Review
https://doi.org/10.61900/SPJVS.2023.02.09

THE USE OF PLATELET-RICH PLASMA TO IMPROVE IN VITRO EMBRYO PRODUCTION AND IMPLANTATION RATE IN MAMMALS

Silviu-Ionuț Borș

Research and Development Station for Cattle Breeding, 707252 Dancu, Iaşi, Romania

bors.ionut@yahoo.com

Abstract
It has been demonstrated that platelet-rich plasma (PRP), a preparation of plasma enriched with a platelet level above the baseline, is essential for the process of tissue regeneration. Over the past ten years, PRP has drawn more attention as an unusual form of therapy. Applications of PRP in animals have demonstrated varying degrees of efficacy in treating a wide range of medical conditions, ranging from ovarian insufficiency to musculoskeletal ailments. Although there are currently few therapeutic PRP uses in farm animals, the encouraging findings of a number of research will likely lead to a rise in interest in PRP use among farmers and veterinarians. In animal reproduction, PRP can be used to enhance follicular growth, oocyte competence, and the uterine environment to boost the implantation rate of the embryos.

Keywords: mammals, reproductive medicine, platelet-rich plasma, embryos

1. Introduction

The history of platelet-rich plasma (PRP) began with the first publication in Nature magazine (Kingsley, 1954), followed by the first article about ten years later to describe the use of PRP in a therapeutic approach (Levin et al., 1964). Platelet-rich plasma has already been used clinically in humans and animals for its healing properties related to increased concentrations of autologous growth factors (Foster et al., 2009). Notable growth factors released from platelets that are involved in the healing process include platelet-derived growth factor (PDGF), transforming growth factor (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and insulin-like growth factor (IGF-1) (Wu et al., 2016). Platelets also release chemicals that promote tissue repair and significantly impact the reactivity of vascular and other blood cells in angiogenesis and inflammation. Apart from the storage pools of growth factors they contain cytokines including proteins such as PF4 (platelet factor 4) and CD40L (Anitua et al., 2004).

In reproductive medicine of dairy cows, platelet-rich plasma (PRP) has recently been used and is based on the knowledge that platelet growth factors can improve the endometrial environment, which is abundant of growth factor receptors, adhesion molecules, cytokines, lipids, and other factors that improve endometrial and embryonic growth. Despite the progress in the field of assisted reproductive technology (ART) multiple embryos fail to implant (Gonçalves et al., 2019).

PRP is simple to obtain, with low cost and is rich in growth factors. Since PRP is an autologous preparation, and thus non-toxic and non-allergenic, it can be used as an adjuvant therapy for traditional treatment under different medical conditions, with usually acceptable results (Molina et al., 2018).

This review offers a brief description of PRP and summarizes what the literature has so far provided of using PRP in reproductive medicine of mammals.

2. Definition of Platelet-rich plasma (PRP)

Platelet-rich plasma (PRP) is a biological product known as a portion of the autologous blood plasma fraction with a platelet level above the baseline (Alves and Grimalt, 2016), enriched in platelet-rich growth factors (GFs), chemokines, cytokines, and other plasma proteins (Lynch and Bashir, 2016). Also, PRP provides not only a high platelet level, but also the clotting factors, which usually remain at their normal physiological levels (Wroblewski et al., 2010).

PRP have been used as an adjuvant for various indications for more than 30 years, resulting in considerable interest in the potential of autologous PRP in regenerative medicine (Everts et al., 2020). Despite its substantial use for therapy in various fields, no standardized procedure has been yet set up for the preparation of PRP. A multitude of protocols is available, implying a simple or double centrifugation (Figure 1), and all of them have shown an increase in the platelet

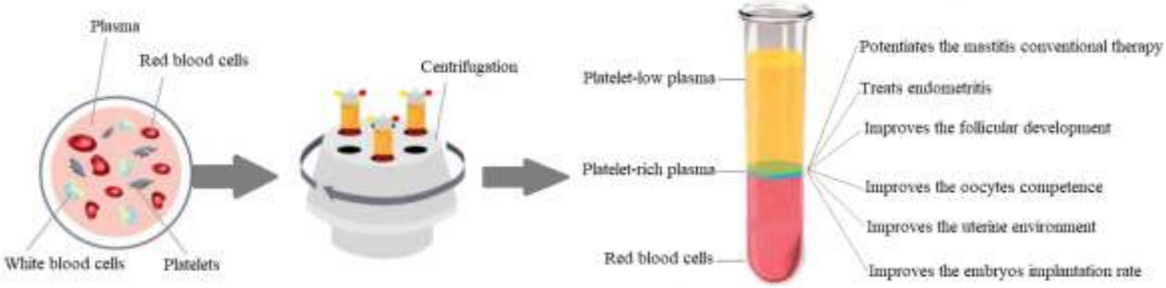

Figure 1. Schematic representation of the Platelet-rich plasma protocol. In the bovine reproductive medicine the PRP can be use to potentiate the mastitis conventional therapy, to treat endometritis and to improve the follicular development, oocyte competence and uterine environment for increasing the embryos implantation rate. Adapted from Gonçalves et al., (2019).

concentration allowing a therapeutic effect (Croisé et al., 2020). However, one must be aware that significant differences in components are observed in different separation methods and may have specific results on treated tissue (Mazzocca et al., 2012). Grossly elevated concentrations of platelet-released GFs may also have inhibitory effects on healing (Collins et al., 2021). Each preparation method is intended to create an end product with a particular bioaction, and consequently, with a specific clinical application and not just one single final blood derivate containing plasma and high concentrations of platelets (Bos-Mikich et al., 2018). Briefly, PRP is a prepared product from blood collected on anticoagulant via a one- or two-stage centrifugation protocol. The collected blood is submitted to a soft spin centrifugation to separate the red blood cells (lower layer, approx. 45%) from the rich in platelets plasma (upper layer, approx. 55%). In between there will be a leucocyte layer (buffy coat, <1%). The upper level can be sampled and used as it is, or submitted to a second, hard spin centrifugation to obtain a platelet concentrated plasma or pure PRP, made of granules, which is represented by the lower layer. This coat is in general subsequently homogenized with plasma (Croisé et al., 2020). There are a range of commercial kits available to ease PRP preparation (Dhurat and Sukesh, 2014).

Growth factors are released from platelets during physiological wound healing as a consequence of activation of the clotting cascade. Most protocols support platelet activation ex vivo to induce growth factor release. The addition of calcium chloride or thrombin, which induces the degranulation of platelets and the release of growth factors, can accomplish this. Degranulation happens instantly and thus quickly after the activation phase. It is important to use triggered PRP soon after preparation (Dohan et al., 2009).

In veterinary medicine, the therapeutic use of PRP is inferior to human medicine. It has been used to promote equine tendon repair (Rindermann et al., 2010), to treat intestinal wound healing in pigs (Fresno et al., 2010), or to cure large cutaneous lesions in dogs (Kim et al., 2009). In the cattle industry, its beneficial effects have been tested against bovine mastitis (Lange-Consiglio et al., 2014), in repeat breeder cows (Lange Consiglio et al., 2015), for the improvement of embryo recovery in Holstein cows treated by intra-ovarian platelet-rich plasma before superovulation (Cremonesi et al., 2020a, Borș et al. 2022a) and in bovine ovarian hypofunction (Cremonesi et al., 2020b).

3. The PRP use to improve embryo production and embryo implantation in mammals

In the field of assisted reproductive technologies, promising results were confirmed by Sills et al., (2018), who performed intraovarian administration of autologous activated PRP in the human ovarian senescence and provided the first clinical data on IVF cycle characteristics following this intervention.

Ovarian senescence is a complex physiological process involving the interaction and gradual accumulation of several factors, of which the most important aspects are the reduction in the number and quality of ovarian follicles (de Vet et al., 2002; Turola et al., 2015). In this context it seems plausible to consider using autologous PRP to improve the ovarian microenvironment – and even interacting with putative ovarian germline stem cells (GSCs) – warrants serious consideration (Sills et al., 2018).

The study of Sills et al., (2018) proposes a new hypothesis in which the PRP-related growth signals established communication with uncommitted ovarian stem cells and provided the appropriate environment needed to induce differentiation of the new oocytes. In this study the ± 25 months with 5 ml of autologous PRP for each ovary under ultrasound guidance via the transvaginal route. A total of 5.3 ± 1.3 mature oocytes were reported at metaphase stage 2 following an in vitro fertilization program, and all patients developed at least one blastocyst appropriate for cryopreservation.

Thibodeaux et al., (1993) used in their study an in vitro culture system of oviductal cells and platelets to establish if any possible effect occurs in vivo during early embryonic development. An interesting aspect of this study is that oviductal cells seem to produce platelet-derived growth factor that stimulates embryonic women with proven infertility were treated for 60

development. Furthermore, the other endometrial cells and even the embryo may produce platelet-activating factors which stimulate the PRP production (Thibodeaux et al., 1993).

In reproductive medicine of dairy cows, Cremonesi et al., (2020a) used for the first time the autologous PRP inside the bovine ovary to improve embryo recovery from eight super-ovulated donor cows. In according with the results of Pantos et al., (2016) and Sills et al., (2018), the studies of Cremonesi et al., (2020a), Vo et al 2020 and Borș et al. (2022a, b) indicates that PRP has a very important impact on follicular development (Figure 2).

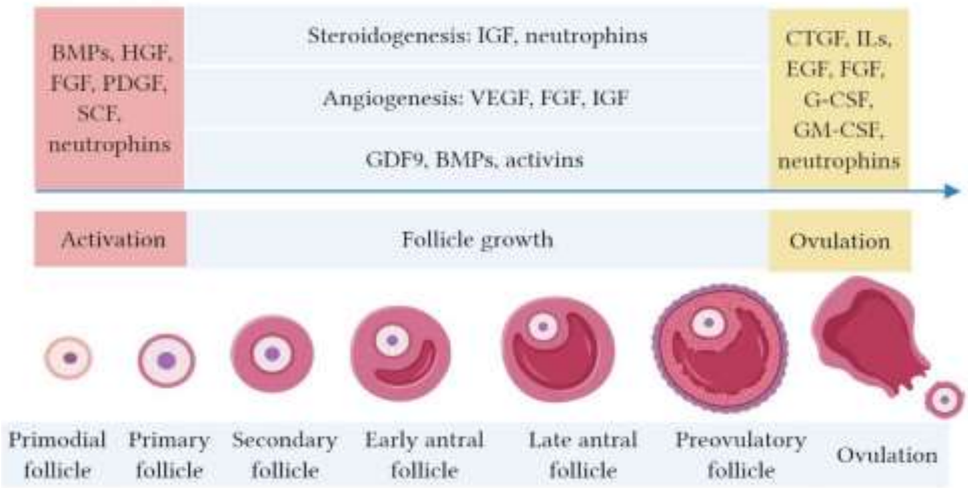

Figure 2. The roles of growth factors involved in PRP in folliculogenesis (Borș et al., 2022b; Vo et al., 2020).

Only few studies described the effects of PRP on morula and blastocysts in vitro production (Thibodeaux et al., 1993; Lange-Consiglio et al., 2015; Ramos-Deus et al., 2020). In the study of Lange-Consiglio et al., (2015) two different percentages of PRP were used (respectively 5% and 10%), as a partial or complete replacement of fetal calf serum (FCS). The blastocyst rate and the total cells number of the blastocysts were statistically increased in the culture medium with 5% PRP when compared to both the control and medium with 10% PRP (Lange-Consiglio et al., 2015; Ramos-Deus et al., 2020). These interesting results were assigned to the growth factors released by the PRP, including FGF, transforming growth factor- β (TGF-β), PDGF and EGF (Siess, 1989; Whal et al., 1989) that can stimulate bovine embryo development (Larson et al., 1992a, Larson et al., 1992b). Munson et al., (1992) demonstrated that TGF-β and PDGF can promote proliferation of both bovine trophoblastic cells and endometrial epithelial cells during in vitro culture. However, the low blastocyst rate obtained in the medium with 10% PRP, compared with control, might be influenced by the excess of growth factors that may have had an inhibitory effect on the embryo development (Lange-Consiglio et al., 2015). The addition of PRP into the oocyte maturation medium is not beneficial (Ramos-Deus et al., 2020).

In 2015, Chang et al., tested for the first time the effect of using PRP to improve endometrial thickness in patients undergoing IVF treatment. From five patients with poor endometrial response after standard IVF protocol, four had a successful pregnancy after PRP infusion, 1–2 times in each cycle. In the next year, Farimani et al., (2016) evaluated the effect of PRP intrauterine infusion on pregnancy rates after frozen–thawed embryo transfer. In this trial, from nine women with a history of recurrent implantation failure, six were diagnosed pregnant (pregnancy rate was 66.6%). Furthermore, in 2017 Farimani et al., reported a successful pregnancy after administration of autologous PRP to improve endometrial receptivity, in a 45 years old woman with primary infertility and two failed IVF cycles. The next two studies confirmed the effect of using autologous PRP in order to improve the endometrial quality and implantation rates in patients with refractory endometrium (with poor response to conventional therapy) (Zadehmodarres et al., 2017; Molina et al., 2018).

These promising results ask for further studies in order to evaluate the PRP effect on dairy cow follicular recruitment, oocyte competence, in vitro fertilization and embryo development. Also, it is necessary to evaluate the presumptive effect of IVF media supplementation with autologous activated PRP on blastocyst formation. The results could offer a new strategy for using the PRP in future embryo transfer programs in human and veterinary medicine, with the aim of improving the embryo production and implantation rates (Figure 3).

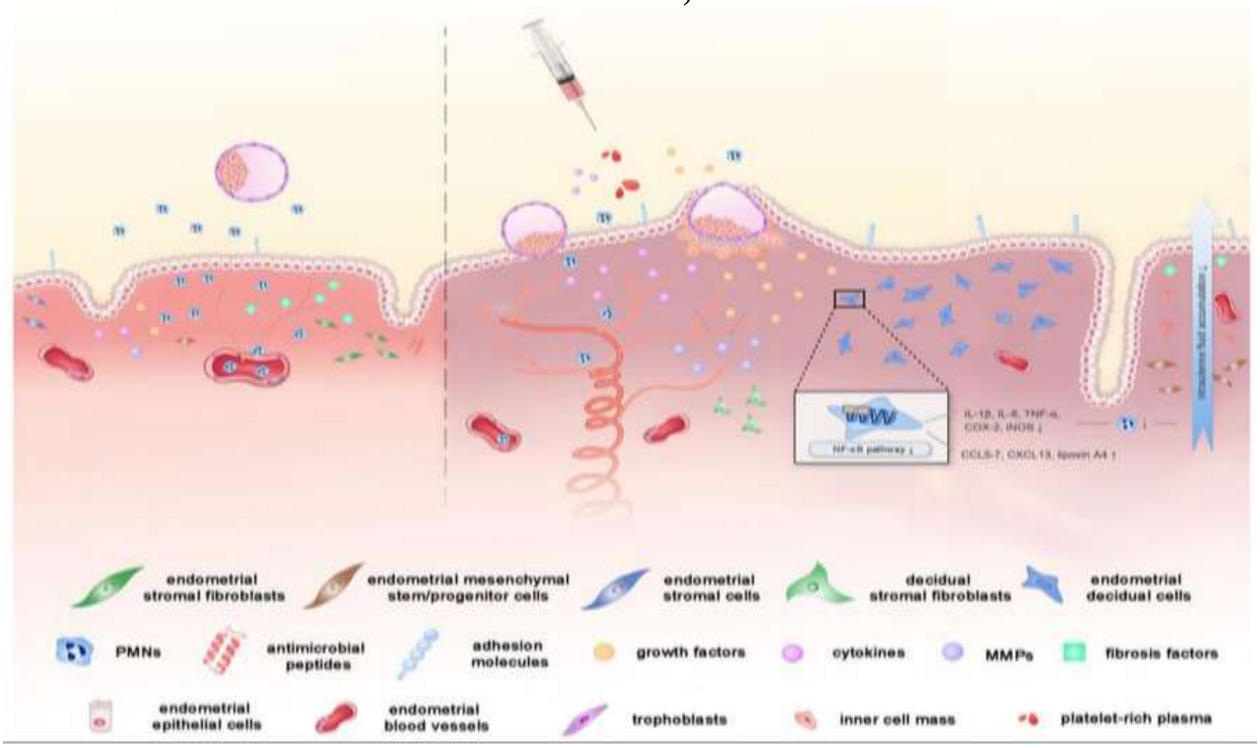

Figure 3. Potential mechanisms of PRP on endometrial receptivity in assisted reproductive technology. PRP might improve the endometrial receptivity through the improvement of cell proliferation, vascularisation, anti-inflammatory properties, and the reduction in the degree of fibrosis, with the help of the concentrated peptides, GFs and cytokines in PRP (Borș et al., 2022b)

CONCLUSIONS

Veterinarians and farmers should show more interest in autologous platelet-rich plasma (PRP) once its effectiveness in tissue regeneration processes is shown, either in conjunction with other therapies or on its own. PRP is an easily available and reasonably priced therapeutic substance derived from blood components.

The lack of standardization concerning preparation techniques hinders consensus on PRP bio-formulations and their application.

While PRP's therapeutic benefits are widely recognized, there are still a number of unanswered concerns regarding its molecular process.

REFERENCES

Anitua, E., Andia, I., Ardanza, B., Nurden, P., Nurden, A.T., 2004. *Autologous platelets as a source of proteins for healing and tissue regeneration*. Thromb. Haemost. 91, 4–15. https://doi:10.1160/TH03-07-0440.

Alves, R., Grimalt, R., 2016. *A randomized placebocontrolled, double-blind, half-head study to assess the efficacy of platelet-rich plasma on the treatment of androgenetic alopecia.* Dermatol. Surg. 42, 491–497. https://doi:10.1097/DSS.0000000000000665.

Borş S.-I., Dascălu D.-L., Borş A., Fahmy H.M., Kandil O.M., Abdoon A.S.S. 2022a. *Intraovarian Injection of Reconstituted Lyophilized Growth-Promoting Factor Extracted from Horse Blood*

Platelets (L-GFequina) Increases Oocytes Recovery and In Vitro Embryo Production in Holstein Cows. Animals.; 12(19):2618. https://doi.org/10.3390/ani12192618.

medicine: Prospective and applications, Reproduction in Domestic Animals, 57(11): 1287-1294, https://doi.org/10.1111/rda.14213.

Bos-Mikich, A., de Oliveira, R., Frantz, N., 2018. *Platelet-rich plasma therapy and reproductive*

Int. J. Clin. Exp. Med. 8, 1286–1290. https://PMCID: PMC4358582.

Collins, T., Alexander, D., Barkatali, B., 2021. *Platelet-rich plasma: a narrative review.* EFORT Open Rev. 6, 225–235. https://doi:10.1302/2058-5241.6.200017.

Cremonesi, F., Bonfanti, S., Idda, A., Lange-Consiglio, A., 2020a. *Improvement of embryo recovery in Holstein cows treated by intra-ovarian platelet rich plasma before superovulation***.** Vet. Sci. 7, 16. https://doi:10.3390/vetsci7010016

Cremonesi, F., Bonfanti, S., Idda, A., Lange-Consiglio, A., 2020b. *Platelet rich plasma for regenerative medicine treatment of bovine ovarian hypofunction*. Front. Vet. Sci. 7, 517. https://doi:10.3389/fvets.2020.00517.

Croisé, B., Pare, A., Joly, A., Louisy, A., Laure, B., Goga, D., 2020. *Optimized centrifugation preparation of the platelet rich plasma:* Literature review. J. Stomatol. Oral Maxillofac. Surg. 121, 150–154. https://doi:10.1016/j.jormas.2019.07.001.

de Vet, A., Laven, J.S.E., de Jong, F.H., Themmen, A.P.N., Fauser, B.C.J.M., 2002. *Antimullerian hormone serum levels: a putative marker for ovarian aging*. Fertil. Steril. 77, 357–362. https://doi:10.1016/s0015-0282(01)02993-4.

Dhurat, R., Sukesh, M., 2014. *Principles and methods of preparation of Platelet-rich Plasma: a review and author's perspective*. J. Cutan. Aesthet. Surg. 7, 189–197. https://doi: 10.4103/0974-2077.150734.

Dohan Ehrenfest, D.M., Rasmusson, L., Albrektsson, T., 2009. *Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF)*. Trends. Biotechnol. 27, 158–167. https://doi:10.1016/j.tibtech.2008.11.009.

Everts, P., Onishi, K., Jayaram, P., Lana, J.F., Mautner, K., 2020. *Platelet-rich plasma: new performance understandings and therapeutic considerations in 2020*. Int. J. Mol. Sci. 21, 7794. https://doi:10.3390/ijms21207794.

Farimani, M., Bahmanzadeh, M., Poorolajal, J., 2016. *A new approach using autologous platelet-rich plasma to treat infertility and to improve population replacement rate.* J. Res. Health. Sci. 16, 172–173. https://PMCID: PMC7191021.

Farimani, M., Poorolajal, J., Rabiee, S., Bahmanzadeh, M., 2017. *Successful pregnancy and live birth after intrauterine administration of autologous platelet-rich plasma in a woman with recurrent implantation failure: A case report.* Int. J. Reprod. Biomed. 15, 803–806. https://PMCID:PMC5816241.

Foster, T.E., Puskas, B.L., Mandelbaum, B.R., Gerhardt, M.B., Rodeo, S.A., 2009. *Platelet-Rich Plasma: From Basic Science to Clinical*

Borş S.-I., Ibănescu I., Borş A., Abdoon A.S.S., 2022b - *Platelet-rich plasma in animal reproductive*

medicine. J. Assist. Reprod. Genet. 35, 753–756. https://doi:10.1007/s10815-018-1159-8.

Chang, Y., Li, J., Chen, Y., Wei, L., Yang, X., Shi, Y., Liang, X., 2015. *Autologous platelet-rich plasma promotes endometrial growth and improves pregnancy outcome during in vitro fertilization. Applications*. Am. J. Sports Med. 37, 2259–2272. https://doi:10.1177/0363546509349921.

Fresno, L., Fondevila, D., Bambo, O., Chacaltana, A., García, F., Andaluz, A., 2010. *Effects of platelet-rich plasma on intestinal wound healing in pigs*. Vet. J. 185, 322–327. https://doi:10.1016/j.tvjl.2009.06.009.

Gonçalves, N.J.N., Frantz, N., de Oliveira, R.M., 2019. Platelet-rich plasma (PRP) therapy: *An approach in reproductive medicine based on successful animal models.* Anim. Reprod. 16, 93–98. https://doi:10.21451/1984-3143-AR2018-093.

Kingsley, C.S., 1954. *Blood coagulation; evidence of an antagonist to factor VI in platelet-rich human plasma*. Nature. 173, 723–724. https://doi:10.1038/173723a0.

Kim, J.H., Park, C., Park, H.M., 2009. *Curative effect of autologous platelet-rich plasma on a large cutaneous lesion in a dog.* Vet. Dermatol. 20, 123–126. https://doi:10.1111/j.1365-3164.2008.00711.x.

Larson, R.C., Ignotz, G.G., Currie, W.B., 1992a. *Transforming growth factor and basic fibroblast growth factor synergistically promote early bovine embryo development during the fourth cell cycle*. Mol. Reprod. Dev. 33, 432–435. https://doi:10.1002/mrd.1080330409.

Larson, R.C., Ignotz, G.G., Currie, W.B., 1992b. *Platelet derived growth factor (PDGF) stimulates development of bovine embryos during the fourth cell cycle.* Development. 115, 821–826. https://PMID:1425356.

Lange-Consiglio, A., Spelta, C., Garlappi, R., Luini, M., Cremonesi, F., 2014. *Intramammary administration of platelet concentrate as an unconventional therapy in bovine mastitis: First clinical application*. J. Dairy Sci. 97, 6223–6230. http://dx.doi.org/ 10.3168/jds.2014-7999.

Lange-Consiglio, A., Cazzaniga, N., Garlappi, R., Spelta, C., Pollera, C., Perrini, C., Cremonesi, F., 2015. *Platelet concentrate in bovine reproduction: effects on in vitro embryo production and after intrauterine administration in repeat breeder cows.* Reprod. Biol. Endocrinol. 13, 65. https://doi:10.1186/s12958-015-0064-6.

Levin, R.H., Freireich, E.J., Chappell, W., 1964. *Effect of storage up to 48 hours on response to transfusions of platelet rich plasma. Transfusion*. 4, 251–256. https://doi.org/10.1111/j.1537-2995.1964.tb02867.x.

Mazzocca, A.D., McCarthy, M.B., Chowaniec, D.M., Cote, M.P., Romeo, A.A., Bradley, J.P., Arciero, R.A., Beitzel, K., 2012. *Platelet-rich plasma differs according to preparation method and human variability*. J. Bone. Joint. Surg. Am. 94, 308–316. https://doi: 10.2106/JBJS.K.00430.

Molina, A., Sánchez, J., Sánchez, W., Vielma, W., 2018. *Platelet-rich plasma as an adjuvant in the* *endometrial preparation of patients with refractory endometrium*. JBRA. Assist. Reprod. 22, 42–48. https:// doi: 10.5935/1518-0557.20180009.

Munson, L., Wilkinson, J.E., Bechtel, M., 1992. *Transforming growth factor-β and platelet-derived growth factor (PDGF) are synergistic mitogens for bovine trophoblastic and endometrial epithelial cells.* Biol. Reprod. 46 Suppl. 1: S67.

Pantos, K., Nitsos, N., Kokkali, G., Vaxevanoglou, T., Markomichali, C., Pantou, A., Grammatis, M., Lazaros, L., Sfakianoudis, K., 2016. *Ovarian rejuvenation and folliculogenesis reactivation in peri-menopausal women after autologous platelet-rich plasma treatment*. Abstracts, ESHRE 32nd Annual Meeting; 3–6 Jul 2016; Helsinki, Finland. Hum. Reprod. 1, i 301**.**

Ramos-Deus, P., Santos Nascimento, P., Vieira, J.I.T., Chaves, M.S., Albuquerque, K.A., Ferreira-Silva, J.C., Grázia, J.G.V., Santos Filho, A.S., Batista, A.M., Teixeira, V.W., Oliveira, M.A.L., 2020. *Application of platelet-rich plasma in the in vitro production of bovine embryos.* Trop. Anim. Health. Prod. 52, 2931–2936. https:// doi: 10.1007/s11250-020-02307-5.

Rindermann, G., Cislakova, M., Arndt, G., Carstanjen, B., 2010. *Autologous conditioned plasma as therapy of tendon and ligament lesions in seven horses*. J. Vet. Sci. 11, 173–175. https://doi:10.4142/jvs.2010.11.2.173.

Sills, E.S., Rickers, N.S., Li, X., Palermo, G., 2018. *First data on in vitro fertilization and blastocyst formation after intraovarian injection of calcium gluconate-activated autologous platelet rich plasma*. Gynecol. Endocrinol. 34, 756–760. https://doi: 10.1080/09513590.2018.1445219.

Thibodeaux, J.K., Del Vecchio, R.P., Broussard, J.R., Dickey, J.F., Hansel, W., 1993. *Stimulation of development of in vitro-matured and in vitro-fertilized bovine embryos by platelets.* J. Anim. Sci. 71, 1910–1916. https://doi:10.2527/1993.7171910x.

Turola, E., Petta, S., Vanni, E., Milosa, F., Valenti, L., Critelli, R., Miele, L., Maccio, L., Calvaruso, V., Fracanzani, A.L., Bianchini, M., Raos, N., Bugianesi, E., Mercorella, S., Di Giovanni, M., Craxi, A., Fargion, S., Grieco, A., Camma, C., Cotelli, F., Villa, E., 2015. *Ovarian senescence increases liver fibrosis in humans and zebrafish with steatosis.* Dis. Model. Mech. 8, 1037–1046. https://doi:10.1242/dmm.019950.

Whal, S.M., McCartney-Francis, N., Mergenhager, S.E., 1989. *Inflammatory and immunomodulatory roles of TGF-β*. Immunol. Today. 10, 258–261. https://doi: 10.1016/0167-5699(89)90136-9.

Wroblewski, A.P., Melia, H.J., Wright, V.J., 2010. *Application of platelet-rich plasma to enhance tissue repair*. Oper. Tech. Orthop. 20, 98–105.

Wu, P.I., Diaz, R., Borg-Stein, J., 2016. *Platelet-Rich Plasma. Phys*. Med. Rehabil. Clin. N. Am. 27, 825–853. https://doi:10.1016/j.pmr.2016.06.002.

Zadehmodarres, S., Salehpour, S., Saharkhiz, N., Nazari, L., 2017. *Treatment of thin endometrium with autologous platelet-rich plasma: a pilot study*. JBRA. Assist. Reprod. 21, 54–56. https://doi:10.5935/1518-0557.20170013.