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Nonsteroid Anti-Inflammatory Drugs to Improve Fertility in Cows

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Additional information is available at the end of the chapter

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1. Introduction

The bulk of milk production in the world is supplied from the cows. Besides the fact that genetic is the most important factor in a cow's milk productivity; feeding and environmental factors are also considered as crucial factors. Because of these points, calving and increasing reproductive performance has become the most fundamental issue for milk industry. For the animal breeders both having milk and obtaining a calf throughout a year is indispensable. To accomplish this, there should not be a problem in a herd from the aspect of reproduction. But many factors like diseases and environmental agents in cows in post partum period cause decrease in fertility. Among the reasons of reduction in fertility; there are factors like indetermination of estrus in time or detection of estrus in wrong time, premature estrus, subestrus, anestrus, delaying of ovulation, failure of ovulation and fertilization, inadequacy of communication between embryo and uterus, poor body condition score, heat stress, dystocia, retained placenta, delayed uterine involution, metritis, endometritis, and other illnesses. Although a great deal of studies has been done to lower the infertility caused by these factors, this problem has not been eradicated completely up to these days [1-6].

The reasons mentioned above are associated with female animals and environmental conditions. Together with this, fertility is dependent not only to the female but also to the male. There should not be a problem in male's genital organs and the male must have the ability to produce sperms to fertilize the ovum. If artificial insemination is carried out, morphologic structures, numbers, motilities of the sperms should be normal [7-10].

Progestagens and Prostaglandin F_2 alpha ($PGF_2\alpha$) have been used to prevent the disorders related to estrus. But observation of estrus is necessary in both administrations. For this reason researchers have dealt with developing protocols without estrus observation. Ovulations have been synchronized with a method developed in Wisconsin University, and this

method was named as Ovsynch. Later, this method has been modified by the combined use of both progestagens and prostaglandins and many modified ovsynch protocols have been derived [11-12].

Nowadays, one of the methods used by the veterinarians to increase the percentage of pregnancy in cows is GnRH and hCG application just before the artificial insemination, together with the insemination or 1st – 15th days after insemination because hormonal balance is very crucial in early embryonic period. Nearly, 25% of cattle embryos die within the first three weeks of pregnancy [4, 13-15]. In this period, continuation of progesterone release by corpus luteum is vital for the life of embryo [16]. For this reason, researchers have strived to keep the progesterone level sufficient enough in early pregnancy by administering GnRH and hCG in different days of estrus cycle. hCG application is done to animals during the insemination or luteal period in order to provide the rupture of Graafian follicle, to abolish functional insufficiencies of corpus luteum and to rise the endogenous progesterone production to the most effective level, and as a result of these applications it has been stated that pregnancy rate has increased in some studies [17-18]. In the same way, with the GnRH application before, during and post insemination in different days, it has been notified that pregnancy rate has been increased by means of stimulating folliculogenesis, ovulation and luteal structures [19-21].

The latest method to regulate maternal and fetal relation, to retard or inhibit luteolysis, to maintain high progesterone levels and as a result; to enhance pregnancy rate is application of Nonsteroid Anti-inflammatory Drugs (NSAID) in critical days of pregnancy. Estrus cycle's hormonal mechanism should be very well known for the good management of this process.

2. Estrus cycle

Cows are polyestrous animals throughout the year. They show estrus within 18-24 day periods only if they are not pregnant. Estrus cycle is controlled and managed by hormones released by hypothalamus, hypophysis, ovaries and uterus [22].

In the start of estrus cycle GnRH plays the most important role [23]. By giving GnRH released from hypothalamus in each 30 – 120 minute periods to hypophysis system, it induces synthesis and release of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). With the FSH effect follicles in the ovaries start to grow. Follicular development is observed in waves. In each wave just one follicle passes to dominant state from the many developed follicles, rarely two follicles passes to preovulatory stage. Follicular development or atresia is not seen in other follicles in follicular wave. While estradiol produced from preovulatory follicle induces LH release, it inhibits FSH release [3, 5, 24]. But FSH release is not only regulated by estradiol and GnRH, inhibin which is an ovary originated peptide also inhibits FSH release just like estradiol. Moreover, activin which is a peptide hormone found in follicular liquid induces FSH release, but follistatin inhibits it [5].

Released estrogen both causes physiological changes in genital canal and emergence of overt estrus signs. Ovulation in cows takes place 24 to 30 hours after the peak of LH. Ovulated follicle undergoes structural and functional change with the effect of LH and metamorphoses to corpus luteum. Developing corpus luteum releases progesterone, and so, it makes a negative feedback to hypothalamus and by hindering FSH and LH release it also follicular activities in ovaries. In the meantime, by inhibiting contractions of uterus and stimulating the glands in endometrium, it causes the liquid so called uterus milk to be released. As a result it prepares a suitable ambient and provides the continuation of gestation [5, 22, 25-28].

If there is not a live embryo in uterus in the 16th -18th days of cycle, PGF₂α is synthesized, and causes the corpus luteum's regression and decreases the progesterone secretion. Decrease in progesterone causes LH peak and this increase in LH results in increase of estradiol level. While luteolysis is progressing, a new preovulatory follicle develops and cycle resumes. If the animal gets pregnant, PGF₂α secretion is blocked and progesterone level stays in the level enough for sustain the gestation [16, 24, 25, 27].

3. Fertilisation

Gestation is a process which starts with fertilisation and completed with birth of the young. Fertilization is the name given to the event of forming a diploid chromosome cell from two haploid chromosome cells by entering of spermatozoon into oocyte [29].

Fertilization takes place in oviduct ampulla in domestic mammals. It happens approximately in 12 hours. In the end, zygote forms [30-31].

4. Early embryonic period

Zygote undergoes a set of mitotic division which is called "segmentation". With the first segmentation division, blastomere which is a two cell embryo forms. When the blastomeres proliferate in countless numbers it is called morula. Then, water diffusion starts in morula and a liquid filled blank which is called blastocoele forms. When this blank forms, embryo is called as blastocyte [30, 32].

When blastocyte undergoes a mitotic division, liquid continues to accumulate in the blastocoele and for this reason pressure inside the embryo increases. Proteolytic enzymes and blastocyte contraction and relaxation movements cause the tear of zona pellucida. When there is a little tear in zona pellucida, blastocyte goes out. This prolapsus which is called as hatching takes place between 9th and 11th days in cows. After this stage, embryo lives freely in uterus until implantation and feeds with uterus milk [14, 24, 30, 32].

15th to 17th days of the gestation is considered as the critical period. Embryonic deaths taking place in this stage causes dramatic economic losses. During this period, unless the signal to prevent the production of PGF₂α is sent, endometrial luteolytic PGF₂α release will be realized. For

the continuation of gestation this endometrial $\text{PGF}_{2\alpha}$ production must be hindered. Biology of this critical period is complex and affected from very different events. Forming of luteolysis or continuation of gestation is dependent on hormonal, cellular and molecular factors belonging to both mother and the embryo. In order to increase the pregnancy rate in artificial insemination and embryo transfer, hCG, eCG and GnRH applications are done in this critical period. In these applications, while increasing progesterone amount, decreasing plasma estradiol 17 beta amounts and inhibiting $\text{PGF}_{2\alpha}$ synthesis from endometrium is aimed [16].

5. Embryo signals and pregnancy recognition

Blockage of luteolysis during the recognition of gestation can be possible by inhibition of estradiol production because existence of estradiol is obligatory for luteolysis. Estradiol induces $\text{PGF}_{2\alpha}$ secretion. When compared with cyclic animals, follicular development and concentration of plasma estradiol are less in pregnant animals. How does estradiol affects $\text{PGF}_{2\alpha}$ secretion in cellular and molecular levels is not known. However, estradiol has got a central role in luteolysis. For this reason; while antiluteolytic strategies are developed, for the retardation or inhibition of luteolysis decrease of estradiol level is aimed [16].

Progesterone amount circulating in cows provides maternal recognition. This situation shows the importance of high level progesterone for the recognition of pregnancy in critical period. Another factor for the pregnancy recognition is bovine interferon-tau which is released by the embryo. Bovine interferon-tau is also known as bovine trophoblast protein-1 (bTP-1). Bovine interferon-tau which is secreted to lumen of uterus inhibits the release of $\text{PGF}_{2\alpha}$ from the endometrium in critical period. Stimulating of progesterone to bovine interferon-tau is another possible mechanism for the maternal recognition. In the cows, which have higher levels of progesterone in the critical period, more bovine interferon-tau is produced by the embryo [16, 32].

Interferon-tau shows its affect by hindering estradiol receptors. Subsequently, oxytocin receptors diminish and cyclooxygenase inhibitors get activated. Interferon-tau insures the production of some endometrial proteins crucial for the life of embryo. The first of these proteins is bovine granulocyte protein-2. Second one is ubiquitin cross-reactive protein (UCRP). UCRP conjugates with cytosolic endometrial proteins in response to pregnancy and interferon-tau. Proteins conjugated with UCRP become a target for processing by proteasome. This affect of interferon-tau is mediated by the induction of signal transducer and activation of transcription 1 (STAT-1), STAT-2, and interferon regulatory factor 1. UCRP, alpha chemokines and induction of these transcription factors procure pregnancy recognition by mother [33].

6. Early embryonic death

Embryonic death is the most important source of reproductive losses. During the first three weeks of pregnancy embryonic deaths occur by means of several factors. If embryonic deaths take place between the 24th and 50th days, it is called as late embryonic death [4, 13].

Even in healthy cows in the first three weeks of pregnancy, more than 25% of the embryos cannot continue its development. While fertilization rate in cows with first service is 90%, calving rate is about 50-60% [4, 14]. In a study associated with this topic, it is reported that calving rate is 70% after insemination and most of the 30% of embryo losses take place in between 6th and 18th days [34]. If embryonic death happens before 16th - 17th days, cows continue to show estrus within normal intervals. However, if embryonic death happens after 16th - 17th days, returning back to estrus cycle takes longer and cycle interval becomes irregular [4].

There are plenty of factors that cause embryonic death in cows. These are; endocrine, genetic, intrinsic and extrinsic environmental factors, climate, stress, age, insemination time, semen quality, infectious agents, nutrition, chromosomal anomalies. Especially, abnormal progesterone and estrogen profiles cause embryonic deaths. Moreover, in high producing cows steroid metabolism is faster because of liver blood circulation increase. And this causes lower levels of progesterone in luteal period of estrus cycle [4, 35].

7. Embryonic losses due to endocrinologic causes

Low progesterone levels lead to death of embryo by causing excessive estradiol and PGF₂α secretion. It is required that luteolytic effects of estradiol and PGF₂α should be decreased in the early period after insemination in order for maternal recognition of pregnancy [36].

Researchers assert that low progesterone concentration before insemination period causes abnormal follicular development, elicit abnormal oocyte development in ovulatory follicle and ultimately, it causes early embryonic death [37-38].

Adequate secretion of progesterone in luteal period is vital for healthy ovulation, nutrition and survival of developing embryo. Low level of progesterone leads to embryonic death for reasons of:

1. Low progesterone levels from ovulation to 6th day after the insemination causes the inhibition of embryo's development.
2. If progesterone is insufficient in pre-estrus period, uterus deprives of progesterone receptors. As a result of this, in 4th – 9th days of post insemination excessive PGF₂α secretion forms, and this makes both an embryotoxic and luteolytic affect.
3. In 14th – 17th days which are the days of pregnancy recognition, cause of low pregnancy rate is progesterone inadequacy and excessiveness of estradiol.
4. Low progesterone levels in late embryonic period indicate imminent embryonic death [36, 39].

Oxytocin produced by corpus luteum stimulates the release of PGF₂α from endometrium. PGF₂α production depends on reaching of oxytocin receptor number to a threshold value. When these receptors in endometrium reaches a sufficient number, pulsatile secretion of

PGF₂α occurs in response to luteal oxytocin secretion and luteolysis goes after. For this reason, maternal recognition of pregnancy must take place before luteolysis [32, 40].

Specific proteins (bTP-1) produced by blastocyst in cows are signals preventing luteolysis. bTP-1, inhibits the endometrium cells' oxytocin receptor production. As a result, oxytocin cannot induce PGF₂α release. In addition to this, bTP-1 increases protein production from uterine glands. These released proteins into uterus lumen provide nutrition of embryo [32].

8. Cyclooxygenase – COX (Prostaglandin endoperoxide synthase)

NSAIDs¹ are the most commonly used drugs for the treatment of pain for centuries. These drugs also have antipyretic and analgesic affects. They act by inhibiting the enzyme cyclooxygenase (also known as Cox inhibitors). In this way, the synthesis of prostaglandins is blocked. Prostaglandins are mediators which ensure the formation of inflammation symptoms as pain, fever and swelling. Arachidonic acid, which is found in cell membrane, is precursor of prostaglandins. Prostaglandins are end products of fatty acid metabolism. With the effect of Phospholipase A₂, Arachidonic acid is synthesized from membrane phospholipids. As soon as arachidonic acid is released Prostaglandin G₂ and Prostaglandin H₂ is synthesized by the effect of cox enzyme. Then, by means of synthase, PGD₂, PGE₂, PGF₂, PGI₂ and TxA₂ are produced [41-45]. See figure 1.

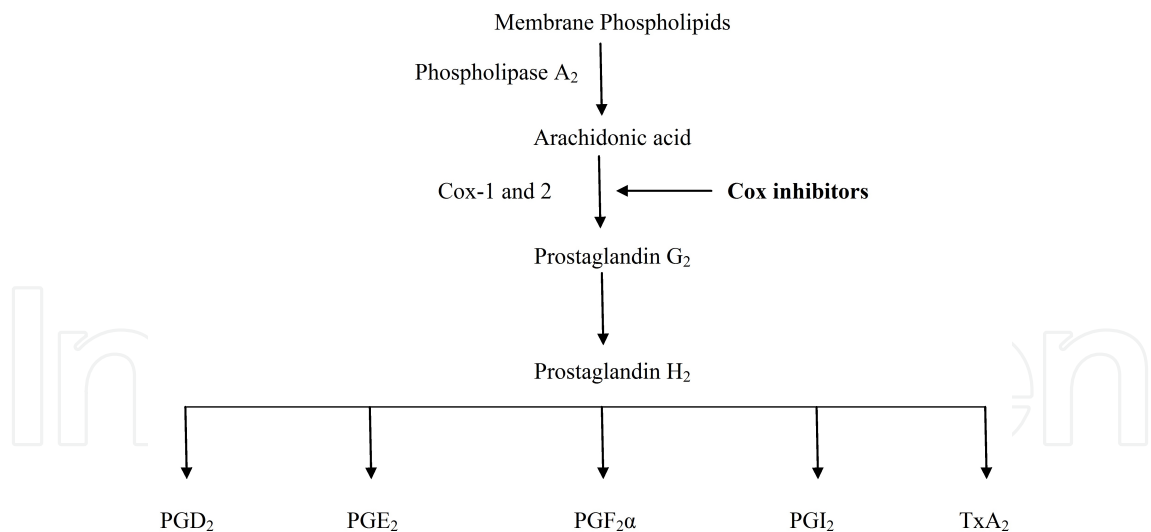


Figure 1. Metabolism of Prostaglandins.

Nowadays, the most known NSAID is aspirin. The past of Aspirin dates back to hundreds of years. The most important step in the discovery of Aspirin is the identification of salicylic acid in 1860. Following this discovery, sodium salicylate in 1875 and phenyl salicylate in

¹ NSAID: Nonsteroid Anti-inflammatory Drugs

1886 were first used. But these drugs formed serious side effects in gastrointestinal system. Aspirin or acetyl salicylic acid was discovered by Felix Hoffman in 1897. It was started to be sold under the name of Aspirin by Bayer Company in 1899 [46].

For the first time, it has been identified that prostaglandin inhibitors prevent product of Cox by John Vane in 1971 [44]. Later studies have shown that Cox enzymes have different isoforms and have different functions. Cox-1 is found in stomach, intestine, kidney and thrombocytes, and Cox-2 is secreted in platelets, macrophages, endothelial cells [41, 47]. While classic NSAIDs inhibit both enzymes, Cox-2 inhibitors inhibit inducible Cox-2. Thanks to this, Cox-2 inhibitors can show anti-inflammatory effect without forming any side effects in gastrointestinal system and in other tissues [43]. Existence of Cox-3 enzyme was discovered by Chandrasekharan et al. in 2002 [48]. See table 1.

Class	Active Ingredient	Effect
Cox-1 Specific Agents	Low Dose Aspirin	It makes COX-1 inhibition without doing COX-2 inhibition.
COX Non-specific Agents	Diclofenac, Ketorolac, Asetaminofen, Flunixin meglumine.	It inhibits both enzymes.
COX-2 Selective Agents	Meloxicam, Nabumetane, Nimesulid, Carprofen.	With Clinic therapeutic doses in human and animals, while doing COX-2 inhibition, in increasing doses they cause COX-1 inhibition.
COX-2 Specific Agents	Celecoxib, Rofecoxib.	They are agents which do not cause COX-1 inhibition even in maximum therapeutic clinical doses.

Table 1. Cox inhibitors are classified as below [41, 49]:

Cox inhibitors are used with different aims in reproductive field. Among these are; blocking of ovulation and implantation, preventing post operative adhesions and hindering of premature births (tocolytic) [50-58].

A lot of studies have been done to understand the importance of Cox enzyme in implantation. It has been found out that COX-2 is produced by uterus luminal epithel and stroma which surround blastocyte during implantation in rats. This situation indicates that COX-2 has a fundamental role in implantation [59-60]. Again in another study, it has been identified that female rats which have COX-1 deficiency have normal fertility and young number. Because in the presence of COX-1 enzyme deficiency, COX-2 supplies this deficit [59]. However, female rats which have COX-2 deficiency are infertile. Because lacking of COX-2 enzyme occurs ovulation, fertilization, implantation and desidualization defects [61].

Parallel with the studies done on experiment animals, studies searching the effects of NSAIDs on pregnancy rates of livestock have also been done. In these studies, flunixin meglumine, meloxicam, and carprofen have been used in order to increase pregnancy rate in cows.

9. Flunixin meglumine

Flunixin meglumine is a derivation of nicotinic acid and is also a non-selective cox inhibitor. It is a potent NSAID to keep the inflammation, pain and fever under control. Especially, it is used in visceral pains. In addition to its analgesic effect, it has antiendotoxic and antipyretic effects. Flunixin meglumine's half-life is between 8 and 12 hours in cows, but it is longer in other animals [49, 62].

Flunixin meglumine is used in cows combined with antibiotics to cure illnesses like; joint ill, transit fever, blackleg, superfoul, mastitis, puerperal metritis, vaginal prolapse, pneumonia, downer cow. Moreover, it is used in pain therapy after small operations [63-64].

Flunixin meglumine is used in cows in ways like intramuscular, intravenous and peros. When it is used orally, the dose is 1 mg/kg. 1.1-2.2 mg/kg dose is used in intravenous way. The most application way is intramuscular injection and the dose is 1.1 mg/kg. This dose of flunixin meglumine is given once in a day or two times by dividing the dose. Flunixin meglumine can be given in 6-8 hour intervals in 0.25-0.50 mg/kg doses. Average therapy period is three days and it can be given 5 days maximum [65-69].

10. Carprofen

Carprofen is a propionic acid derivative NSAID and a selective cox-2 inhibitor. The drugs in this group take -fen suffix (e.g. ibuprofen, ketoprofen). Carprofen is the safest drug in this group because its peripheral prostaglandin inhibition is weak. It is a long effective NSAID with a clinical effect time of 12 hours. Carprofen in cows administered subcutaneous, in dose of 1.4 mg/kg to body weight [49, 65, 70-72].

11. Meloxicam

Meloxicam is a selective cox-2 inhibitor. It is an oxicam group NSAID. It has anti-inflammatory, analgesic and antipyretic effects. Half-life is 13 hours in cows. It is used in cows by intramuscular, intravenous and subcutaneous ways in single doses of 0.5 mg/kg [65, 70-71].

12. NSAID use after insemination

In many studies about usage of flunixin meglumine, carprofen and meloxicam in different times after post insemination, decreasing $\text{PGF}_2\alpha$ release, increasing luteal progesterone level and preventing early embryonic deaths are aimed [72-75].

In some of these studies [74-77], deserved pregnancy rates have been accomplished, on the other hand in some other studies [78-80] pregnancy rates have not changed.

In a study [75], in order to prevent early embryonic deaths in cows which are exposed to transportation stress, flunixin meglumine was given. In the study animals were divided into 3 groups as; control, stress (S) and stress +flunixin meglumine (SFM). After the synchronization of the cows' estrus with MGA - PGF₂α, insemination was done by observing the estrus. Animals were exposed to stress 14 days after the insemination. 1.1 mg/kg dose of flunixin meglumine was given to SFM group before transportation. Just transportation stress was formed in S group. When looked at the pregnancy rates (Control 76%, Stress 69% and SFM 84%), it is seen that there is a positive relation between pregnancy rates and flunixin meglumine application.

Merrill et al [76] searched the effects of 1.1mg/kg dose of flunixin meglumine administration on embryonic mortality of stressful and unstressed cows. They used 259 heifers and 127 cows. They designed the application groups as; control, control + flunixin meglumine, stress and stress + flunixin meglumine. In the first experiment, they used 259 angus crossbred heifers. All the heifers were synchronized with Controlled Internal Drug-Release (CIDR®) and PGF₂α. In the second experiment, they used 127 angus crossbred cows. All the cows were synchronized with MGA and PGF₂α. Applications started 14 days after artificial insemination. While pregnancy rate of animals exposed to transportation stress is 62%, unstressed animals had 64% pregnancy rate. While the pregnancy rate of flunixin meglumine cured animals was 69%, it was 59% in others. In the first experiment they reported that flunixin meglumine given animals had more pregnancy rate than others which were not given. In the second experiment, it was reported that flunixin meglumine applied animals had higher pregnancy rates than others (80% vs. 66%).

In another study, single dose of flunixin meglumine injection (1.1 mg/kg) was done on the 14th day after the insemination to animals which were exposed to transportation stress. The effect of this application on early embryonic deaths and prostaglandin in circulation and cortisol levels were searched. Researchers used 483 beef cows and animals were divided into 4 groups. They designed the groups as; first group transport, second group transport + flunixin meglumine, third group no transport (n=130) and the last group no transport + flunixin meglumine. After the application, transport + flunixin meglumine group had higher pregnancy rate than flunixin meglumine free group (74% vs. 66%) without looking at transportation. Just flunixin meglumine administered cows' pregnancy rates were found higher than non-flunixin meglumine cows (71% vs. 61%). Cortisol concentration in cows exposed to transportation stress got increased but pregnancy rate did not change. In flunixin meglumine given subjects prostaglandin concentration was found lower than not givens. As a result researchers came to conclusion that NSAID applications would increase the pregnancy rate [77].

Odensvik et al [81] have reported that application of flunixin meglumine both orally and parentally supports luteal function. They administered 2, 3 or 4 oral doses 2.2 mg/kg flunixin meglumine to heifers. They started the 9 day-therapy period 14-15 days of the estrus. As a result, they have found that estrus cycle is prolonged in groups of 3 and 4 doses administration. Luteolysis have taken place when 2 or 3 doses of flunixin meglumine have been applied. But in 4 dose give groups luteolysis have been postponed. The first cycle of the

animals was evaluated as control and the 2nd cycle was evaluated as therapy cycle. Before the experiment, cycles of the animals were synchronized by $\text{PGF}_2\alpha$.

Dogruer et al. [82] synchronized repeat breeder heifers by applying two dose $\text{PGF}_2\alpha$. 48 hours after $\text{PGF}_2\alpha$, they administered GnRH (buserelin acetate) and after 12 – 14 hours they made fixed time artificial insemination. Then, they divided the heifers into two groups randomly and they injected a group flunixin meglumine on the 15th and 16th days. They used the other group as control. They made a pregnancy test to animals on the 29th day and in the end, they identified 50% pregnancy rate in therapy group, and 20% in the control group.

Güzeloğlu et al [74] gave GnRH on the 48th hour after synchronisation with $\text{PGF}_2\alpha$ to 52 Holstein heifers and they inseminated them after 12-14 hours. Following this application, they administered 1.1 mg/kg dose of flunixin meglumine after artificial insemination on 15th days evening and 16th days morning via intramuscular way. Pregnancy test was done on the 29th day and, 20 pregnant animals in the treatment group and 13 pregnant animals in the control group was found.

In a study by Lucacin et al [78], they administered 1.1 mg/kg dose of flunixin meglumine to animals between the estrus cycle's 11th and 16th days. Saline solution was given to animals in the control group. The estrus cycle of the animals was synchronized by the applications of estradiol benzoate + CIDR + $\text{PGF}_2\alpha$ and then, fixed time artificial insemination was done. Researchers did not find any difference between progesterone concentrations and pregnancy rates of treatment group and control group.

Rabaglino et al. [79] synchronized the heifers with Cosynch+CIDR protocol, they gave half of them double dose of flunixin meglumine (400 mg) on the 15th and 16th days after artificial insemination. At the end of this application, 59.4% pregnancy was reached in control group, 59.5% pregnancy rate was reached in Flunixin meglumine given group.

Geary et al. [80] searched the effects of flunixin meglumine on the pregnancy rates in a study done on Angus heifers. In the first experiment they synchronized the animals with MGA and $\text{PGF}_2\alpha$. Animals were inseminated 12 hours after the observation of estrus. 13 days after the artificial insemination they injected single dose of flunixin meglumine to animals. While pregnancy rate was 72% in control group, it remained 66% in flunixin meglumine group. In the second experiment, Angus cows were synchronized via Select Synch or Select Synch + CIDR method. After that, they were inseminated by observing estrus. Around 13 days after artificial insemination they were injected flunixin meglumine. In the pregnancy test done on the 47th day, no difference was observed between the control and subject group (57% vs. 58%). In the third experiment both the heifers and the cows were used as materials. While Heifers were synchronized through Select Synch + CIDR protocol, cows were synchronized with Co-Synch + CIDR protocol. Pregnancy test was done on the 29th day; it was confirmed on the 75th day in heifers and 99th day in cows by ultrasound examination. As the conclusion of the experiment, no difference was found between flunixin meglumine and control group (50% vs. 48%).

Kruger and Heuwer [83] made a study to assess the carprofen and flunixin meglumine's effect on pregnancy rate of dairy cattle. They injected animals with carprofen and flunixin

meglumine on 14th, 15th and 16th days after the insemination. In the first experiment, 413 Holstein-Friesian heifers were used. The cycles of these animals were synchronized with PGF₂ α and they were inseminated by observing their estrus. 2.2 mg/kg dose of flunixin meglumine was given to therapy group animals after the insemination's 14th-15th days or 15th-16th days. No application was done to animals in the control group. At the end of this experiment, pregnancy rate in the control group was 58.7% and 58.6% in the treatment group. Serum progesterone levels on 14-15 days and 21-22 days after insemination were compared in both pregnant and non-pregnant animals. It was observed that on the 21-22 days progesterone levels of pregnant animals were higher. In the second experiment researchers used 380 Holstein cows and these animals were synchronized by ovsynch protocol. After 16 hours from the second GnRH injection, fixed time artificial insemination was done. 1.4 mg/kg dose of carprofen was given via subcutaneous on the 15th day after the insemination to the treatment group. No therapy was applied to control group. It was identified that while pregnancy rate in carprofen given group was 33%, it was 35.5 in control group. Researchers come to the idea that NSAID application does not affect the reproductive performance.

Heuwieser et al. [72] made a study on 970 cows. They divided the animals into three groups. They administered 1.4 mg/kg dose of carprofen subcutaneous following artificial insemination. 1.4 mg/kg dose of carprofen was given into the uterus 12-24 hours after the insemination to the 2nd group. 3rd group was left as control. After the first insemination the pregnancy rates were found as 42.2%, 38.3% and 45.1%, respectively. As a result they reported that, subcutaneous carprofen therapy did not affect the pregnancy rate but intrauterine therapy had a negative effect on the pregnancy rate.

Amiridis et al. [73] applied flunixin meglumine, ketoprofen and meloxicam to heifers. In the end, they came to conclusion that meloxicam administered animals have the longest estrus cycle and meloxicam is much more potent than other NSAIDs. The same researchers made a study on repeat breeder cows; 1st group was GnRH, 2nd group was progesterone, 3rd group was meloxicam and the 4th group was GnRH + progesterone + meloxicam. They reported that the highest pregnancy rates were seen in 4th group.

In another study on Holstein heifers, 0.5 mg/kg dose of meloxicam was administered subcutaneous on the 15th day following the insemination. Finally, it was identified that pregnancy rate was 24.3% in meloxicam cured group and 52% in control group. In the light of these data, researchers reported that meloxicam application during the time of maternal recognition will be harmful to pregnancy [84].

In a study aimed at increasing pregnancy rate and progesterone synthesis by inhibiting prostaglandin synthesis, a fixed time artificial insemination was done by synchronising cycles of Nelore cows. Researchers divided the animals into 8 groups and they designed the groups as follows. 1st group constitutes the control and given saline on 7th and 16th days; to the 2nd group, saline on the 7th day and flunixin meglumine on the 16th day; to the 3rd group, bST on the 7th day and saline on the 16th day, to the 4th group, bST on the 7th day and flunixin meglumine on the 16th day, to the 5th group, hCG on the 7th day and saline on the 16th day, to the 6th group, hCG on the 7th day and flunixin meglumine on the 16th day, to the 7th group, bST + hCG on the 7th day and saline on the 16th day, to the last group, bST + hCG on the 7th

day and flunixin meglumine on the 16th day were administered. It was found out that the group only cured with hCG on the 7th day showed a higher rate of pregnancy [85].

Tek et al. [86] searched the effects of flunixin meglumine and oxytetracyclin combinations on the cows diagnosed with subclinical endometritis. They applied intramuscular flunixin meglumine (2 mg/kg) and oxytetracyclin (300 mg). They inseminated the animals in the first estrus seen after the application. When compared with the control group, pregnancy rates were higher in flunixin meglumine and oxytetracyclin administered group (25% vs. 55%).

In another study, animals with puerperal metritis were injected with ceftiofur (CEF) and/or flunixin meglumine. CEF was given to the first group for three days. A single dose of flunixin meglumine (2.2 mg/kg) was given intravenous in addition to CEF to the animals in the second group. At the end of the study, researchers came to a conclusion that flunixin meglumine application does not have a beneficial effect on clinical recovery and reproductive performance [87].

13. NSAID use before embryo transfer

Preparing a suitable environment inside uterus is aimed with NSAIDs applied in different times before embryo transfer to cows and heifers. In most of these studies, while flunixin meglumine or ibuprofen applications just before the embryo transfer increase the pregnancy rate has been reported [88-90], in a study it has been reported that it is ineffective [91], and in another study [91] it has been reported that pregnancy rate has diminished.

Elli et al. [88] investigated whether ibuprofen application increases implantation rates during embryo transfer in cattle. In their study done on 100 heifers, they gave half of them 5 mg/kg dose of intramuscular ibuprofen 1 hour before embryo transfer. Pregnancy rate in the treatment group reached 82% but stayed 56% in control group.

Purcell et al. [89], in a study they made on beef cattle applied either 500 mg dose of flunixin meglumine 2-12 minute before embryo transfer or they inserted CIDR shortly after the embryo transfer. The first of four groups was remained as control group, CIDR to 2nd group, flunixin meglumine to 3rd group, both flunixin meglumine given and CIDR inserted to 4th group. Pregnancy rates were found as 65%, 60.7%, 74.7% and 69.8%, respectively. The average pregnancy rates of flunixin meglumine administered animals (3rd and 4th group) and unapplied animals (1st and 2nd group) were identified as 72.3% and 63%.

In another study [90], 10 ml flunixin meglumine was injected to beef cattle 2-5 minutes before the embryo transfer and when it was compared with control group, it was found that pregnancy rate was higher in flunixin meglumine given group (51.1% vs. 63.8%).

McNaughtan [91] injected 10 ml flunixin meglumine to heifers just before the embryo transfer. He identified that during the pregnancy examination 90 days after the embryo transfer, the difference between the therapy and control group (n: 165) was nonsignificant (50% vs. 45%).

Bulbul et al [92] gave 500 mg flunixin meglumine intramuscular five minutes before embryo transfer in a study done on 39 brown Swiss. As a result of the pregnancy examination on the 30th day by means of ultrasound, they reported that pregnancy rate in the flunixin meglumine given group was lower in comparison to control group (50% vs. 52.6%).

14. Conclusions

Artificial insemination is the first biotechnologic application used in domestic animal. It was first performed by Ivanow in 1899 in Russia on farm animals. This procedure was adopted in 1940s by animal breeders and then it has become prominent all over the world. Such associated technologies as cryopreservation, invitro fertilization and embryo transfer have then started to develop and they have resulted in successful pregnancies (93). NSAID implementations have been used in recent years among the assisted reproductive technologies. NSAIDs are applied as a new strategy to increase the pregnancy rates of cows in artificial insemination. Nevertheless, the results obtained from the previous studies conflict with each other. Especially, there are different studies stating that flunixin application increases, does not change or decreases the pregnancy rate. For this reason, NSAIDs relation with interferon tau and endometrial proteins should be investigated in a more detailed way. Thus, from where the difference in pregnancy rates originate can be found and taking of necessary precautions can be possible.

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