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Research Article

# The Effect of Human Chorionic Gonadotropin and Ketoprofen Applications on Pregnancy Rates in Dairy Cows After Artificial Insemination

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**A B S T R A C T**

The presented study aims to investigate the effect of creating a luteotropic effect on the establishment and maintenance of pregnancy and suppressing the luteolysis mechanism during the maternal recognition process on pregnancy rates in dairy cows. For this purpose, the ovulations of 96 Holstein cows aged between 2-8 years were synchronised with the Ovsynch protocol. After artificial insemination, the cows were divided into four groups. The group receiving Human Chorionic Gonadotropin (hCG) on the 6$^{th}$ day after artificial insemination (H, n=20), the group receiving Ketoprofen on the evening of the 15$^{th}$ day, and the morning of the 16$^{th}$ day after artificial insemination (K, n=24), the group receiving both hCG on the 6$^{th}$ day and Ketoprofen on the 15$^{th}$ day evening and 16$^{th}$ day morning after artificial insemination (HK, n=25), and the control group (C, n=27) that did not receive any post-insemination treatment were established. Pregnancy checks were conducted on the 30$^{th}$, 45$^{th}$, and 60$^{th}$ days through rectal and ultrasonographic (USG) examinations. According to the results of the pregnancy examinations, it was determined that 9 out of 20 cows (45.00%) in group H, 10 out of 24 cows (41.67%) in group K, 16 out of 25 cows (64.00%) in group HK, and 12 out of 27 cows (44.44%) in group C were pregnant. No statistically significant difference (P>0.05) was observed among the groups. The study found that separately providing luteotropic support with hCG or suppressing the luteolysis mechanism with Ketoprofen after artificial insemination did not improve pregnancy rates in cows. However, when both interventions were combined, there was a 20% increase in the pregnancy rate, indicating that different treatment approaches may be required. Further research with larger sample sizes and detailed hormone profiles is needed to optimise and utilise these combination therapies to enhance pregnancy rates.

***Keywords:*** *Human chorionic gonadotropin, ketoprofen, pregnancy rate, cow.*

# Sütçü İneklerde Suni Tohumlama Sonrası İnsan Koryonik Gonadotropin ve Ketoprofen Uygulamalarının Gebelik Oranına Etkisi

**Ö Z E T**

Sunulan çalışmanın amacı, sütçü ineklerde gebeliğin oluşum ve devamlılığında luteotropik etki oluşturmanın ve maternal kabul sürecinde luteolizis mekanizmasını baskılamanın gebelik oranı üzerine etkisini araştırmaktır. Bu amaçla yaşları 2-8 arasında değişen 96 adet Holstayn ırkı ineğin Ovsynch protokolü ile ovulasyonları senkronize edildi. Suni tohumlama sonrası inekler dört gruba ayrıldı. Suni tohumlama sonrası 6. günde İnsan Koryonik Gonadotropin (hCG) uygulanan grup (H, n=20), suni tohumlama sonrası 15. gün akşamı ve 16. gün sabahı Ketoprofen uygulanan grup (K, n=24), suni tohumlama sonrası 6. günde hCG ve 15. gün akşamı ile 16. gün sabahı Ketoprofen uygulanan grup (HK, n=25) ve suni tohumlama sonrası herhangi bir uygulama yapılmayan kontrol grubu (C, n=27) oluşturuldu. Gebelik kontrolleri 30., 45. ve 60. günlerde rektal ve ultrasonografik (USG) muayenelerle yapıldı. Gebelik muayenelerinin sonuçlarına göre, H grubundaki 20 inekten 9'u (%45,00), K grubundaki 24 inekten 10'u (%4,67), HK grubundaki 25 inekten 16'sı (%64,00) ve C grubundaki 27 inekten 12'si (%44,44) gebeydi. Gruplar arasında istatistiksel olarak anlamlı bir fark (P>0,05) bulunmadı. Çalışmada, suni tohumlamayı takiben hCG ile luteotropik destek sağlamanın veya Ketoprofen ile luteolizis mekanizmasını baskılamanın gebelik oranı üzerinde olumlu bir etkisinin bulunmadığı ancak birlikte uygulandığında gebelik oranında %20 artış olduğu belirlendi. Belirlenen bu artış ineklerde gebelik oranını artırmak için farklı tedavi protokollerinin gerekebileceğini işaret etmektedir. Ancak, gebelik oranlarını artırmak amacıyla bu ve benzeri kombinasyonlu tedavilerin optimize edilmesi ve kullanılması için materyal sayısı artırılarak hormon profilleri ile birlikte daha ayrıntılı yeni çalışmalara ihtiyaç olduğu düşünülmektedir.

***Anahtar kelimeler:*** *İnsan koryonik gonadotropin, ketoprofen, gebelik oranı, inek.*

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## Introduction

In order to increase the conception rate in insemination or embryo transfer practices in dairy cows, new approaches to reduce pregnancy losses based on coordinated and timely physiological interactions that occur among the embryo, uterine and corpus luteum (CL) during early pregnancy are still being tried to be developed (Kirbas et al., 2014; Wiltbank et al., 2014)primarily by the corpus luteum (CL). One of these strategies is to improve the function of the CL and/or to improve the concentration of peripheral progesterone ($P_4$) and, consequently, embryonic development through the creation of a new CL or direct application of exogenous $P_4$. For this purpose, one of the hormones that have been widely tested in cows is the Human Chorionic Gonadotropin (hCG) (rensis et al., 2010) induces ovulation throughout the estrous cycle, promotes the formation of accessory corpora lutea when applied in the early luteal phase, and modifies follicular wave dynamics increasing the frequency of three-wave dominant follicular cycles. As hCG acts on ovarian cells independently of the pituitary gland and its effect is longer lasting than that produced by endogenous LH release, use of hCG rather than gonadotropin-releasing hormone (GnRH). Many studies have reported increased pregnancy rates and $P_4$ levels after hCG administration in cows in the days following insemination (Santos et al., 2001; Alnimer and Shamoun, 2015). However, some studies report that $P_4$ levels increase after hCG administration, but there is no positive effect on the pregnancy rate (Hanlon et al., 2005; Rossetti et al., 2011; Stevenson and Pulley, 2012; Kucukaslan et al., 2022). A detailed analysis determined that the preference for hCG administration after insemination to improve fertility should be focused on cows that are expected to have low or moderate fertility, as well as on the day and dose of administration (Besbaci et al., 2020).

One of the strategies to improve pregnancy rates in dairy cows is to prolong the life of the CL by using non-steroidal anti-inflammatory drugs just before the time of possible onset of luteolysis, giving time for embryos with developmental delay to produce sufficient amounts of interferon tau (IFNT) (Binelli et al., 2001). One of the most basic conditions for the formation and continuity of pregnancy in cows during early pregnancy is the initiation of endometrial anti-luteolytic functioning, which causes lysis of the CL in order to maintain a high concentration of $P_4$ in the peripheral blood and endometrial function in order to prevent the formation of new estrus and improve implantation (Bazer et al., 2017; Tinning et al., 2023). This process is initiated by IFNT, which is secreted by the conceptus and blocks the synthesis of Prostaglandin $F_2$ alpha ($PGF_{2\alpha}$) in the endometrium during maternal recognition of pregnancy in cows, at about the 16$^{th}$ day in cows (Sánchez et al., 2018; Wiltbank et al., 2023) the oocyte is fertilized. Therefore, pharmacological strategies aimed at inhibiting the synthesis of $PGF_{2\alpha}$ are used to prevent the onset of endometrial $PGF_{2\alpha}$ release between the 15$^{th}$ and 17$^{th}$ days, which are defined as the critical period for the recognition of pregnancy (Binelli et al., 2001; Paksoy and Das, 2013; Alkan and Erdem, 2018). Non-steroidal anti-inflammatory drugs prevent the formation of luteolytic functioning by inhibiting cyclooxygenase enzymes (COX-1, COX-2) involved in the synthesis of $PGF_{2\alpha}$. Non-steroidal anti-inflammatory drugs, such as flunixin meglumine, carprofen, ketoprofen, and meloxicam, are mainly used for their pain-relieving properties (Smith et al., 2008). However, non-steroidal anti-inflammatory drugs have also been tried to improve fertility by reducing $PGF_{2\alpha}$ release due to manipulations performed during artificial insemination and embryo transfer in cows (Bülbül et al., 2010; Gaievski et al., 2022), or by preventing luteolysis by inhibiting endometrial $PGF_{2\alpha}$ synthesis on the 15-16$^{th}$ days after insemination (Guzeloglu et al., 2007; Erdem and Guzeloglu, 2010; Dursun, 2011). Using non-steroidal anti-inflammatory drugs after insemination it was reported to have a positive effect on the pregnancy rate in some studies (Guzeloglu et al., 2007; Bülbül et al., 2010), but there was no positive effect on the pregnancy rate in some studies (Erdem and Guzeloglu, 2010; Von Krueger and Heuwieser, 2010). It has been stated that various factors such as the type, dose, number of applications, time, and frequency of non-steroidal anti-inflammatory agents, were effective in the formation of these results. In addition, the use of these drugs to improve conception in cows that produce milk should be carefully evaluated (Daeseleire et al., 2003). Ketoprofen is a non-steroidal anti-inflammatory agent that inhibits both COX enzymes. Compared to flunixin meglumine, which tested the effect on pregnancy rate in more studies, ketoprofen stays in circulation for a shorter time than flunixin meglumine, has a narrower volume of distribution, is eliminated from the kidneys in a short time. and It has fewer side effects but is more expensive (Smith et al., 2008). The fact that there are few studies examining the advantages of ketoprofen over other non-steroidal anti-inflammatory drugs and their effects on fertility compared to other non-steroidal drugs suggests that its use should be investigated to improve fertility in lactating cows.

The aim of this study is to investigate the effect on the pregnancy rate in dairy cows of providing luteotropic support with hCG or suppressing the luteolysis mechanism with Ketoprofen separately or in combination after artificial insemination.

## Materials and Methods

The study was conducted with the approval of the Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (ADU-HADYEK) under the protocol number 64583101/2023/32 and date 09/03/2023.

The study was performed on a private dairy farm located in Aydin province, Türkiye. The cattle in the farm were fed with a diet consisting of self-prepared concentrated ration, hay, and silage. Water was provided through automatic water troughs ad libitum. A total of 96 Holstein breed cows, aged between 2 to 8 years, without any puerperal problems (such as retained placenta, uterine

infections, ovarian cysts) as determined during examinations, were included in the study. The study was conducted between the months of March to May.

*Experimental Design*

All cows were synchronised using the classical Ovsynch method for ovulationsynchronisation. In the Ovsynch method, cows were initially administered 10 mcg of Buserelin acetate (Receptal®, 0.004 mg/mL Buserelin, MSD), which is a GnRH analogue, IM. Seven days later, a PG-$F_{2\alpha}$, 500 mcg of cloprostenol (Eustramate®, 500 mcg/2mL cloprostenol, MSD), was administered IM. The second GnRH analogue injection was administered 48 hours later. Artificial insemination was performed on all cows 16-20 hours after the second GnRH injection, and this day was recorded as Day 0 of the study.

To determine the effects of hCG and ketoprofen applications on pregnancy rates at different time intervals, all synchronised cows were randomly divided into four groups: hCG (H), ketoprofen (K), hCG and Ketoprofen (HK), and Control (C). In the H (n=20) group, cows were given 1500 IU of hCG (Chorulon®, Intervet) IM on the 6$^{th}$ day after artificial insemination, as specified by Singh et al. (2020). In the K (n=24) group, cows were administered 3 mg/kg of Ketoprofen (Ketojezik®, 100 mg/mL ketoprofen, Teknovet) IM in the evening of the 15$^{th}$ day and the morning of the 16$^{th}$ day after artificial insemination, as specified by Richards et al. (2009). In the HK (n=25) group, cows received hCG on the 6$^{th}$ day and ketoprofen injections on the evening of the 15$^{th}$ day and the morning of the 16$^{th}$ day after artificial insemination. The C (n=27) group did not receive any additional treatments. Pregnancy examinations were performed in all cows using USG examination (Kaixin 5100v, Hasvet) between days 35 and 45 after artificial insemination. Pregnancy outcomes were confirmed on day 60 through rectal and USG examinations.

*Statistical Analysis*

Statistical analyses were conducted using the SPSS 15.0 software package (SPSS Inc., Chicago, Illinois, USA). Pregnancy rates obtained in the groups were evaluated using the chi-square test.

## Results

In the pregnancy examination conducted, it was determined that 9 out of 20 cows (45.00%) in the H group, 10 out of 24 cows (41.67%) in the K group, 16 out of 25 cows (64.00%) in the HK group, and 12 out of 27 cows (44.44%) in the C group were pregnant. No statistically significant difference (P>0.05) was observed among the groups. In the presented study, the pregnancy rates obtained in the groups were presented in Table 1 and Figure 1.

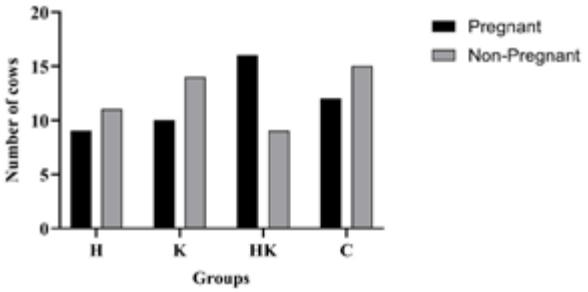


**Figure 1.** Number of pregnant and non-pregnant cows in the study groups (P>0.05). H; Human Chorionic Gonadotropin (hCG) group, K; Ketoprofen group, HK; hCG and Ketoprofen group, C; Control group

## Discussion

In this study, it was evaluated whether the single or combined use of hCG and ketoprofen affected the pregnancy rate at the first insemination in lactating dairy cows. So far, various studies have been conducted to increase plasma $P_4$ levels or prevent luteolysis aim to reduce pregnancy losses during early pregnancy in dairy cows. This study that was anti-luteolytic administration at the time of possible onset of luteolysis following luteotropic hormone administration to increase of plasma $P_4$ level was slightly different from other studies aimed only at increasing $P_4$ levels or preventing luteolysis from affecting the pregnancy rate. To achieve this, hCG was injected on the 6$^{th}$ day after insemination, and ketoprofen was injected on the 15$^{th}$ and 16$^{th}$ days in lactating cows. One of the original aspects of this study is the twice-daily application of ketoprofen, at a dose used in very few studies, to increase the pregnancy rate by stopping luteolysis during the probable onset period of luteolysis 12 hours apart. The study's main finding was that the administration of hCG on the 6$^{th}$ day after insemination and two ketoprofen injections at 12-hour intervals on the 15$^{th}$ and 16$^{th}$ days had a positive effect on the pregnancy rate, although not at a statistically significant level. Another important finding is that hCG administration on only the

**Table 1.** Percentages of pregnant and non-pregnant cows in the study groups [n (%)]

| Groups | Age* | n | Pregnant | Non-pregnant | P value |
|---|---|---|---|---|---|
| H | 4.64±0.36 | 20 | 9 (45.00%) | 11 (55.00%) | 0.37 |
| K | 4.91±0.25 | 24 | 10 (41.67%) | 14 (58.33%) | |
| HK | 4.96±0.24 | 25 | 16 (64.00%) | 9 (36.00%) | |
| C | 5.04±0.21 | 27 | 12 (44.44%) | 15 (55.56%) | |

H; Human Chorionic Gonadotropin (hCG) group, K; Ketoprofen group, HK; hCG and Ketoprofen group, C; Control group, *; Ages of the groups were presented as Mean±SEM (Standard Error of Mean)

6th day resulted in a very small increase in pregnancy rate compared to the pregnancy rates of the control group and the ketoprofen-treated groups, whose pregnancy rates were very close to each other.

It has been shown in some previous studies that the formation of a new CL by ovulation of the dominant follicle of the first wave with hCG administration on the 4th-7th days after insemination in cows or the peripheral $P_4$ concentration is increased by improving the functions of the luteal cells of the existing CL (Santos et al., 2001; Fantini Filho et al., 2004; Walker et al., 2005; De Rensis et al., 2008). It is well known that $P_4$, also known as the pregnancy hormone, plays an essential regulatory role in the formation and maintenance of pregnancy, both before and after insemination. However, it is also stated that the course and timing of the increase in $P_4$ concentration after ovulation is more important than the maximum $P_4$ level in the luteal phase for the formation and maintenance of pregnancy in cows (Mann and Lamming, 1999). Other studies have also shown that the level and profile of $P_4$ in the early period after fertilisation have a more important and positive relationship with embryonic viability than in the late period (Diskin et al., 2006; McNeill et al., 2006; Starbuck et al., 2006). Pandey et al. (2016) stated hCG on the 5th day after insemination improved both the conception rate and the plasma $P_4$ concentration. In this study, the effects of hCG administration on the formation of new CL or $P_4$ levels could not be investigated. However, compared to the pregnancy rates in the ketoprofen group and the control group, higher pregnancy rates were obtained in the hCG and hCG with ketoprofen groups. This result corresponds to the statements that, as previously reported in some studies, elevated $P_4$ levels in early pregnancy benefit the embryo from the effects of $P_4$ on embryo development for a more extended period until the critical period of maternal acceptance, allowing it to grow enough to trigger the anti-luteolytic mechanism. In this study, the pregnancy rate obtained in the ketoprofen group was very similar to that in the control group. It was also stated that hCG administration can delay luteolysis if the dominant follicle of the first wave ovulates and can provide an additional effect that increases the chance of survival by growing more if a live embryo is found. Moreover, it is thought that stopping endometrial $PGF_{2\alpha}$ synthesis with ketoprofen application, which has been exposed to the effect that makes the uterus microenvironment more suitable for conceptus development and the ability to develop larger conceptuses to produce more IFNT that can block $PGF_{2\alpha}$ synthesis, thus increases the likelihood of pregnancy continuation. A similar result as in our study was obtained in a study in which GnRH was injected on the 5th day after insemination, and ketoprofen was injected on the 11th day (Kraevskiy et al., 2020). However, in the other study, it has been reported that the pregnancy rate in Nelore cows administered hCG on the 7th day after insemination and flunixin meglumine on the 16th day did not differ from other groups. In addition, in the same study, it was reported that the pregnancy rate of the group in which hCG was applied on the 7th day and flunixin meglumine on the 16th day was remarkably higher than the pregnancy rate of the group in which only flunixin meglumine was administered and that hCG application in the early pregnancy period was more effective in preventing pregnancy losses (Rossetti et al., 2011).

In the past, various kinds of non-steroidal anti-inflammatory drugs have been studied extensively to prevent endometrial $PGF_{2\alpha}$ synthesis and reduce pregnancy losses by applying them just before the time of possible luteolysis in inseminated cows. However, some studies have reported that these drugs positively affect the pregnancy rate while they do not affect or even negatively affect others. The different results obtained in studies showed it put up various factors, including the variable inhibitory effects on COX-1 and COX-2 of these drugs, the dose, number and time of application of the drug, and animal-dependent factors, etc.

Ketoprofen is a short-acting, non-selective COX-1 and COX-2 inhibitor. In this study, administration of ketoprofen at twice the prospectus dose on days 15 and 16 after insemination did not positively affect the pregnancy rate compared to the control group. In a conducted study (Dursun, 2011), it was reported that a single dose of ketoprofen administered on the 15th day after artificial insemination did not have a positive impact on the pregnancy rate in non-lactating cows, and only a slight increase in pregnancy rate was observed, consistent with the findings of our own study. Ketoprofen's short half-life and the 12-hour interval between two drug applications may have led to the no complete inhibition of $PGF_{2\alpha}$ synthesis and, as a result, to the regression of the CL. A similar view has also been put forward in a study in which flunixin meglumine was applied twice every 24 hours (Von Krueger and Heuwieser, 2010).

## Conclusion

In this study, it was determined that after artificial insemination providing luteotropic support with hCG on the 5th day or suppressing the luteolysis mechanism with ketoprofen applications on the 15th and 16th days at 12-hour intervals did not have a positive effect on the pregnancy rate. However, in the group where both applications were performed after artificial insemination (hCG on the 5th day and ketoprofen on the evening of the 15th day and morning of the 16th day), there was a 20% increase in the pregnancy rate. These results are believed to be attributed to the combination of hCG supporting luteal function in the early stages of pregnancy, allowing the embryo to develop sufficiently until maternal acceptance, and the inhibitory effect of ketoprofen on $PGF_{2\alpha}$ synthesis. It has been concluded that more comprehensive studies should be conducted by determining hormone profiles during the early embryonic period with a larger number of animals.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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