIPLE

- TRACER---------- ADDEDBY INJECTION/INHALATION----------
- CO BINDS TO HB-----

4 Binding

Sites….. Absorbed CO …. $nCO_{absorbed}$

HBCO = % HBCO post - % HBCO pre

- Since no redistribution of CO occurs between red blood cells, only the red blood cells passing the pulmonary capillaries within the rebreathing period can be tagged, which slows the distribution of CO.

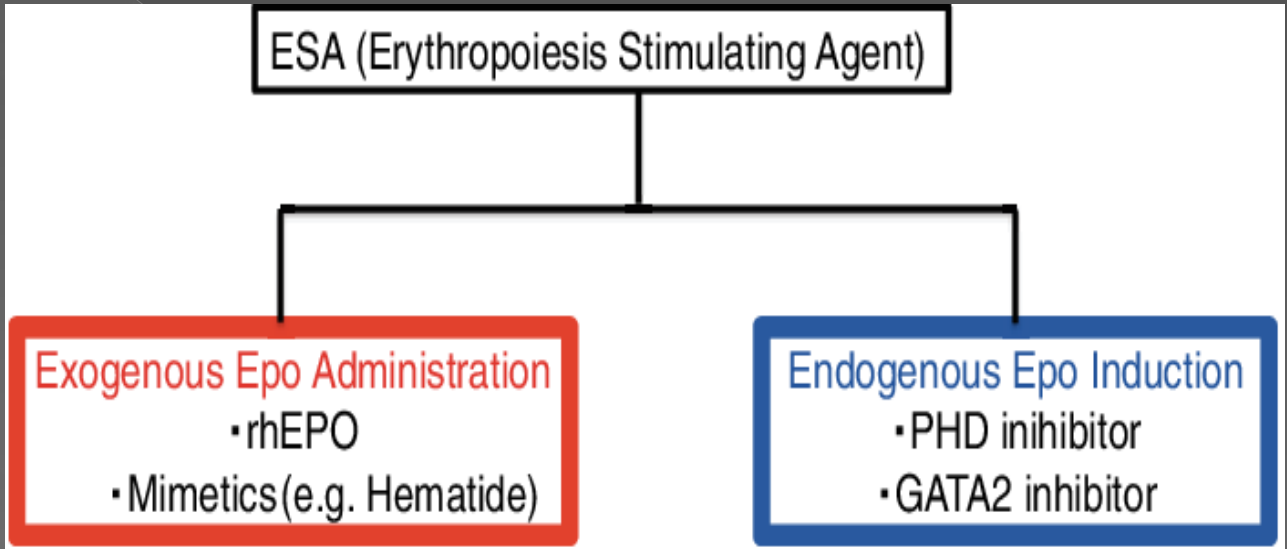


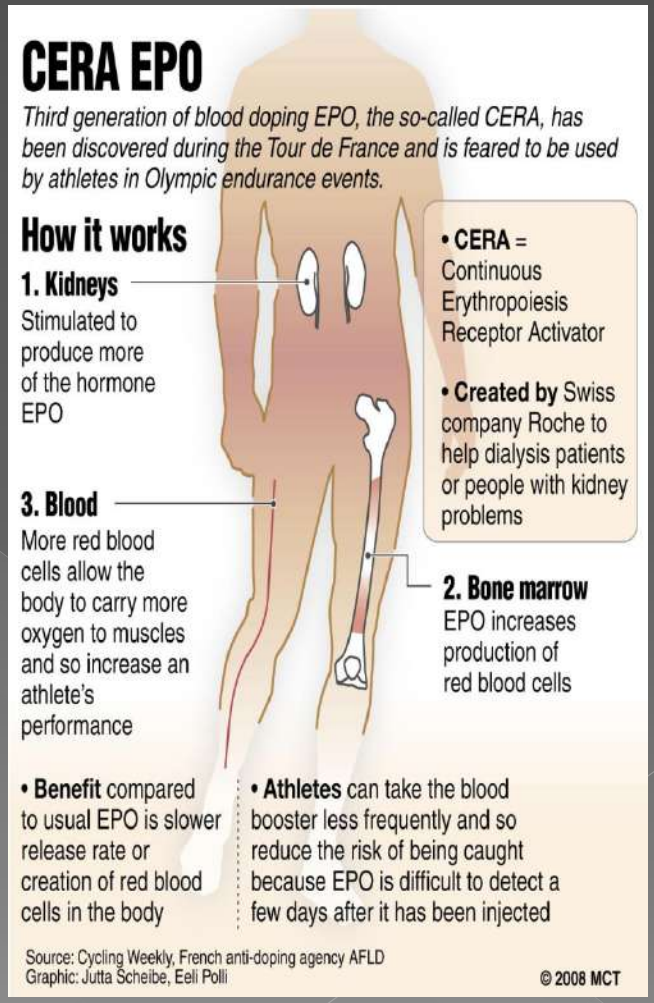
Fig. 1. Classification of erythropoiesis-stimulating agents (ESAs). ESAs can be classified into two categories depending on their mechanisms of action. One mechanism of ESA action is exogenous administration of recombinant human

Peptidic ESAs- ERYTHROPOIESIS STIMULATING AGENT

- rhEpo preparations (epoetins) are produced in Epo cDNA transfected Chinese hamster ovary (CHO) or baby hamster kidney (BHK) cell cultures.

CERA- Continuous Erythropoietin Receptor Activator

- third-generation ESA
- Trade Name- MIRCERA



Riccardo Riccò

- Italian professional road bicycle received a 24-month suspension to Riccardo Riccò after he tested positive for a new Erythropoietin (EPO) drug, Mircera,

Cryo-RBC

- Because cold-stored (+1° to +4°C) blood is viable for a maximum of 42 days, during which time the athlete's physical performance is compromised from blood donation, and erythropoietin is readily detected;
- Blood doping method of choice for athletes is transfusion of one´s own, long-term cryopreserved red blood cells (cryo-RBC).
- Upon transfusion, increases are immediately observed in circulating RBC count, hemoglobin mass ,blood volume and oxygen carrying capacity, resulting in enhanced physical performance.

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DIFFERENT STORAGE CONITIONS

COMP LIFE	TEMP	SHELF
RED CELL	4-6 ° C	35-42 DAYS
FFP	-40° C	1 YEAR
PLATELETS	20-24 °C	5 DAYS
CRYO	-40°C	1 YEAR

Types of Blood Doping

- Blood transfusions
- Injections of erythropoietin (EPO)
- Injections of synthetic oxygen carriers

ATHLETES

- 1. **MARION JONES**
- 2. **LANCE ARMSTRONG**

- **BEN JOHNSON**

Platelet derived preparations (PRP)

- Platelet derived preparations (PRP) are not prohibited. Despite the presence of some growth factors, platelet-derived preparations were removed from the Prohibited List as recent studies on PRP do not demonstrate any performance enhancement beyond a potential therapeutic effect.
- Note that individual growth factors from any other source remain prohibited under S.2.

Platelet derived preparations (PRP)

- Autologous blood fraction with platelet concentration exceeding above baseline.

Bone and soft tissue healing enhancement has been shown using PRP with 1,000,000 platelets/µl, it is this concentration of platelets in a 5-ml volume of plasma which is the working definition of PRP today.

- Platelet number "more not necessarily better" 1.5million/ µl may be optimal, above this has a catabolic effect.
- Timing: During or after inflammatory stage of healing is optimal

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PLATELETS

- Platelets are cytoplasmic fragments of megakaryocytes formed in bone marrow.(2-3micrometre)
- They lack nuclei but contain mitochondria, microtubules and granules…. ALPHA, DELTA & LAMBDA
- Alpha granules bound by membrane(200-500nm)
- 50-80 granules per platelet = 30 bioactive protein= tissue healing.

- Alpha-granules ---the most cited intra-platelet structures because of the presence of coagulation factors, a large number of PDGFs, and regulators of angiogenesis but minimal thrombotic functions.
- The dense granule constituents like ADP, serotonin, polyphosphates, histamine, and epinephrine are more implicit as modifiers of platelet activation and thrombus formation.

RBCs in PRP ???

In response to oxidative stress----RBC cell membrane disintegrates and releases toxic hemoglobin (Hb) , measured as plasma-free hemoglobin (PFH), hemin, and iron
PFH and its degradation products (heme and iron) collectively lead to detrimental and cytotoxic effects to tissues, causing more oxidative stress, loss of nitric oxide, activation of inflammatory pathways, and immunosuppression
Microcirculatory dysfunction, local vasoconstriction with vascular damage, and significant tissue injury.
Most importantly, when C-PRP containing RBCs is delivered to tissues, it causes a local response called eryptosis, which triggers the release of a potent cytokine, macrophage migration inhibitory factor
This cytokine inhibits the migration of monocytes and macrophages. It exerts profound pro-inflammatory signals to surrounding tissues that inhibit the migration of stem cells and fibroblast proliferation and causes significant local cellular dysfunction.

Leukocytes in PRP

- Eosinophils and basophils are not measurable in PRP formulations as their cell membrane is too fragile to withstand the centrifugal processing forces.
- PRP rich in neutrophils could result in a higher collagen type III to collagen type I ratio, adding to fibrosis and decreased tendon strength.
- Mononuclear T and B lymphocytes are more concentrated than any other leukocytes by Cell-mediated cytotoxic adaptive immunity.

Types of PRP

Preparation	Acronym	Leukocytes	Fibrin density
Pure platelet-rich plasma	P-PRP	Poor	Low
Leukocyte- and platelet-rich plasma	L-PRP	Rich	Low
Pure platelet-rich fibrin	P-PRF	Poor	High
Leukocyte- and platelet-rich fibrin	L-PRF	Rich	High

- The active secretion of these growth factors by platelets begins within 10 min after activation, with more than 95% of the pre-synthesized growth factors secreted within 1 h.

- NEXT 5-7 DAYS PLATELETS SECRETE AND SYNTHESIZE ADDITIONAL GFS

Activation causes the granules present in platelets to fuse to its cell membrane (also called degranulation) where the secretory proteins are transformed to a bioactive state by the addition of histones and carbohydrate side chains.

The active proteins are then secreted, binding to transmembrane receptors of target cells, which include mesenchymal stem cells, osteoblasts, fibroblasts, endothelial cells and epidermal cells.

These agonists bound transmembrane receptors then activate an intracellular signal protein that causes the expression of a gene sequence that directs cellular proliferation, matrix formation, osteoid production, collagen synthesis etc. thus provoking tissue repair and tissue regeneration.

PLATELET ACTIVATION

- **IN VIVO**
  - ✓ Anti coagulated PRP,
  - ✓ Earlier bovine thrombin used…risk of coagulopathy
  - ✓ CACL2 AND AUTOLOGOUS PREPARED THROMBIN
- **IN VITRO**
  - ✓ Application of PRP enhances gene expression of ECMP, COLLAGEN PRODUCTION and Tenocyte proliferation
  - ✓ Promote cell proliferation and synthesis of angiogenic factors

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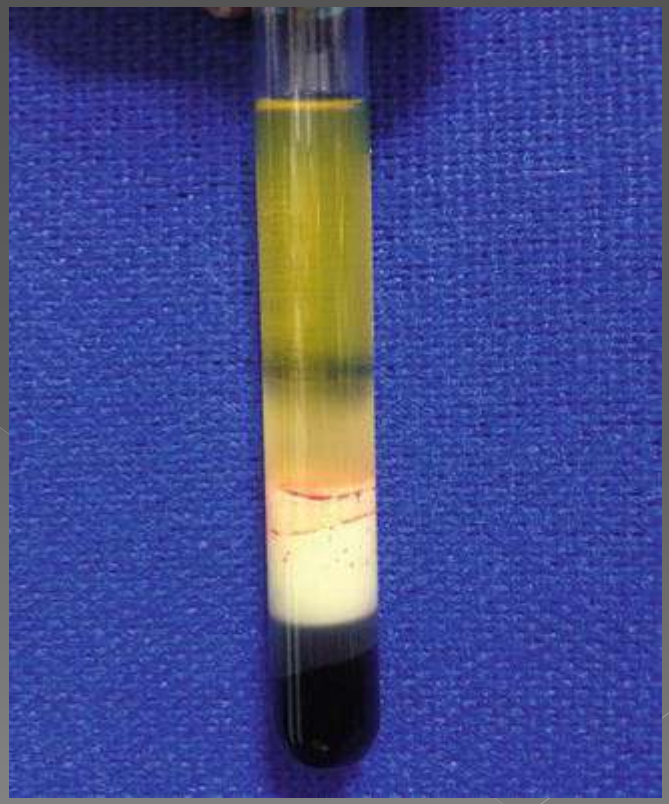
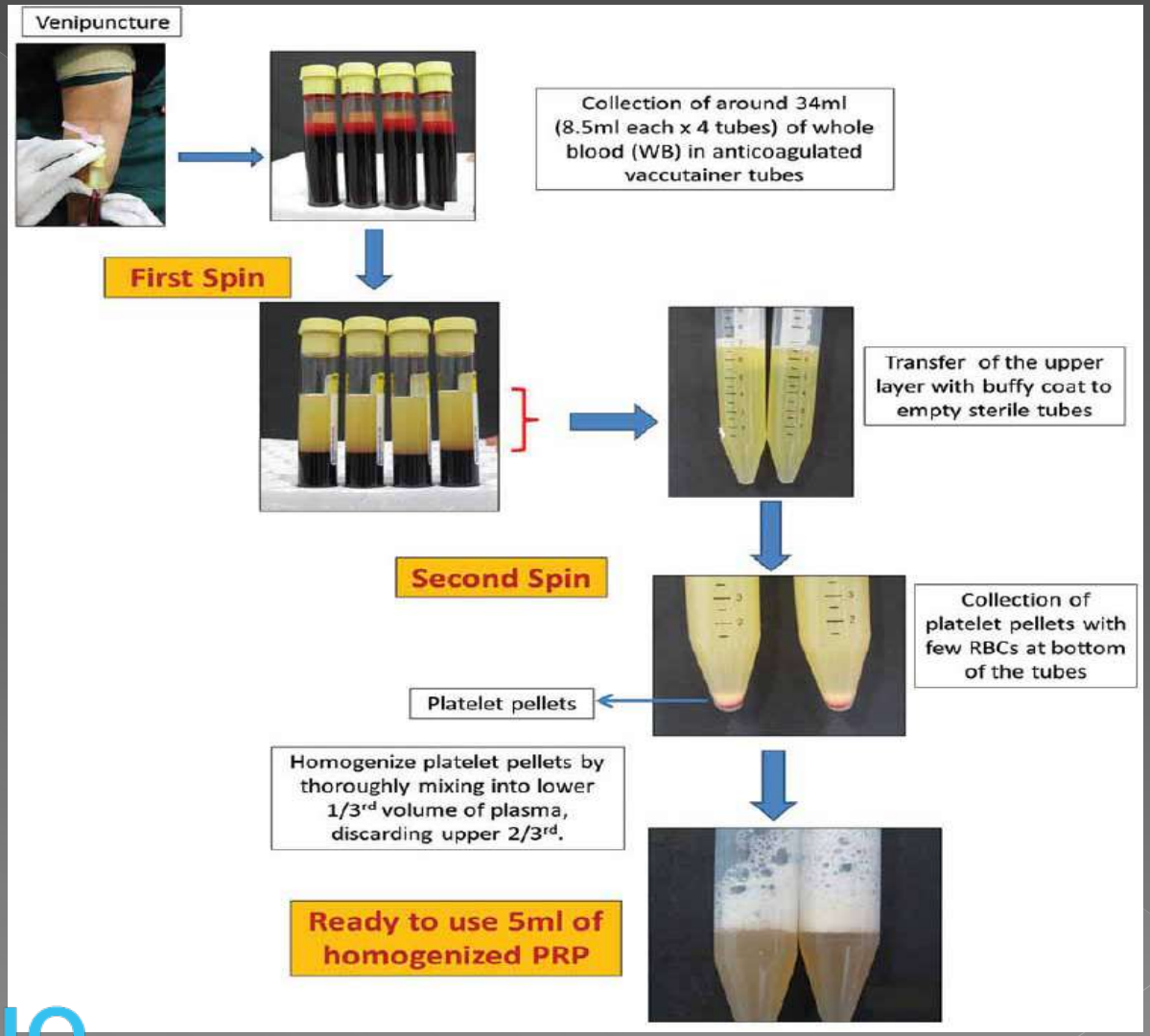
PRINCIPLES OF PRP PREPARATION

PRP is prepared by a process known as **differential centrifugation….**

Acceleration force is adjusted to sediment certain cellular constituents based on different specific gravity.

- ✓ It can be prepared by the PRP method or by the buffy-coat method.





Obtain WB by venipuncture in acid citrate dextrose (ACD) tubes
Do not chill the blood at any time before or during platelet separation.
Centrifuge the blood using a 'soft' spin.
Transfer the supernatant plasma containing platelets into another sterile tube (without anticoagulant).
Centrifuge tube at a higher speed (a hard spin) to obtain a platelet concentrate.
The lower 1/3rd is PRP and upper 2/3rd is platelet-poor plasma (PPP). At the bottom of the tube, platelet pellets are formed.
Remove PPP and suspend the platelet pellets in a minimum quantity of plasma (2-4 mL) by gently shaking the tube.

BUFFY COAT METHOD

WB should be stored at 20°C to 24°C before centrifugation.
Centrifuge WB at a 'high' speed.
Three layers are formed because of its density: The bottom layer consisting of RBCs, the middle layer consisting of platelets and WBCs and the top PPP layer.
Remove supernatant plasma from the top of the container.
Transfer the buffy-coat layer to another sterile tube.
Centrifuge at low speed to separate WBCs or use leucocyte filtration filter.

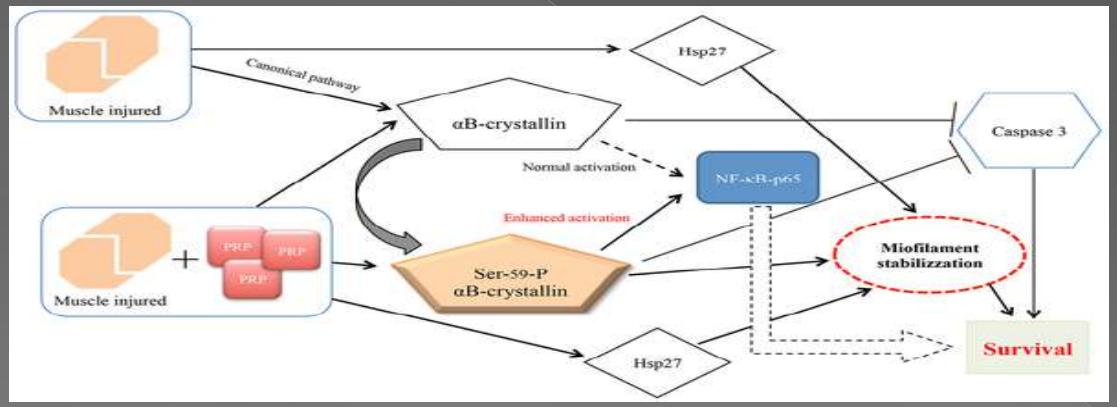
REGULATION

- WADA had temporarily banned PRP in 2009-2011.
- Currently not a banned substance but individual growth factors still banned.
- FDA approved use for bone graft substitute, office use is off label.
- No regulation by NCAA, NHL, NFL & NBA
- 2008 IOC consensus documented its evidence on connective tissue and muscle injury healing there is anecdotal evidence.

PRP in Muscle Healing

- PRP treatment increased myocyte proliferation, growth factor expression (e.g., PDGF-A/B and VEGF), leukocyte recruitment, and angiogenesis in muscle models when compared to control groups.

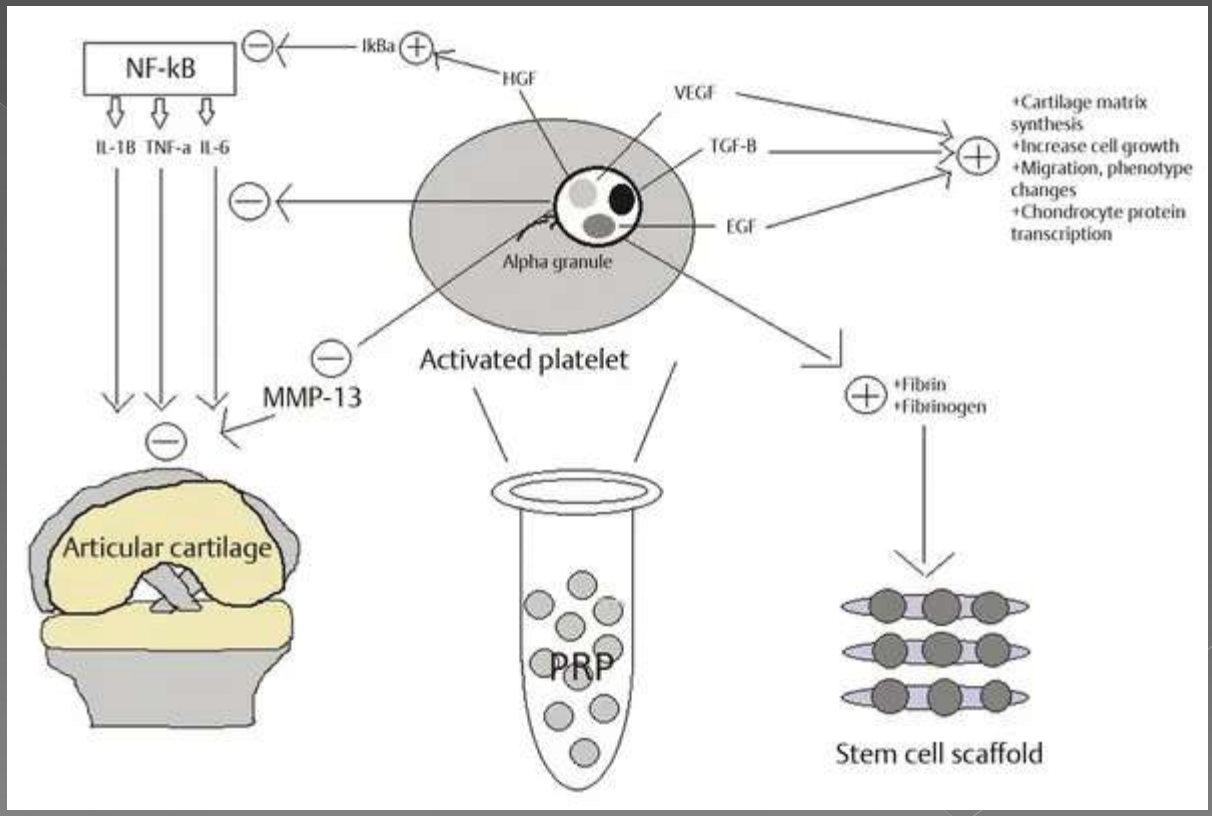
- PRP preparation techniques remain inconsistent across studies in the basic science literature



PRESENCE OF PRP ENHANCES PHOSPHORYLATION OF SER-59 OF AB-CRYSTALLIN, WHICH BINDS MYOFILAMENTS AND THE INACTIVE PRECURSOR OF CASPASE 3, CAUSING THEIR STABILIZATION AND INHIBITION OF APOPTOSIS.
PHOSPHO SER-59 AB-CRYSTALLIN ENHANCES NF-KB-P65 ACTIVATION WHICH MAY CONTRIBUTE TO INCREASED CELL SURVIVAL DURING REGENERATION PROCESS.

PRP in Cartilage

- Increase synthetic capacity of chondrocyte.
- Increase gene regulation through upregulation.
- Increase proteoglycan production.
- Increase deposition of type 2 collagen.
- Inhibit catabolic effect of IL-B, TNF-alpha on chondrocytes.



Systematic effects of PRP

- Increase in serum IGF, BFGF and VEGF levels.
- Activate biological pathway causing increase in GF levels.
- VEGF levels increase upto 4 weeks post injection and serve as testing marker.

(Wasterian et al AJSM, 2013)

The DEPA classification

1.Dose of injected platelets

: calculated by multiplying the platelet concentration in PRP by the obtained volume of PRP. According to the injected dose (measured in billions or millions of platelets), it should be categorized into

(a) very high dose of injected platelets of >5 billion

(b) high dose of injected platelets, from 3 to 5 billion

(c) medium dose of injected platelets, from 1 to 3 billion,

(d) low dose of injected platelets, <1 billion.

2. Efficiency of the production

: corresponds to the percentage of platelets recovered in the PRP from the blood. It is categorized as follows:

(a) high device efficiency, if the recovery rate in platelets is >90%

(b) medium device efficiency, if the recovery rate in platelets is between 70 and 90%

(c) low device efficiency, if the recovery rate is between 30 and 70%, and

(d) poor device efficiency, if the recovery rate is <30% and corresponds to the relative composition of platelets, leucocytes, and RBCs in the obtained PRP.

- 3. **Purity of the PRP obtained**:
  - correlates to the relative composition of platelets, leucocytes, and RBCs in the obtained PRP. It is described as

(a) very pure PRP, if the percentage of platelets in the PRP, compared with RBCs and leucocytes, is >90%;

(b) pure PRP, between 70 and 90% of the platelets;

(c) heterogeneous PRP, if the percentage of platelets is between 30 and 70%, and

(d) whole-blood PRP, if the percentage of platelets in the PRP is <30% compared with RBCs and leucocytes.

- 4. **Activation process**:
- if an exogenous clotting factor was used to activate platelets, such as autologous thrombin or calcium chloride

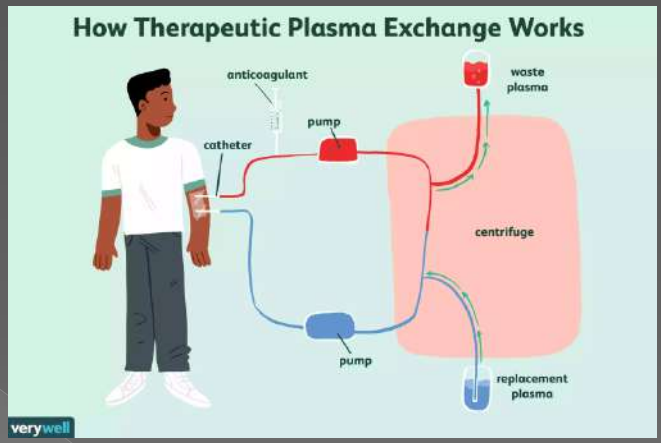
Factors influencing PRP yield

The clotting process is influenced from the time of the draw. To avoid unintentional activation of platelets, most protocols use large bore needles (>22) to draw the blood…. downward trend in platelet counts with longer draw time. \
CENTRIFUGATION…..
Temperature 21°C-24°C for centrifugation of blood for obtaining PRP…. 12°C-16°C for storage
1. Anticoagulants…EDTA because could damage the platelet membrane. anticoagulants with citrate and dextrose of sodium citrate are recommended. ACD-A & Na-Citrate…. Vials difference in PH
Activation of PRP…. PRP can be activated exogenously by thrombin, calcium chloride or mechanical trauma. Collagen is a natural activator of PRP, thus when PRP is used in soft tissue, it does not need to be exogenously activated

BANNED OR NOT BANNED???

PLASMAPHERESIS

- The status of plasmapheresis is different for plasma donors and recipients:
- For the **PLASMA DONOR**, plasmapheresis is prohibited under section M1.1 because the donor's own red blood cells (and other blood components) are being reintroduced back into their own circulatory system after the plasma or blood components have been separated outside of the person's body.
- For the **PLASMA RECIPIENT,** who is receiving plasma from a different donor, plasmapheresis is not prohibited under M1.1 or M1.3 as the patient receives only plasma, but not whole blood or red blood cells.
- **For the plasma recipient, plasmapheresis would only be prohibited under M2.2 if it is not legitimately received in the course of hospital treatment when the volume is more than 100 mL per 12 hour period.**



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Intravenous Laser Therapy

- Intravenous laser therapy is prohibited under M1.3 as defined by "Any form of intravascular manipulation of blood…"

Methylhexaneamine (MHA)

TARGET ORTHO

- MHA is known by many different names, including, but not limited to, dimethylamylamine, 1,3-dimethylamylamine, dimethylpentylamine, methylhexamine, methylhexanamine, 1,3-dimethylpentylamine. It is prohibited In-Competition only as a specified stimulant under Section 6.b.
- .

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Colostrum

- Colostrum is not specifically prohibited, however it contains certain quantities of IGF-1 and other growth factors which are prohibited and may influence the outcome of anti-doping tests.
- Therefore, WADA does not recommend the ingestion of this product.

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Dialysis a Prohibited Method?

TARGET ORTHO

- Dialysis (also known as hemodialysis) is a medical treatment for patients with kidney failure.
- Dialysis is a prohibited method under M1.1, as blood is taken out from the patient and filtered, before being reintroduced back into the patient's circulatory system.
- An athlete needing dialysis treatment requires a Therapeutic Use Exemption.

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Mannitol

- Mannitol by INHALATION IS PERMITTED e.g. to perform bronchial provocation testing in asthma.
- Mannitol is only prohibited when administered intravenously.

EYE DROP

- Carbonic anhydrase inhibitors DORZOLAMIDE and BRINZOLAMIDE, when administered topically in the eye, are not prohibited.
- Do not have a diuretic effect when topically applied.
- Eye drops containing BETA-BLOCKERS **ARE** PROHIBITED IN PARTICULAR SPORTS under section P1 because the ophthalmic administration of beta-blockers results in systemic concentrations of the drugs similar to when the medication is taken orally.

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EYE DROP

- Anti-inflammatories
  - Non-steroidal anti-inflammatories, antihistamines, and mast cell stabilisers are permitted for all routes of administration.

- Mydriatics and cycloplegics
  - Atropine, cyclopentolate, homatropine, and tropicamide are all permitted.
  - Phenylephrine is listed in the 2012 Monitoring Programme as a stimulant with the potential for abuse, but it is not currently prohibited.

Local Anaesthetics

- Lidocaine, bupivicaine, proxymetacaine, and tetracaine are permitted for all routes of administration.
- Cocaine is prohibited in-competition.
- Adrenaline is permitted only for local administration (for example, ophthalmologic, nasal) or when co-administered with a local anaesthetic; it is otherwise prohibited in-competition.
- Tear deficiency, ocular lubricants, and astringents----All artificial tears, ocular lubricants, and astringents are permitted.

EYE DROP-Treatment of glaucoma

- Beta-blockers are prohibited by all routes in- and out-of-competition in the following sports: archery and shooting.
- They are prohibited in-competition only in the following sports: aeronautics, automobiles, billiards, boules, bridge, darts, golf, ninepin and tenpin bowling, powerboating, skiing and snowboarding (in ski jumping, freestyle aerials/halfpipe and snowboard halfpipe/big air).
- All prostaglandin analogues and prostamides are permitted.
- The sympathomimetics apraclonidine and brimonidine are permitted.
- Topical preparations of brinzolamide and dorzolamide are not prohibited.

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Mohd Asif- Unintended Doping

- Pakistan pacer Sanctioned ban for 1 year after tested positive for Nandrolone
- He was taking keratyl", an eye drop containing Nandrolone, and this was the reason for his positive test.

Difference between a "delivered" vs "metered" dose from asthma inhaler?

- For beta-2-agonists, given by ANY device, the amount of drug can be expressed in two ways:
- **Metered Dose**– the quantity of drug substance contained in the delivery device (inhaler)
- **Delivered dose**– the amount of drug that is available to the lungs; delivered from the mouthpiece of the inhalation device.

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- The Prohibited List refers to the

- **DELIVERED DOSE FOR FORMOTEROL**
- **METERED DOSE FOR SALBUTAMOL, SALMETEROL AND VILANTEROL**

Vitamin B12, as it contains cobalt?

- Vitamin B12 (cyanocobalamin) is not prohibited because the cobalt present does not have the same effects as elemental cobalt or cobalt salts.
- The amount of cobalt that is naturally contained in food is not significant and would not be enough to act as a doping agent.
- If a dietary supplement includes cobalt, for example inorganic cobalt or cobalt salts, then it would be considered prohibited.Ø

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Are hypoxic chambers permitted?

TARGET ORTHO

- Hypoxic chambers artificially induce hypoxic conditions.
- Their use is not prohibited by WADA, however some sporting authorities ban the use of hypoxic chambers during competitions under their sport rules.
- Athletes must check the rules that apply to hypoxic chambers with the sporting authorities governing the events they compete in.

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What is the status of supplemental oxygen?

- Supplemental oxygen administered by INHALATION, BUT NOT INTRAVENOUSLY, is permitted.

Trimetazidine (TMZ)

- Antianginal agent trimetazidine increases cell tolerance to ischaemia by maintaining cellular homeostasis.
- It has Cytoprotective activity which limit myocyte loss during ischaemia in patients with angina pectoris.
- Generic name- 1-(2,3,4-trimethoxybenzyl)piperazine
- The drug is currently listed as a "metabolic modulator" and WADA prohibits athletes from using the drug in or out of competition.

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Sun Yang- Chinese Swimmer

- First male swimmer in history to earn Olympic and World Championship gold medals at every FREESTYLE distance from 200 to 1500 metres.
- In May 2014, the Sun YANG was banned for three months after he tested positive for trimetazidine, that was banned four months earlier and classified as a stimulant at the time by the WADA.

CURIOIS CASE OF SUN YANG AND BAN

2018 testing incident and 2020 ban

- He was banned eight years in a doping case stemming from destroying a drug-test sample with a hammer in September 2018.
- Sun was previously suspended three months in 2014 for a banned stimulant.
- Hammer incident to be his second violation, thus a stiffer penalty.

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MANIPULATION OF BLOOD AND BLOOD COMPONENTS- M1

- ALL TIMES
- All prohibited methods in this class are non-*Specified* except methods in M2.2. which are *Specified Methods*.