ISSN: 1220-3173; E-ISSN: 2457-7618

OVARIAN HYPOFUNCTION IN THE CARPATHIAN INDIGENOUS BUFFALO, AS INFERTILITY FACTOR

HIPOFUNCȚIA OVARIANĂ LA BIVOLUL INDIGEN CARPATIN, FACTOR DE INFERTILITATE

Ş. CIORNEI[1)], D. DRUGOCIU[1)], P. ROŞCA[1), *)], Liliana GHINEȚ (CIORNEI)[1)]

ABSTRACT | REZUMAT

From a veterinary sanitary point of view, aim of this paper is to increase the productivity of buffaloes by diagnosing general diseases, especially for the genitalia. Uterine and ovarian pathology in buffaloes is inextricably linked to feed quality, microclimate conditions, veterinary medical act quality, but also to the drugs quality that are used.The importance of the female reproductive system anatomy, and knowledge of buffalo female anatomical features are important, among others, for the diagnosis of ovarian and uterine malfunctions, for the use of reproductive techniques such as pregnancy diagnosis, artificial insemination, superovulation, egg collection, and embryo transfer. The female genital tract includes the ovaries, which produce gametes and hormones; oviducts, which are extensions of the uterus and provide transport functions for oocytes, sperm and secretions; the uterus, which is the site of embryonic and foetal development; the vagina, which is the channel for the sexual intercourse and the foetus delivery, and the external genitalia. Regarding the evolution and outcome of follicular waves, ovarian inactivity evolves with ovarian hypofunction type III, II and I. The study was based on the diagnosis of ovarian hypofunction in all gynaecological diseases (uterine diseases and other ovarian diseases) in two buffalo farms in Romania, with a population of over 300 animals each. The biological material used was the indigenous Carpathian buffalo, from the Mediterranean line. During the 2 years, the ovarian hypofunction (OHf) represented 19.25% of the total gynaecological diseases encountered in buffaloes. After the treatment described and applied to buffaloes with type III ovarian hypofunction (OHf) all presented oestrus within 3 days of administration of eCG. Treatment of type III ovarian hypofunction was effective in 62.2% of treated cases, which showed oestrus immediately after treatment, and in 37.8% of cases oestrus appeared after 18 days. Type I ovarian hypofunction was considered ovarian atrophy and was not treated because the ovarian response is weak or non-existent and treatment is expensive.

Keywords: buffalo, infertility, ovarian hypofunction, ultrasonography

Din punct de vedere sanitar veterinar, se urmărește creșterea productivității bubalinelor prin diagnosticarea afecțiunilor generale, dar mai ales ale aparatul genital. Patologia uterină și ovariană la bivoliță este indisolubil legată de calitatea furajării, condițiile de microclimat, calitatea actului medical veterinar, dar și de calitatea produsului medicamentos sau hormonal utilizat. Importanța anatomiei sistemului reproductiv femel, cunoașterea particularităților anatomice ale bivolițelor este importantă, printre altele, pentru diagnosticul malfuncțiilor ovariene și uterine, pentru utilizarea tehnicilor de reproducere, cum ar fi diagnosticarea gestației, inseminarea artificială, superovulația, prelevarea ovocitelor și transferul de embrioni. Aparatul genital femel include ovarele, care produc gameți și hormoni; oviductele, care sunt extensii ale uterului și oferă funcții de transport pentru ovocite, spermă și secreții; uterul, care este locul dezvoltării embrionare și fetale; vaginul, care este canalul pentru copulare și asigură trecerea fătului și organele genitale externe. Hipofuncția ovariană este considerată o inactivitate ovariană și se manifestă în trei tipuri de la I la IV. Referitor la evoluția și deznodământul valurilor foliculare, inactivitatea ovariană evoluează cu hipofuncție ovariană de tipul III, II și I. Studiul s-a bazat pe diagnosticul hipofuncțiilor ovariene din totalul afecțiunilor ginecologice (afecțiuni uterine și alte afecțiuni ovariene) în două ferme de bubaline din România, cu o populație de peste 300 de animale fiecare. Materialul biologic folosit a fost bivolul indigen carpatin, din linia Mediteraneeană. Pe parcursul celor doi ani luați în studiu hipofuncțiile ovariene au reprezentat 19,25% din totalul afecțiunilor ginecologice întâlnite la bivolițe. După tratamentul descris și aplicat bivolițelor cu hipofuncție ovariană de gradul III, toate au prezentat estru în 3 zile de la administrarea PMSG-ului. Tratamentul hipofuncției ovariene de gradul II a fost eficient la 62,2% din cazurile tratate, care au manifestat estru imediat după tratament. La 37,8% din cazuri estrul a apărut după 18 de zile. Hipofuncția de gradul III a fost considerată atrofie ovariană și nu a fost supusă tratamentului deoarece răspunsul ovarian este slab sau inexistent, iar tratamentul costisitor.

Cuvinte cheie: bivoliță, hipotrofie ovariană, infertilitate, ecografie

1) Iași University of Life Sciences "Ion Ionescu de la Brad" Faculty of Veterinary Medicine, Iași, Romania

*) *Corresponding author: petru1065@yahoo.com*

A healthy genital tract is a prerequisite for a buffalo to maintain its ability to reproduce and as a result to produce milk. Ovarian dysfunction is common during the postpartum period. Ovarian abnormalities impair the development of ovarian follicles and corpus luteum and subsequently oestrous cycle and pregnancy in buffaloes. In Romania, the growth of buffaloes must be interpreted by their economic value, related to the area of formation and spread, to the possibilities of expansion and capitalization of territories in which cattle cannot adapt and do not provide appropriate production. Buffaloes account for about 10% of the world's cattle population. In countries with large numbers of buffaloes, the economic contribution of this species is significant, given that other species of cattle are difficult to adapt, or achieve much lower levels of production. Romania has around 62,000 buffaloes and a milk production around 1,700 litres per lactation, with 132 kg of pure fat and a fat percentage of 7.49% (2, 7, 27, 30). Buffalo milk has a complex composition and is characterized by a high share of dry matter and fat, superior to milk produced by all other species of farm animals. It is also high in vitamins and minerals (27).

Although it is a polyoestrous female, the sexual cycle has a strong seasonal character, especially as a manifestation: in lowland and hill areas, the sexual cycle is more intense in April and September, while in mountainous areas, the intensity is higher in June and July (10,25). Seasonality is marked by ambient temperature, photoperiod and specificity of nutrition. The effect of the photoperiod is similar to that of cows. During the summer, the ambient temperature and the photoperiod have a maximum level (the prolactin titre increases), as well as the plasma progesterone are low (12). High temperature also contributes to seasonal depression in males, the heat stress results in reduced seminal quality, lower sperm viability and weak expression of libido (21,29). The multitude elements that can determine the phenomenon of anaphrodisia and the pathologies that precede it, include hereditary, bacterial, fungal, metabolic and last but not least, hormonal factors (4,9). Although performing a correct clinical examination and a manual transrectal examination on the buffalo genital tract, these will not be able to be completely replaced in the future, and the ultrasound exam comes as a modern method of investigation, complementary, which will contribute to the specification and/or completion of the diagnosis. As an imaging method, it is the only one that can provide images of the pathological and physiological structures of the buffalo ovary, on the pregnant uterus, even in the first 30-40 days of pregnancy.

The application of therapy, but especially the fight against reproductive disorders by classical methods, involve very high costs, therefore the interest to be able to reduce the service period at the buffalo by implementing ultrasound as a modern and current diagnostic method and by this to increase productivity is even higher (1,7). The normal or pathological evolution of the reproduction and reproductive function is influenced by a series of exogenous and endogenous factors such as feeding, hygiene, and stress. These factors cause a variety of disorders that prolong the interval of conception, or often, conception becomes impossible, which leads to great economic losses.

Ovarian dysfunction is common in the puerperal period and is an important clinical entity because it affects an animal's ability to reproduce and produce milk. Ovaries and ovarian structures in buffalo are significantly smaller compared to cows, especially in seasonal ovarian hypofunction, and ovarian pathologies limit the reproductive value of this species (22).

There are very common ovarian diseases in buffaloes, they are diagnosed throughout the year, but especially in the cold season, in poor feeding conditions, or during the spring-summer inactivity (11).

Ovarian trophic disorders are common in ruminants after puberty, both in primiparous and multiparous. The ovary is an organ with high sensitivity, having a double function, endocrine and germinal. Trophic disorders are the consequence of various food deficiencies, trauma, infections, endocrine disorders, hypophyseal gonadotropic secretory insufficiency (FSH and LH), or hormonal treatments performed incorrectly (10,18,24). Sterility in trophic disorders is due to changes in metabolic processes. The modification of these ovarian cellular activities results in the reduction of the volume of active cells, the modification of the already formed structures or of the hormonal receptors (5,6,13).

The diagnosis depends on the severity of ovarian hypofunction and utero-ovarian changes.

Fate of the first dominant follicle for the restoration of cyclicity and the recovery of reproduction functionality has been described by several authors like Hopper (2015) in Bovine reproduction (8). The fate of the first dominant follicle in the postpartum cow is determined by the pulsatile LH support it receives and by its own steroidogenic capacity to produce oestradiol. Depending on these factors, the first dominant follicle may: (a) ovulate and form a corpus luteum (CL); (b) become anovulatory, possibly developing into a cystic follicle; (c) regress, making way for a new dominant follicle (3,8,26).

In the buffalo-cow species, Purhoit (2015) present as possible causes of anaesthesia (anaphrodisia) a group of diseases called -ovarian functional disorders. In ovarian functional disorders are described: sub-estrum and silent heat as negative results of ovarian hypofunction (ovarian inactivity).

Sub-estrum produced by ovarian inactivity, as silent heat is a clinical condition in which in spite of the

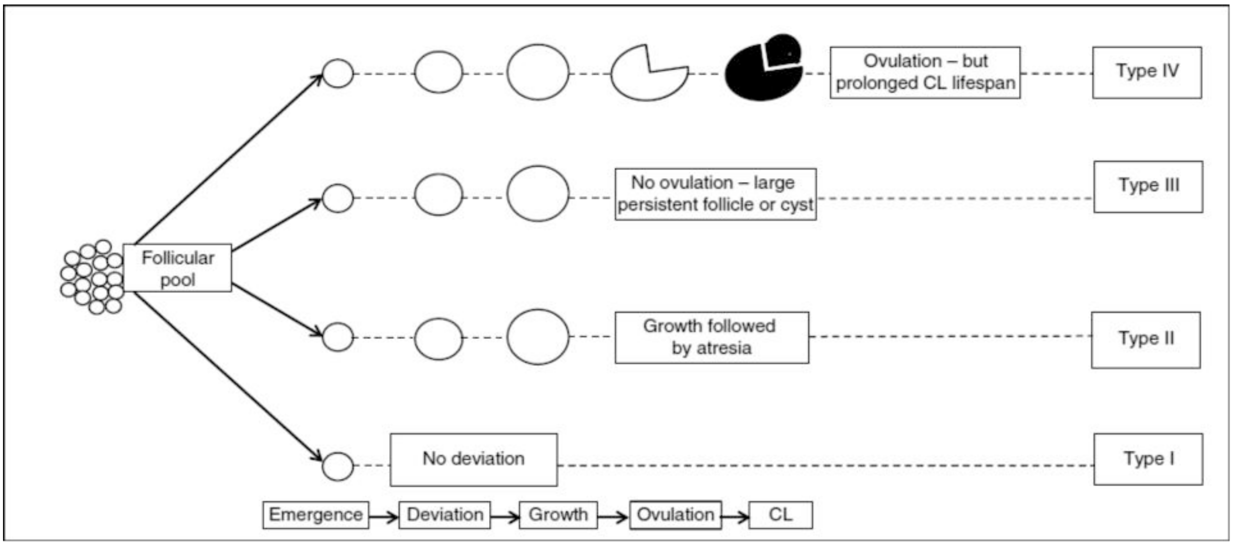

Fig. 1. Schematic representation of types of anoestrous conditions based on ovarian follicular dynamics (8,31). Ovarian hypofunction types

normal ovarian cycle, i.e., periodic follicular growth, ovulation and luteinization, overt oestrus does not occur normally. Because behavioural signs of oestrus are too weak, the animal in heat is missed and not inseminated or brought to the bull and the reproductive cycle is missed. Categorizing anoestrus based on ovarian follicular dynamics (ovarian hypofunction /ovarian inactivity), classifications have been published. The classification of anovulatory conditions in cattle based on three functionally critical follicular diameters relating to emergence (~4 mm), deviation (~9 mm), and ovulation (10–20 mm) has been described (31). With the above classification as the basis, four types of anoestrus, types I to IV, were described by Peter *et al.* (2009) (19) (Fig. 1).

In **type I anoestrus**, there is growth of follicles up to emergence but no deviation occurs resulting in lack of selection of a dominant follicle. This type of anoestrus is presumed due to extreme malnutrition, which could exert a negative effect on FSH production suppressing follicular growth, although other factors could also be involved. Ovaries under this type may be described as either "inactive" or "smooth" as a reference to the lack of palpable ovarian structures during reproductive examinations per rectum.

In **type II anoestrus** both follicular deviation and growth occur, followed by regression, in some cases, after a follicle attained dominance. Regression of a dominant follicle is usually followed by the emergence of a new follicular wave, 2–3 days later. In this type of anoestrus, there may be sequential emergence of follicular waves prior to the eventual occurrence of the first ovulation.

In **type III anoestrus**, deviation, growth, and establishment of a dominant follicle takes place, but the dominant follicle fails to ovulate, becoming a persistent structure which may either linger as an anovulatory follicle or continue to grow and develop into a cystic follicle.

In **type IV anoestrus,** a dominant follicle ovulates and forms a CL, but the luteal phase is prolonged due to the absence of timely luteolysis followed by CL regression. Aberrant follicular growth patterns resulting in the absence of an estrogenic dominant follicle at the ideal time to trigger luteolysis could be a reason for this condition of persistent CL (8,31).

MATERIALS AND METHODS

The study was based on the diagnosis of ovarian hypofunction in all severity of ovarian diseases (uterine diseases and other ovarian diseases) in two buffalo farms in Romania. Farm I, located in the N-E area, and Farm II in the centre of the country. The biological material used was the indigenous Carpathian buffalo, from the Mediterranean line. The absence of oestrus in buffaloes is most often caused by ovarian hypofunctions. The period in which the ovarian hypofunctions were diagnosed in the buffaloes from the two farms was the cold season, respectively the months of November 2016 - April 2017 for Farm I and the months of November 2017- February 2018 for Farm II. In our study, we refer only to anoestrus (anaphrodisia) given by the lack of complete development of follicular waves, as an ovarian hypofunction in Romanian buffalo-cow. Anoestrus type III (Type III- ovarian hypofunction), II (Type II - ovarian hypofunction) and I (Type I- ovarian hypofunction).

A working protocol was designed to establish the diagnosis of ovarian hypotrophy as follows:

- Consultation of zootechnical records;
- Carrying out the gynaecological investigation (anamnesis);

- Transrectal gynaecological examination of the ovaries;
- Their re-examination at a certain time interval;
- Ultrasound examination of the ovaries.

Following the consultations of the zootechnical records, the age of the cows was established, the parity, milk production, other gynaecological diseases, what treatments were performed, the date of oestrus after therapy and fertile breeding / IA (Fig. 2).

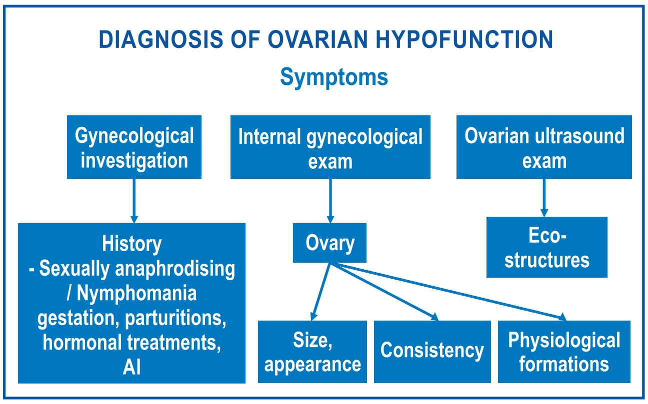

Fig. 2. Scheme of the protocol used for the diagnosis of ovarian inactivity in buffaloes

The anamnesis, as part of the gynaecological investigation, established data on the manifestation of sexual cyclicity of the buffaloes studied.

Thus, the date of the last oestrus was established, date of natural breeding was performed, whether the sexual cycle is regular or not, as were the oestrous signs as clinical expression.

By transrectal gynaecological examination was examined the ovaries which were appreciate several benchmarks such as: size (length and width in cm), appearance (flattened, globular), consistency (elastic, firm) and finding the formations developed on the ovaries (follicles and bodies luteal), according to the methodology described by Ciornei (2012) (9).

The diagnostic approaches for ovarian dysfunction and ovarian pathologies include transrectal palpation and transrectal ultrasonography.

Due to the small size of the ovaries and therefore the ovarian structures, transrectal ultrasound examination have become a suitable tool to improve the efficiency of the diagnosis of ovarian conditions affecting female buffaloes.

Ultrasonography has gained popularity in buffalo reproduction during recent years as it has many research and clinical applications such as evaluation of ovarian function (14,28). The Honda HS - 1600V® portable ultrasound scanner (Japan), was used for our studies with 7.5 MHz transrectal probe.

The treatment of ovarian hypotrophy was specific to each degree of the condition.

RESULTS AND DISCUSSION

Type III ovarian hypofunction was manifested by the lack of oestrus or oestrus without obvious manifestations, associated with discrete changes in the genital tract. At the transrectal examination, a slight decrease of the ovaries in volume was observed, the consistency is close to normal, and the identification of some ovarian functional formations with incomplete evolution (follicles) (fig. 3).

Type II ovarian hypofunction was a more severe ovarian disease. The internal examination discovered slightly reduced ovaries in volume, increased consistency and the absence of functional formations (follicles or corpus luteum), the disease is also called "smooth ovaries".

Type I ovarian hypofunction, also called ovarian atrophy, was characterized by anaphrodisia and ovarian changes. Internal palpation shows a decrease in volume of the ovaries (between 0.5-1 cm) with increased consistency (hard), lack of functional formations on it (smooth ovaries).

Endometrial hypoplasia occurs in the uterus.

On transrectal palpation of the ovaries with type III hypofunction, small, dense ovaries were found, lacking surface formations, follicles, corpus luteum, and the genital tract have hypotrophy.

Ultrasound showed a hyperechoic formation, well circumscribed, ovoid, with dimensions between 0.8 and 1.8 cm, sometimes with the presence of a small, anechoic spot, which represents an atretic follicle (fig. 3 and 4). The uterus was reduced in volume, flaccid, or in a barely perceptible tone, and the cervix was small, dense, slightly hyperechoic.

To determine the diagnosis by transrectal route, several examinations were required, which allowed the differentiation from congenital ovarian hypoplasia. If from one examination to another, the ovaries do not show evolutionary changes (volume, consistency, and follicles / CL). It means that the female buffalo is in ovarian hypofunction type I or ovarian atrophy.

And if the female did not have any parturition and no oestrus was observed, the diagnosis of congenital hypoplasia is established.

The results obtained from our investigations on ovarian hypofunction as a factor of infertility in CIB (Carpathian Indigenous Buffalo) are presented in Table 1.

By the total gynaecological diseases encountered in buffaloes during the two years studied, they highlighted the fact that ovarian hypofunction accounted for a share of 19.25% (31/161). By farm categories, 19.17% for Farm I (14/73) and 19.32% for Farm II (17/88). At Farm I, in the first year 25% (9/36) were diagnosed, and in the second year 13.5% (5/37) out of a total of 37 gynaecological diseases diagnosed.

Table 1

Percentage of ovarian hypofunction in the Carpathian Indigenous Buffalo in the two farms in Romania, for two years of supervision

		Farm I		Farm II		Total	
No animals (buffalo-cow)		(14/73)		(17/88)		(31/161)	
Percentage		19.17%		19.32%		19.25%	
by year	2017	25%	(9/36)	16.67%	(6/36)	20,83%	15/72
	2018	13.5%	(5/37)	21.15%	(11/52)	17,97%	16/89

At Farm II, in the first year was 16.67% (6/36), and in the second year there was an increase in the number of ovarian diseases represented by hypofunctions to 21.15% (11/52).

Fig. 3. Ultrasound image of buffalo with type I ovarian hypofunction. The ovary with a size of 1.92/1.17 cm and the presence of two hypoechoic evolutionary follicles is observed

Fig. 4. Ultrasound image of buffalo with type II ovarian hypofunction. You can see the ovary with a size of 2.02/1.1 cm without follicles

The treatment of type III hypofunction was aimed at improving the ration, neuro-reflex therapy and specific hormonal treatment. The ration administered during treatment contained easily digestible fodder administered at discretion with vitamin and mineral supplements. Considering that type III ovarian hypofunction is characterized by a deficiency of pituitary gonadotropic hormones, especially FSH, by supplementing and improving the feed ration, the adenohypophysis will be able to secrete normal titre of gonadotropic hormones, given that they are glycoprotein hormones.

Neuro-reflex therapy consisted of repeated 2-3 days of uterine and ovarian massage, which leads to local congestion, aiming at better blood circulation and improved local metabolism. These physiological effects lead to better functioning of the genital tract and ovarian function.

Specific treatment consisted of serum gonadotropins 10 days after diagnosis. Serum gonadotropin has the main effect of FSH, and the hormonal preparation used on the farm was eCG (Folligon, MDS, Netherlands). The administration was performed at a dose of 1000 IU, intramuscularly 10 days after diagnosis.

After treatment of the ovarian hypotrophy grade I, all buffaloes had oestrus 3 days after administration of PMSG. We consider the treatment applied successfully and recommend it in the therapy of these diseases.

Treatment in type II hypofunction, aims the ovaries trophicization and a forced induction of follicular growth to the stage of mature follicle and ovulation. Drug treatment was based on a combination of progesterone, prostaglandin, serum gonadotropin and chorionic (Table 2).

Of the total buffaloes with type II hypofunction, diagnosed in Farm II for two years (8 cases, 47.06%) and treated by the protocol described in table no. 2, five of them showed oestrus immediately, in 3-5 days after treatment, and the rest went into heat 18 days after treatment.

We consider that the treatment of type II ovarian hypofunction was effective in 62.2% of the treated cases that showed oestrus immediately after treatment, and in 37.8% of the cases of oestrus appeared later, after 18 days. The disadvantage was the time elapsed until the buffaloes recovered and the cost of therapy.

Table 2

The specific therapeutic protocol in type II ovarian hypofunction in the Romanian buffalo

No.	Drug formula	Active substance	Route of administration / dose	Rhythm of administration
1	PRIDE DELTA	Progesterone 1.55g	Intravaginal	5-9 days
2	Estrumate	PGF2α	i.m. / 2 ml	Day 10
3	Folligon	PMSG Serum gonadotropins	i.m. / 1000 UI	Day 11
4	Chorulon	Chorionic gonadotropins	i.m. / 1500 UI	On oestrus (12-13th day)
5	Neuroreflex therapy			Once, every 2-3 days
6	Correction of nutrition and vitamin-mineral supplements			With 10 days before treatment and during

In the case of type I hypofunctions, the ovaries are acyclic and reactive with a clinical manifestation of anaphrodisia. Taking into account the seasonality of buffaloes and that the ovaries are small, but with high reactivity, type III and II hypofunction can be treated by specific hormonal therapy associated with a balanced diet and neuroreflex therapy.

Type I ovarian hypofunction was considered atrophy, ovarian hypoplasia and was not treated because the ovarian response is weak or non-existent.

The overall incidence of ovarian pathologies in the buffalo varies from 8.58% to 48%. Adult buffaloes are more likely to suffer from abnormalities of the ovaries compared to buffalo heifers (15,22).

An Israeli study from the 1980s reported an incidence of 8.5% inactive ovaries from 7751 lactations. While type I anoestrus is not widely seen in dairy cattle of developed countries, this type of anoestrus could be more prevalent in regions of the world where balanced energy-dense rations are unavailable to dairy cattle (15). One clinical study recorded an incidence 7.85% for sub-oestrus in buffaloes (16).

Ovarian hypofunction in the buffalo is strongly affected by seasonal changes nutrition and diseases such as trypanosomiasis and should therefore be carefully evaluated (17,23).

CONCLUSIONS

Out of the total gynaecological diseases encountered in buffaloes during the two years studied, they highlighted the fact that ovarian hypofunction represented a share of 19.25%. Treatment of type II ovarian hypofunction was effective in 62.2% of treated cases, which showed oestrus immediately after treatment, and in 37.8% of cases oestrus appeared later, after 18 days. The ovaries of the Romanian buffalo are small, but with high reactivity, type III and type II ovarian hypofunctions can be treated with specific hormone therapy associated with a balanced diet and neuroreflex therapy.

REFERENCES

1. *Awasthi M.K.,* (2013), Follicular dynamics in the female buffalo. In: Purohit GN Eds, Bubaline Theriogenology, (Ed.) International Veterinary Information Service, La Jolla, California, USA
2. *Baraitareanu S., Vidu L.*, (2019), The preventive medicine of bovine viral diarrhoea - mucosal disease in dairy farms: a review. Revista Romana de Medicina Veterinara, 29(2):61-64
3. *Beam S., Butler W.,* (1999), Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. J Reprod Fertil, 54:411-424
4. *Ciornei Ș.G.*, (2021), Embryo Transfer [Online First], IntechOpen, DOI: 10.5772/intechopen.99683. Available at: https://www.intechopen.com/online-first/78116 (Accessed: April27, 2021)
5. *Ciornei S., Drugociu D., Rosca P., Ciornei L.,* (2020), Increasing the genetic potential in a nucleus of Romanian Buffaloes, by artificial insemination with sexed semen, after stimulation with Ov Synch protocol – A Case Report. 36th Annual Meeting of the Association of Embryo Technology in Europe (AETE). Anim Reprod, 17(3)
6. *Ciornei Ş., Drugociu D., Rosca P., Ghinet (Ciornei) L.,* (2020), Caesarean section in uterine torsion at Buffalo (RIB) – a case study. Revista Romana de Medicina Veterinara, 30(2):53-56
7. *Ciornei S., Drugociu D., Rosca P., Liliana Ciornei,* (2018), Polyovulatory response and *in vivo* embryo production at the romanian indigenous buffalo, out of breeding season in N-E of Romania, Lucrări Științifice - Medicină Veterinară, Universitatea de Științe Agricole și Medicină Veterinară "Ion Ionescu de la Brad" Iași,61(4):286-291
8. *Divakar J.,* (2015), Ambrose postpartum anestrus and its management in dairy cattle Ch 52, In: Hopper R.M (Edit.), Bovine Reproduction, 1st edit., (Ed.) John Wiley & Sons, Hoboken, NJ, USA, 456

9. *Ghinet (Ciornei) L.,* (2021), Research on the implementation of biotechnical methods for stimulating the reproductive function of caw-buffaloes (indigenous Carpathian buffalo), PhD-Thesis, USAMV Iasi, Romania
10. *Groza I.Ș., Muntean M.*, (2000), Elements of animal reproductive physiology (in Romanian), (Ed.) Academic Press, Cluj-Napoca, Romania
11. *Groza I.Ş., Tomai G., Cenariu M., Ciupe S.*, (2012), Ultrasonography a modern tool for pregnancy diagnosis and identification of fetal structures in domestic buffloes. Bulletin UASVM Veterinary Medicine, 69(1-2):1-8
12. *Hafez B., Hafez E.S.E.*, (2000), Reproduction in farm animals, 7th ed, (Ed.) Lippincott Williams & Wilkins, Philadelphia, USA
13. *Hanzen C., Gautam G., Nakao T.*, (2009), Prevalence of urovagina and its effect on reproductive performance in Holstein cows. Theriogenology, 71(9):1451-1461
14. *Honparkhe M., Gandotra V.K., Nanda A.S.*, (2004), Ultrasonographic measurements in comparison with the rectal palpation and echotexture of reproductive organs of buffaloes (*Bubalus bubalis*) during different stages of the oestrous cycle. Asian Austr J Anim Sci, 17:919-923
15. *Marai I.F.M., Habeeb A.A.M.*, (2010), Buffaloes reproductive and productive traits as affected by heat stress. Trop Subtrop Agro Eco Systems, 12: 193-217
16. *Modi L.C., Patel P.A., Patel SP, Patel G., Joshi A., Suthar D.,* (2011), Prevalence of reproductive problems in buffaloes in Mehsana milk-shed area of Gujarat. International J Agro Vet Med Sci, 5:424-428
17. *Narnaware S.D., Sirothia K.A., Gawande T.R.,* (2008), Ovarian pathology, hematobiochemistry and serum mineral profile in trypanosome infected Nagpuri buffaloes. Royal Vet J India, 4:38-40
18. *Nechifor F., Drugociu D., Roșca P., Ciornei Ș.,* (2016), Elements of pathology for the diagnosis and therapy of dystocia in cows (in Romanian), (Ed.) Ion Ionescu de la Brad, Iași, Romania
19. *Peter A, Vos P, Ambrose D.,* (2009), Postpartum anestrus in dairy cattle. Theriogenology, 71:1333-1342
20. *Phogat J.B., Pandey A.K., Singh I.*, (2016), Seasonality in buffaloes reproduction. Int J Plant Sci, 6(2):2231-4490
21. *Purhoit G.N.*, (2015), Ovarian pathologies in the buffalo, In: Purohit G.N., Bubaline Theriogenology, (Ed.) International Veterinary Information Service, Available at: https://www.ivis.org/library/bubaline-theriogenology/ovarian-pathologies-buffalo (Accessed: January 14, 2019)
22. *Purohit G.N.*, (2014), Ovarian and oviductal pathologies in the buffalo: Occurrence, diagnostic and therapeutic approaches. Asian Pacific J Reprod, 3(2):156-168
23. *Qureshi SM, Habib G, Samad HA, Siddiqui M.M., Ahmad N., Syed M.*, (2002), Reproduction-Nutrition relationship in dairy buffaloes, I. Effect of intake of protein, energy and blood metabolites levels. Asian Austr J Anim Sci, 15:330-339
24. *Runceanu L., Ciornei Ş,G., Drugociu D., Roşca P.,* (2008), Biosafety of artificial insemination by microbiological spermogram. Cattle and pigs (in Romanian), (Ed.) Ion Ionescu de la Brad, Iaşi
25. *Runceanu L., Groza I.Ș., Șonea A., Zamfirescu S., Drugociu D., Roșca P., Ciornei Ș., Runceanu M., Iuga P., Drugociu D., Nechifor F.*, (2016), Seasonality of animal reproduction (in Romanian). (Ed.) Ion Ionescu de la Brad, Iași, Romania
26. *Savio J., Boland M., Hynes N., Roche J.,* (1990), Resumption of follicular activity in the early postpartum period of dairy cows. J Reprod Fertil, 88: 569-579
27. *Tataru M., Papuc I., Vidu L., Raducu C., Chirila F., Dan S., Marza M.S., Purdoiu C.R., Lacatus R., Miresan V.*, (2019), Qualitative evaluation of raw milk of buffalo from the Romanian Murrah breed. Revista Romana de Medicina Veterinara, 29(3):11-20
28. *Terzano G.M.*, (2012), Ultrasonography and reproduction in buffalo. J Buffalo Sci, 1:163-173
29. *Vale W.G.*,(2007),Effects of environment on buffalo reproduction. Italian J Anim Sci, 6(2):130-142
30. *Vidu L., Bota A.,* (2014), Phenotypic, genotypic and breeding peculiarities of the native buffalo (in Romanian), (Ed.) Pim, Iași, Romania
31. *Wiltbank M., Gümen A., Sartori R.,* (2002), Physiological classification of anovulatory conditions in cattle. Theriogenology, 57:21-52.