Practical Ovsynch Programs

J. Richard Pursley
Department of Animal Science, Michigan State University, East Lansing, MI 48824

Abstract

Reproductive efficiency is a major factor contributing to efficiency, profitability and sustainability of dairy farms. Estimates of economic loss due to reproductive inefficiencies range from $1 to 5 billion per year. Given the decreasing trend of conception rates during the past 30 years, it can be anticipated that fertility of lactating dairy cows will continue to be a paramount challenge to profitable dairy herd management. The exact physiological reasons for poor fertility of lactating dairy cows are unknown. This presentation focuses on correcting limitations of Ovsynch in addition to understanding the fertility potential of an ovulatory follicle by controlling its age, size, and function. Ovsynch can improve pregnancy rates when estrus detection rates are poor and inaccurate, leading to improper timing of artificial insemination in relation to ovulation. However, inadequate synchronization rate of Ovsynch limits its effectiveness. The focus of our research program is to determine if fertility of lactating dairy cows can be increased by modifying Ovsynch to control emergence of the ovulatory follicular wave and initiate ovulation at the prime stage of development of the synchronized ovulatory follicle. Our ultimate goal is to devise an exogenous hormonal control strategy using gonadotropin-releasing hormone (GnRH) and prostaglandin F2α ($PGF_{2\alpha}$) that reduces age, yet optimizes size and function, of the ovulatory follicle of Ovsynch. Based upon strong published data, it is our contention that control of ovulation to the first GnRH of Ovsynch, appropriate timing of luteolysis and the final GnRH-induced luteinizing hormone (LH) surge are essential for the synchronous emergence, development, and synchronized ovulation of the ovulatory follicle.

Résumé

La performance en reproduction est une composante majeure de l'efficacité, de la rentabilité et de la durabilité des élevages laitiers. Les pertes économiques liées à l'inefficacité en reproduction se situent approximativement entre 1 et 5 milliards $ par année. Étant donné la tendance à la baisse des taux de conception au cours des trente dernières années, il est prévisible que la fertilité des vaches laitières en lactation continue d'être un défi de taille pour la réalisation d'une gestion rentable des troupeaux laitiers. Les causes physiologiques exactes de la faible fertilité des vaches laitières en lactation ne sont pas connues. Cette présentation s'intéresse à la correction des limites du protocole Ovsynch, en plus d'expliquer que le potentiel de fertilité d'un follicule ovulatoire peut être influencé par le contrôle de son âge, de sa taille et de sa fonction. Ovsynch peut accroître les taux de gestation lorsque les taux de détection des chaleurs sont faibles et inexacts, ce qui conduit à une mauvaise synchronisation de l'insémination artificielle relativement à l'ovulation. Cependant, le taux inapproprié de synchronisation Ovsynch restreint son efficacité. Notre programme de recherche vise à déterminer si la fertilité des vaches laitières en lactation peut être accrue de manière à modifier le protocole Ovsynch pour contrôler l'émergence de la vague folliculaire et déclencher l'ovulation au premier stade de développement du follicule ovulatoire synchronisé. Notre objectif ultime est de concevoir une stratégie de contrôle hormonal exogène faisant appel à l'hormone de libération des gonadotrophines (GnRH) et à la prostaglandine F2 alpha (PGF_{2a}), stratégie qui réduise l'âge, mais optimise la taille et la fonction du follicule ovulatoire du Ovsynch. En se basant sur des données publiées fiables, nous soutenons que le contrôle de l'ovulation à la première GnRH du Ovsynch, le choix du moment propice pour la lutéolyse et la poussée soudaine finale de l'hormone lutéinisante produite par la GnRH sont essentiels à l'émergence synchrone, au développement et à l'ovulation synchronisée du follicule ovulatoire.

Introduction

Successes of synchronization programs are primarily dependent on compliance, but even when compliance of injections is being met there are a number of physiological factors that may influence the likelihood of a pregnancy. Our long-term research and extension goals are to improve reproductive efficiencies of lactating dairy cows and thereby increase profitability of dairy operations. Due to the acceptance and high usage of Ovsynch by dairy producers, it is critical that this program be optimized for the greatest conception rate possible. This presentation will focus on the potential impacts of age and size of the ovulatory follicle on fertility. In addition, a new concept will be introduced for optimizing ovulation to the first injection of Ovsynch at the most optimal stage of the estrous cycle.

The Problem

Reproductive inefficiency is an obstacle to dairy farm profitability and sustainability.[13] During the past 50 years, reproductive efficiency of lactating dairy cows progressively decreased due primarily to low and steadily decreasing estrus detection and conception rates.[9,29] Current reports indicate that estrus detection rate is approximately 34% in lactating cows (MI DHIA 2005, yearly summary) and 70% in heifers.[23] The dramatic change in estrus detection rate from the transition of heifer to cow may be attributed to changes in circulating hormonal concentrations[25,30] as well as differences in environment, with cows spending more time on concrete[3,12] and being more susceptible to heat stress.[26] Conception rates in cows decreased from approximately 70%[7,10] to 35%[11,14] while conception rates in heifers remained steady at approximately 70%.[10,23] It appears that increased steroid metabolism in lactating cows may be the key reason why circulating hormonal concentrations may be different in cows vs. heifers.[25] This could be a key reason why ovulatory follicles are older and larger in cows as compared to heifers. Interestingly, during an estrous cycle high producing cows may need several more days of proestrus follicular development in order to acquire sufficient circulating concentrations of estradiol to cause estrus and an LH surge. The impact of this added time most likely negatively impacts fertility.

The effect of prolonging the lifespan of the ovulatory follicle

Treatments based on maintenance of sub-luteal levels of P_4 (1-2 ng/ml; P_4) for an extended period are known to cause prolonged growth and dominance of the dominant follicle (DF). This follicle is described as a persistent DF.[15] When the persistent DF is allowed to ovulate, fertility is decreased when compared with younger ovulatory follicles.[5,15] It was proposed that the negative effect of prolonged growth and dominance of the ovulatory follicle on fertility is associated with the hormonal environment in which the follicle is induced to persist. Sub-luteal levels of P_4 during a prolonged period cause increased frequency of LH pulsatility, preventing a preovulatory LH surge from occurring. As a result, the oocyte resumes meiosis while still contained in the follicle and starts undergoing premature nuclear maturation dissociated from follicular maturation and ovulation. Histological characteristics of these persistent DF indicate that the oocyte undergoes early germinal vesicle breakdown and continues a progression through the cell cycle towards metaphase I or II. By the time of ovulation of the persistent follicle, the oocyte has already matured and aged, which results in lower fertility that may be explained either by low fertilization rates, high early embryonic mortality or both. Also, persistent follicles are known to maintain a high and sustained production of estradiol, which may alter intrafollicular, oviductal and/or uterine environments, thus compromising sperm and/or oocyte transport and embryonic development.[15]

The effect of an abbreviated lifespan of the ovulatory follicle

Follicles that are induced to ovulate before reaching physiological maturity are less fertile. Several studies have repeatedly shown the relationship between smaller follicular size at the time of induced ovulation with GnRH and lower conception rate (CR).[14] In a recent study from our laboratory, cows treated with Ovsynch that responded to a GnRH-induced LH surge with ovulatory follicles < 12 mm in diameter had lower CR (36% for ovulatory follicles > 12 mm vs 27% for ovulatory follicles < 12 mm; P<0.01).[14] They also comprised nearly 34 % of the cows in the study (n = 1424). Perry showed a quadratic relationship between follicular size and fertility in beef heifers, with a maximum CR of 71 % at a follicular size of ~13 mm at the time of the LH surge. Follicles less than 11.5 mm or greater than 16 mm were less likely to support pregnancy than the optimum-sized follicle.

Compromised oocyte quality and subsequent luteal function are reasons proposed why reduced age, size and/or physiological status of follicles reduces CR. Lower circulating P_4 concentrations or early induction of luteolysis can impair embryo recognition, development and/or implantation. Another possibility could be related to a non-synchronized, and therefore incorrect, timing of endocrine signals controlling final follicle and oocyte maturation, leading to ovulation of an immature oocyte. Reduced estradiol concentrations due to a younger DF may also alter uterine and oviductal environment, affecting either gamete or embryo survivability.

Follicular wave pattern and fertility

It is widely accepted that ovarian follicles primarily develop in two- or three-wave patterns throughout the estrous cycle of cattle.[8] Lactating dairy cows generally have two waves of follicular growth per cycle.[2] In either wave pattern, the DF of the final wave is the one intended to ovulate. The emergence of the ovulatory wave occurs earlier for two- vs. three-wave cows (approximately day 10 vs. day 16 of the cycle) leading to different life spans of the ovulatory follicles for each wave pattern. So, at the time of ovulation, a difference in age can be observed between the pre-ovulatory follicle coming from a two-wave (10 to 11 days) versus a three-wave (6 to 7 days) pattern of follicle development.[8] Therefore, the two-wave pattern that predominates in lactating dairy cows is generally characterized by a larger and older DF reaching ovulation, compared with three-wave patterns.[8] Moreover, second-wave pre-ovulatory follicles were found to be approximately three days older than those of a third wave when the interval from emergence to estrus was measured.[2] This difference in lifespan between second- and third-wave ovulatory follicles was explained by a longer period of follicular dominance, rather than by the interval of follicle growth from emergence to dominance, which remained the same in both cases.[2]

The older age of a second-wave ovulatory follicle could be expected to have a negative impact on fertility. If most dairy cows have two waves, then a high proportion of the population could be expected to be ovulating an older DF, thus providing a possible explanation of the low CR problem in lactating dairy cows. Only a few studies compared fertility between three- vs. two-wave cows,[2] with significant trends towards greater CR in three- vs. two-wave cows. Unfortunately, sufficient numbers of three-wave cows are difficult to procure due to the fact that most lactating dairy cows have two follicular waves during a normal estrous cycle.

Supporting effect of duration of dominance of the ovulatory DF on fertility

There appears to be a stronger association between fertility and length of ovulatory follicle lifespan and/or duration of physiological dominance, than the number of follicular waves and fertility. The interval from emergence of the ovulatory follicle to estrus was approximately one day shorter in cows diagnosed pregnant compared to cows diagnosed not pregnant (7.8 ± 0.2 d versus 8.6 ± 0.2 d, respectively; $P < 0.01$) regardless if they had two or three follicular waves.[2] A key result from the same study was a significant inverse relationship between follicle lifespan and dominance duration, and CR. The longer the ovulatory follicle remained in the ovary waiting for the ovulation trigger, the lower the probability of establishing a pregnancy.

Furthermore, a recent study found differences in follicular development between lactating cows and nulliparous heifers.[31] The latter had a significant trend towards a 1.2-day shorter duration of dominance of the ovulatory follicle when compared to cows. The relevance of this issue becomes clear when considered together with the current CR for both groups: 35% for lactating dairy cows vs. 70% for dairy heifers.

The ovulatory follicle plays a key role in reproductive success by coordinating hormonal interactions, final maturation of the oocyte and ovulation. An increasing number of studies reinforce a consistent association between physiological characteristics of the ovulatory follicle and fertility of lactating dairy cows, including follicular age, size, and function (E_2 production capacity). Although Ovsynch was developed to synchronize the time of ovulation to allow for timed-AI, few studies have focused on how Ovsynch can be utilized to maximize age, size and function of the ovulatory follicle to enhance the fertility outcomes of this program.

The Ovsynch Protocol: Strengths and Limitations

Pursley *et al*[21] developed Ovsynch to control time to first and subsequent AI, thus maximizing service rate and improving overall estrus detection rate. Ovsynch is based on three scheduled hormonal injections (Figure 1). The first injection of GnRH is intended to cause ovulation of any functional DF present in the ovary and induce subsequent emergence of a new follicular wave approximately 1.5 days later.[21] Then, a new DF develops and undergoes selection and dominance during the next seven days. If a follicle does not respond to this GnRH, it is likely the cow is in the early stages of a follicular wave (first three to four days of the wave) in which the future DF does not yet have LH receptors. In this case the DF will soon deviate from the subordinate follicle(s) and develop, but this follicle will either be older, or may reach atresia, by the time of the $PGF_{2\alpha}$ of Ovsynch. On day seven, $PGF_{2\alpha}$ is administered to induce luteolysis, thus allowing for further growth and maturation of the DF, now a pre-ovulatory follicle. Finally, a second GnRH injection is administered 48 hours after $PGF_{2\alpha}$ to induce a pre-ovulatory LH surge that triggers ovulation between 24 and 32 hours later.[21]

Cows treated with Ovsynch yield overall conception rates similar to those obtained after breeding to detected estrus (37 vs. 39%, respectively; $P > 0.05$).[23] Yet, a major limitation to Ovsynch is the wide variability of synchronization rates (defined as having a regressed CL and ovulation following the final GnRH). Up to 30% of cows may not have an ovulation synchronized with Ovsynch programs.[6,14,17,19,28] It is likely that non-synchronized cows will not be inseminated at an appropriate time relative to ovulation, thereby decreasing their chances of becoming pregnant. Thus, improving synchronization rates alone could have a dynamic impact on reproductive performance. Published research from our laboratory indicates that synchroniza-

Figure 1. Description of the orignal Ovsynch program utilizing GnRH and PGF_{2a} to control the time of ovulation in lactating dairy cows.

tion rates, and subsequent conception rates, can be greatly improved (discussed later).[1] Vasconcelos *et al*[28] attributed most of the variability in synchronization rate in cows to the stage of the estrous cycle in which Ovsynch was initiated. Cows started on Ovsynch at mid-cycle (days five to nine of cycle) had a greater probability of synchronizing compared to late estrous cycle, and therefore had a greater chance of conception.[16,28]

Compelling New Data

New published data from our laboratory[1] indicate that when lactating dairy cows are treated with a presynchronization program utilizing $PGF_{2\alpha}$ then GnRH, more cows have an ovulatory response to that first GnRH. Cows that ovulate to the first GnRH likely initiate emergence of a new follicular wave. Thus, these cows have a greater percentage of follicles in an ideal stage of development at the time of the final injection of GnRH with regard to the relationship between age, size, and E_2 concentration and the increased probability of a pregnancy.

Attaining consistent ovulation in response to first GnRH of Ovsynch constitutes the first key step to optimizing synchronization of ovulation to Ovsynch in lactating dairy cows.[1,28] Ovulation to first GnRH of Ovsynch is followed by emergence of a new follicular wave, from which the ovulatory follicle of Ovsynch develops.[21] Thus, variation in response to first GnRH leads to extreme variation in the timing of emergence of the ovulatory follicle of Ovsynch. This, in turn, translates into substantial variation in age and size of ovulatory follicles at the time of the final GnRH of Ovsynch.[1] This variation may lead to a reduced chance of conception.

Our laboratory tested a Pre-Ovsynch program that maximizes ovulation to first GnRH of Ovsynch, which decreased the variability in size of the preovulatory follicle and increased synchronization rate to Ovsynch.[1] Based on previous data, we designed an injection scheme using $PGF_{2\alpha}$ and GnRH prior to Ovsynch in which cows were treated with 25 mg $PGF_{2\alpha}$, then two days later 100 µg of GnRH. Then, either four (G4G), five (G5G) or six (G6G) days later, cows received the first GnRH of Ovsynch, and continued the Ovsynch program. Controls received only Ovsynch. Figure 2 describes the primary outcomes of these data.[1] Compared to Ovsynch alone, G6G dramatically improved the percentage of cows ovulating to first GnRH (A), percentage of cows responding to $PGF_{2\alpha}$ by luteolysis (B), and percentage of cows with both a luteolytic response to $PGF_{2\alpha}$ and ovulation to the final GnRH of Ovsynch (C). These data support the concept that enhanced percentage of cows ovulating to first GnRH of Ovsynch holds potential to increase the percentage of cows pregnant to Ovsynch.

Additional data reveal that cows ovulating in response to the first GnRH of Ovsynch, regardless of treat-

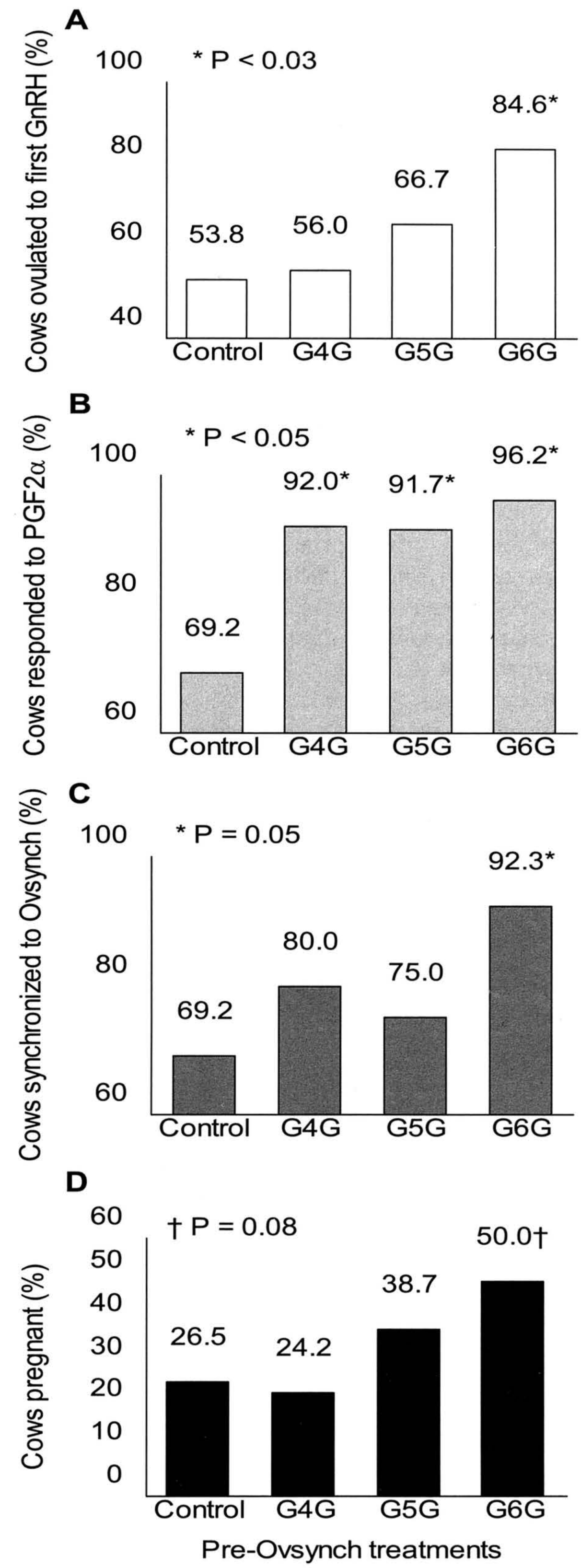

Figure 2. Effect of Pre-Ovsynch treatment at 4, 5, or 6 days prior to Ovsynch on % ovulating following Ovsynch, CL regression during Ovsynch, synchronization rate to Ovsynch, and % cows pregnant (n = 130).

ment, yielded significantly less variability in pre-ovulatory follicle size at the final injection of GnRH (Figure 3), a greater chance of luteolysis in response to the $PGF_{2\alpha}$ of Ovsynch, and a greater chance of ovulating to the final GnRH.[1] Also from this study,[1] a positive linear relationship was detected between concentrations of E_2 at the final GnRH of Ovsynch and the probability of a pregnancy (Figure 4). In addition, a quadratic relationship was also detected between ovulatory follicle size at final GnRH and probability of a pregnancy (Figure 5). Thus, it is of critical importance to optimize the age and size of the ovulatory follicle to allow these follicles to secrete as much E_2 as possible at the time of the final injection of GnRH of Ovsynch.

Conclusions

Low fertility of high producing lactating dairy cows may be caused, at least in part, by a compromised oocyte that has become over-stimulated during a prolonged follicular phase due to inadequate concentrations of estradiol in circulation. In order to solve this problem, control over the age and size of the ovulatory follicle utilizing Ovsynch technology may be necessary. It is clear that ovulation to the first GnRH of Ovsynch is critical to initiate the follicular wave that includes the new pre-ovulatory follicle. By controlling this process, the age of the ovulatory follicle can be reduced and the size can be maximized. Further work is under way to understand new ways to increase the growth rate of the ovulatory follicle and whether a greater reduction in age of the ovulatory follicle, while still maximizing function (estradiol production), will translate into greater fertility.

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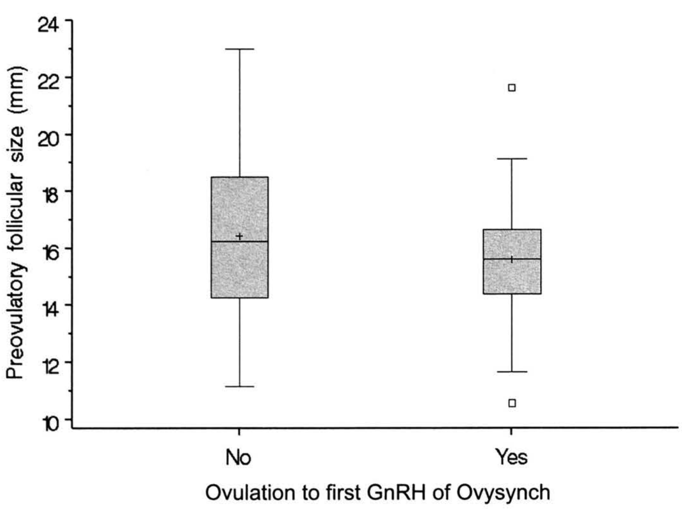

Figure 3. Box plot for follicle size at final GnRH of Ovsynch in cows that did or did not ovulate to 1st GnRH of Ovsynch. The central box represents the inter quartile range (IQR) bounded by the first and third quartile (25th and 75th percentile, respectively). The whiskers are drawn between the quartiles and the corresponding extreme value of the group. A group with larger box and whiskers has associated a larger residual variance.

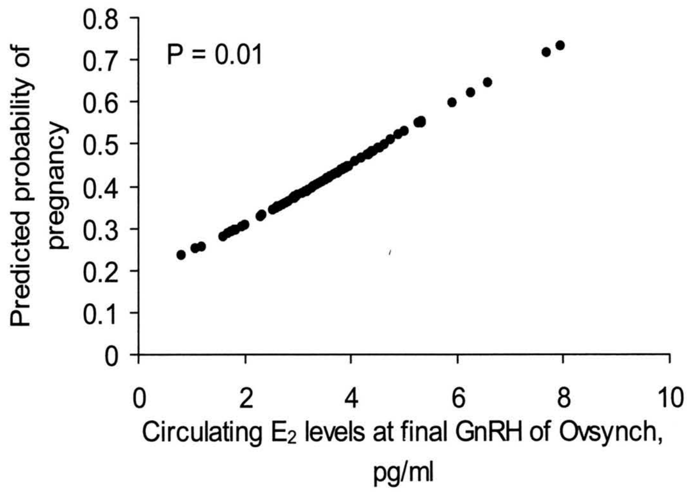

Figure 4. Association between circulating concentrations of E_2 at final GnRH of Ovsynch and predicted probability of pregnancy 35 d after AI in lactating Holstein dairy cows.

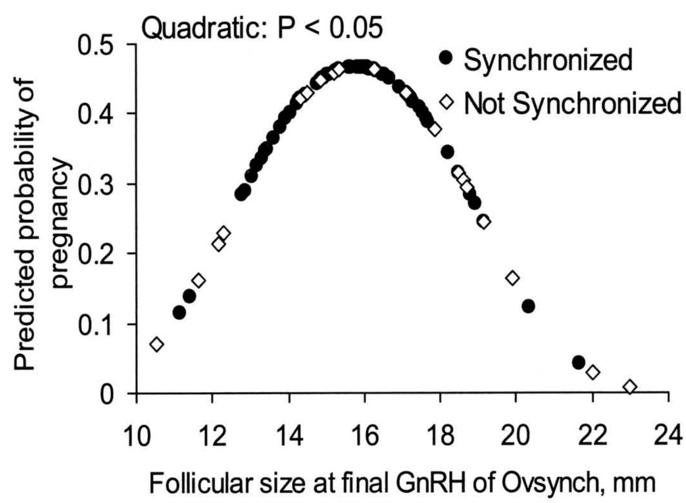

Figure 5. Association between follicle size at final GnRH of Ovsynch and predicted probability of pregnancy 35 d after AI in lactating Holstein dairy cows. Synchronized cows were cows that responded to both PGF_{2a} (by regressing a functional CL) and final GnRH of Ovsynch (by ovulating).

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