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**Comparison of synchronisation protocols on pregnancy rate in dairy cows and heifers: A systematic review and network meta-analysis**

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# Comparison of synchronisation protocols on pregnancy rate in dairy cows and heifers: A systematic review and network meta-analysis

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**ABSTRACT:** Estrus synchronisation protocols are used as an essential strategy in the reproductive management of dairy herds, including Ovsynch, Presynch, Selectsynch, Heatsynch, Cosynch, Double-Ovsynch, and some other combinations of these, while the optimal protocol is still uncertain. We performed a network meta-analysis (NMA) to compare the efficacy of the synchronisation protocols in dairy cows and heifers. Pubmed, Scopus, Web of Science, Cabi Direct, and The Cochrane Library were searched up to May 13, 2019. The comparison of synchronisation protocols in terms of pregnancy rate was done by combining direct and indirect evidence. Ranking the synchronisation protocols were conducted by using surface under the cumulative ranking curve (SUCRA). 48 randomized trials involving 9 different protocols were included. Compared with the control group, none of the synchronisation protocols were more effective in pregnancy rate at first insemination. Presynch+Ovsynch was found to be more efficient than Ovsynch (RR=1.21, 95% CI:1.04-1.40), also Presynch+Ovsynch and Presynch+Heatsynch were superior to Presynch+Selectsynch (RR=2.01, 95% CI: 1.03-3.93 and RR=1.91, 95% CI: 1.21-3.01). In conclusion, presynchronisation strategies such as Presynch+Ovsynch increase the effectiveness of pregnancy rate at first insemination compared to other synchronisation protocols. Nevertheless, the non-superiority of the synchronisation protocols against not applying any hormonal treatments should be considered in reproductive management in dairy herds.

*Keywords*: dairy cow; dairy heifer; estrus synchronization; network meta-analysis; reproduction

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## INTRODUCTION

High reproductive performance is a core element of profitability in commercial dairy herds. In order to achieve high pregnancy rate, understanding the estrus cycle is crucial. In cows, estrus cycle has between two and four waves (Tibary et al., 2019, Lindley et al., 2021). Follicular growth in cattle emerge in different wave patterns according to the estrus, anestrus and pregnancy stage of the cows (Tibary et al., 2019, Kasimanickam, 2021). In general, while a great deal of heifers shows three waves, cows might show two waves per cycle (Lindley et al., 2021). Depending on waves occurs in the cycle, and the length of the cycle itself each wave indicate 7-10 or 6-12 days length (Kasimanickam, 2021). Altough the hormonal mechanism of estrus is well defined, the difficulty of estrus detection and thereby low fertility is one of the reasons that reduces the efficiency of reproduction (Pursley et al., 1995, 1997; Stevenson, 2006). Several synchronisation protocols based on timed AI have been developed by synchronizing the ovulation to overcome the limitation of estrus detection. By means of this, the requirement of estrus detection has disappeared, so reproductive management of dairy herds has become optimized (DeJarnette et al., 2001). One of these synchronisation programs, *Ovsynch* is a hormonal protocol of synchronized ovulation using $PGF_{2\alpha}$ and two doses of GnRH and cows are inseminated 16 h after the second injection of GnRH without estrus detection (Pursley et al., 1997). *Cosynch* was developed as an alternative synchronisation protocol to Ovsynch, in which insemination was performed along with the second injection of GnRH eliminating the 24 h waiting period as Ovsynch (DeJarnette and Marshall, 2003). *Heatsynch* protocol differs from Ovsynch using Estradiol Cypionate instead of second GnRH injection (Pancarci et al., 2002). *Selectsynch* is another alternative synchronisation protocol to Ovsynch, in which the second injection of GnRH was not administrated and insemination was performed after the $PGF_{2\alpha}$ injection when estrus was detected (Cartmill et al., 2001). *Double-Ovsynch* is a relatively new synchronisation protocol performing two Ovsynch protocols 7 d apart in a row (Souza et al., 2008). A presynchronisation protocol, *Presynch*, was developed in which two injections of $PGF_{2\alpha}$ was administrated 14 d apart (Moreira et al., 2001). This protocol not only may be applied alone to synchronize estrus, but also before any other synchronisation protocol as a combined treatment (e.g. Presynch+Ovsynch, Presynch+Heatsynch).

There are many studies assessing the effectiveness of various synchronisation protocols within the context of reproductive performance parameters such as pregnancy rate. Several pairwise meta-analyses have been performed to compare the effects of some of these protocols or hormonal administrations on reproductive parameters (Rabiee et al., 2004, Rabiee et al., 2005, Bisinotto et al., 2015, Yan et al., 2016, Borchardt et al., 2017). However, conventional pairwise meta-analysis allows only head-to-head comparison and can not combine all the evidence of more than two comparators. Thus, further research is still required for the comparison of all the protocols together. Network meta-analysis (NMA) is a statistical method, which allows estimating the relative treatment effects of interventions that have not been compared against each other in a study by combining direct and indirect evidence (Cipriani et al., 2013). NMA not only compares more than two comparators, but also creates a hierarchy between them. The aim of this study was to conduct a NMA evaluating comprehensively the efficacy of synchronisation protocols based on timed AI on pregnancy rate.

## MATERIALS AND METHODS

### Search strategy and selection criteria

A systematic review and NMA was conducted to explore the effectiveness of synchronisation protocols to improve reproductive parameters in dairy cows and heifers. Relevant literature was identified through comprehensive searches of Pubmed, Scopus, Web of Science, Cabi Direct, and The Cochrane Library up to May 13, 2019. The electronic searching strategy was based on these keywords: [("dairy cow" OR "dairy cattle" OR "dairy heifer" OR "dairy *bos taurus*" OR "dairy herd" OR "dairy farm" OR holstein) AND (ovsynch OR cosynch OR "co-synch" OR heatsynch OR selectsynch OR presynch OR "double-ovsynch" OR synchron*) AND (pregnancy OR pregnant OR "pregnancy rate" OR "P/AI")]. This search algorithm was suitably adapted for every electronic database. In addition, reference lists of eligible studies and published meta-analyses on this topic were manually searched. Two reviewers (PA and MOY) undertook screening of every article independently.

Articles published only in English or Turkish were included and no geographical restriction was applied. Conference abstracts were not included in the study if full texts were not available. Dairy cows' breeds (e.g. Holstein, Friesian, Jersey) and their crossbreeds were included. Beef cows and crossbreeds of cow and

zebu breeds *(Bos Taurus x Bos Indicus)* were excluded from the study. Healthy animals were included in the study while animals were excluded which were diagnosed with any reproductive abnormalities such as endometrisis, mastitis, pyometra, or ovarian cysts. Additionally, trials performed with acyclic cows, repeat breeder cows, and cows in the early postpartum period (<45 d pp) and studies in which authors indicated the existence of heat stress were excluded.

**Study selection and data extraction**

Randomized trials that compared the effect of synchronisation protocols on pregnancy rate in dairy cows and heifers were included. As a numerous combinations of hormonal treatments might be investigated in the studies, we only focused on injectable and timed AI-based synchronisation protocols described previously which are Ovsynch, Cosynch, Double-Ovsynch, Heatsynch, Presynch, and Selectsynch (Lane et al., 2008). Additionally, we included studies that applied a combination of any synchronisation protocols together with Presynch. We accepted any minor modifications to the dose and application time of hormones in accordance with the protocol. When a treatment arm received no hormonal treatment and the animals were inseminated according to observational estrus detection, it was labeled as a control group. Treatment arms with Presynch protocol were included when the intervention was applied on at least two different injections of PGF2α. Data on study-level characteristics (author, year, and country of the study), animal-level characteristics (parity, postpartum day), treatment-level characteristics (synchronisation protocol, application period of the protocol, details of the protocol, dose of hormones, AI protocol, pregnancy diagnosis days after AI) and outcome measure were extracted from each study. Two reviewers (PA and MOY) selected the studies and extracted the data independently. In any case of disagreement, a third reviewer (RV) achieved a consensus.

**Outcomes**

The primary outcome was pregnancy rate. Pregnancy rate is considered in this NMA as the number of pregnant cows divided by the total number of cows in each group as previously described by Rabiee et al. (2005). We assumed that the first pregnancy diagnosis to be on 32 d (28-42) for pregnancy rate.

**Risk of bias assessment**

We assessed the risk of bias of each study according to the Cochrane risk of bias assessment tool which contains the following items: adequate sequence generation, allocation concealment, blinding, incomplete outcome data selective outcome reporting and other bias (Higgins and Green, 2008). Because of the nature of the trials, we deemed blinding only for the responsible person who performed the AI (e.g. veterinarian, technician) or/and farm owners. Two independent reviewers (PA and MOY) assessed the risk of bias levels of every included study as "low risk", "unclear risk" and "high risk" for each item.

**Statistical analysis**

In this study, a random-effects NMA was performed using a frequentist approach (White, 2015). By performing a NMA, one can both compare more than two treatments at once and rank these treatments in terms of their effectiveness on the relevant outcome (Mavridis et al., 2015). NMA also allows to estimate the relative treatment effects of interventions that have not been compared head-to-head synthesizing both direct effects and indirect estimates in a mixed estimate (Cipriani et al., 2013). We used the Stata routines and self-programmed commands to perform NMA and visualize the results with graphs (Chaimani et al., 2013; Chaimani and Salanti, 2015; White, 2015). Risk ratio (RR) was used as an effect measure. The structure of the network was shown with a network plot in which nodes represent the treatments and the edges represent the direct comparisons between treatments. Furthermore, the size of nodes is weighted by the number of the studies that evaluated each treatment and the thickness of edges is weighted by the number of studies that compared each pair of treatments in the network plot (Cipriani et al., 2013; Chaimani and Salanti, 2015).

Transitivity is one of the assumptions of NMA and is defined as the similarity of the distributions of the potential effect modifiers across the comparisons. Another principal assumption, which is actually the statistical manifestation of transitivity, is consistency that is defined as the agreement between the direct and one or more indirect estimates in the network (Salanti, 2012). We evaluated the consistency assumption first through the global approach using the Wald test statistics, which follows a $\chi^2$ distribution (Higgins et al., 2012) and then through the loop-specific approach (Bucher et al., 1997; Dias et al., 2010;

Veroniki et al., 2013). In loop-specific approach we demonstrated the inconsistency factors (IF) accompanied by the 95% confidence intervals for each loop with the inconsistency plots. IFs closer to one indicate that the consistency assumption holds.

League table was drawn for the outcome to present the results of relative effectiveness and their uncertainty for each pair of synchronisation protocols (Mavridis et al., 2015). In addition, to present the rank probabilities of the synchronisation protocols we used the surface under the cumulative ranking (SUCRA) and rankogram plots (Salanti et al., 2011). The SUCRA values represent the ratio of the effectiveness of a treatment in reference to a theoretical treatment that always performs the best with no uncertainty and the larger SUCRA values indicate the better treatment. Rankograms visualize the rank probabilities against the possible ranks for all competing treatments. Thus, we showed the probability of 'being the best, second best, third best etc.' for each treatment (Salanti et al., 2011; Mavridis et al., 2015).

Finally, we did a sensitivity analysis by excluding studies suspected to cause inconsistency and performed another sensitivity analysis by excluding the studies or trials which are conducted with heifers to determine whether the effects of the hormonal treatments in heifers differ from the effects in primiparous or/and multiparous cows. In all analyses, we used a 5% level of statistical significance.

## RESULTS

### Study characteristics

Forty-eight studies published between 1974 and 2018 were included in present study. The PRISMA flow chart in Fig 1 shows the electronic searching process (Hutton et al., 2015). Three studies provided two independent datasets from different experiments or herds which were evaluated separately. Forty two-arm and eleven multi-arm trials were enrolled to the present study. The studies were carried out in 16 different countries: 18 USA, 11 Turkey (6 of them were published in Turkish journals and 3 of them were in Turkish), 4 Iran, 2 in Canada, Germany and Italy and 1 in Australia, England, Egypt, France, Greece, Hungary, Japan, Jordan and Romania. We presented the summary of study characteristics in Table S1.

### Risk of bias assessment

The risk of bias assessment across the included studies was presented in Fig 2. We were not able to evaluate "allocation concealment" and "selective outcome reporting" items due to the lack of any information about the process of allocation or the study protocol in any of the studies. Hence, we scored the two items as unclear risk of bias for all studies. Four studies scored (Burke et al., 1996; Aréchiga et al., 1998; Lean et al., 2003; Tenhagen et al., 2004) as high risk of bias for "adequate sequence generation" item because randomization were done according to the birth dates or ear tag numbers of cows. The "blinding" item could not be assessed for the participants (cows) because of the nature of the studies but we considered blinding of veterinarian, technician who performed the AI or farm owner. We only scored two studies (Cordoba and Fricke, 2001, 2002) as low risk of bias in "blinding" item and the rest were under unclear risk of bias due to the absence of any information about blinding. "Incomplete outcome data addressed" mostly scored as low risk of bias. To make a general as-

**Table 1.** League table demonstrating the relative effectiveness for each pair of comparison for pregnancy rate.

| Control | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1.07 (0.87-1.33) | Selectsynch | | | | | | | |
| 1.81 (0.92-3.58) | 1.69 (0.83-3.42) | P+S[1] | | | | | | |
| 0.90 (0.77-1.05) | 0.84 (0.66-1.07) | 0.50 (0.26-0.96)* | P+O[2] | | | | | |
| 0.95 (0.57-1.58) | 0.89 (0.52-1.52) | 0.52 (0.33-0.83)* | 1.06 (0.65-1.72) | P+H[3] | | | | |
| 1.05 (0.90-1.22) | 0.98 (0.79-1.20) | 0.58 (0.29-1.15) | 1.17 (0.97-1.40) | 1.10 (0.66-1.85) | Presynch | | | |
| 1.08 (0.96-1.22) | 1.01 (0.83-1.23) | 0.60 (0.30-1.18) | 1.21 (1.04-1.40)* | 1.14 (0.69-1.89) | 1.03 (0.90-1.18) | Ovsynch | | |
| 1.19 (0.89-1.59) | 1.11 (0.79-1.55) | 0.66 (0.31-1.36) | 1.32 (0.97-1.80) | 1.25 (0.70-2.22) | 1.13 (0.83-1.54) | 1.10 (0.83-1.44) | Heatsynch | |
| 1.16 (0.82-1.63) | 1.08 (0.73-1.58) | 0.64 (0.30-1.36) | 1.28 (0.90-1.84) | 1.22 (0.67-2.22) | 1.10 (0.77-1.57) | 1.07 (0.77-1.47) | 0.97 (0.63-1.49) | Cosynch |

Estimates are presented as RR (95% CI). RRs larger than 1 favor the treatment in the column and RRs smaller than 1 favor the treatment in the row.
* indicates statistically significant difference between relevant synchronisation protocols
[1] Presynch+Selectsynch
[2] Presynch+Ovsynch
[3] Presynch+Heatsynch
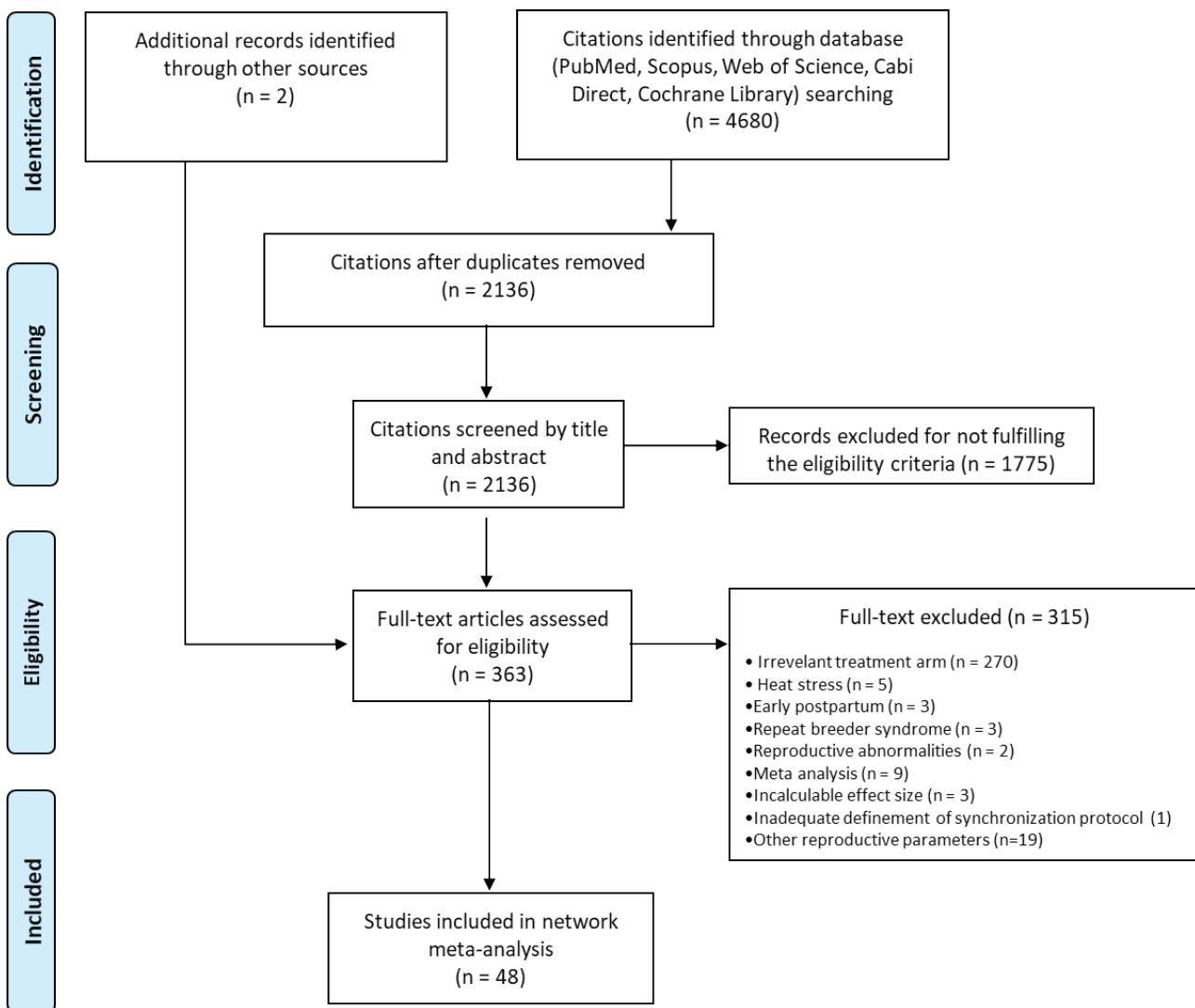
**Figure 1.** Flow chart
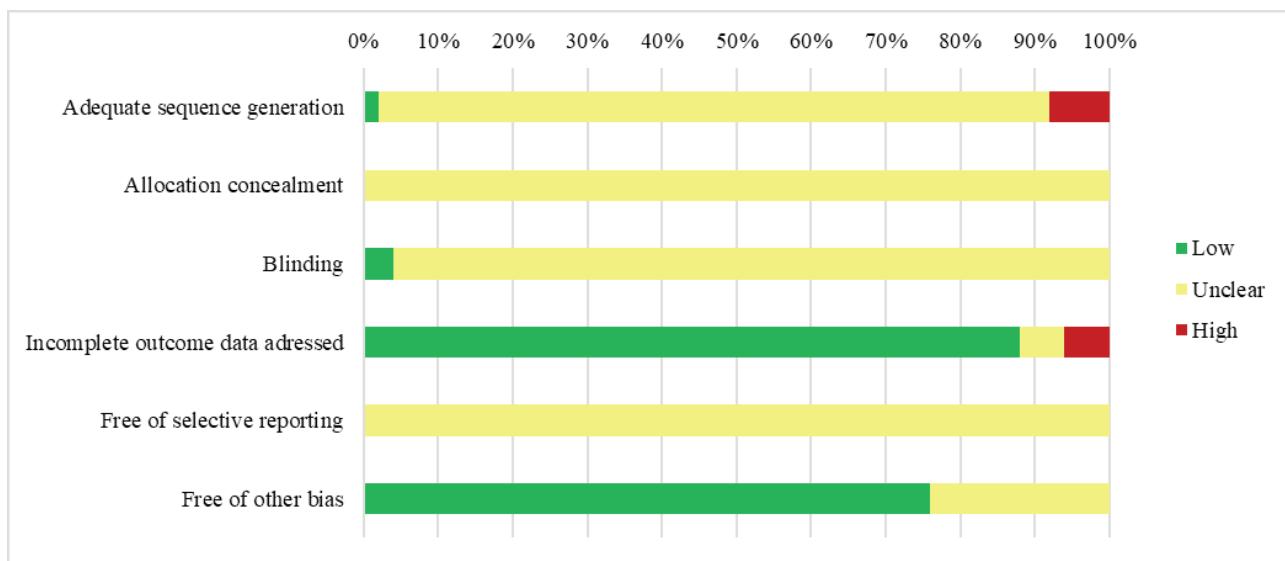


**Figure 2.** Risk of bias assessment

sessment for every study, we used the following algorithm: 1) A study was scored as high risk of bias if at least two of the items were scored as high risk of bias and less than two of the items were scored as low risk of bias, 2) A study was scored as low risk of bias if at least three of the items were scored as low risk of bias and none of the items were scored as high risk of bias, 3) Otherwise we scored as unclear risk of bias. Consequently, two studies were scored as low risk of bias out of 48 trials and the rest was at unclear risk of bias.

**Synthesis of the results**

The overall results of the random effects pair-wise meta-analysis were reported for pregnancy rate in Table S2. We presented the geometry of the networks in network plots (Fig 3). We investigated studies to check the transitivity assumption and didn't observe any incompatibility among the potential effect modifiers which are stated commonly in the included studies.

The total sample size was 19.416 cows and heifers. Cosynch, Heatsynch, Ovsynch, Presynch, Presynch+Heatsynch, Presynch+Ovsynch, Presynch+Selectsynch, Selectsynch, and control group were compared in the pooled analysis. We obtained a connected network with ten loops, in which direct and indirect estimates can be compared (Fig 3). According to the size of nodes, Ovsynch was the most frequently compared

synchronisation protocol and according to the thickness of edges Control vs. Ovsynch was the most common comparison. There was no significant difference noted between control group and any synchronisation protocols for the pregnancy rate (Table 1). Presynch+Ovsynch and Presynch+Heatsynch were significantly better than Presynch+Selectsynch [RR=2.01, 95% CI (1.03-3.93); RR=1.91, 95% CI (1.21-3.01); respectively]. Additionally, Presynch+Ovsynch was significantly superior to Ovsynch [(RR=1.21, 95% CI (1.04-1.40)]. Presynch+Ovsynch had the highest probability of being the best protocol with respect to the SUCRA values and rankogram (Fig 4, Fig S1). The global test for inconsistency was significant ($\chi^2$ (20)=32.07, p=0.043). According to the loop-specific approach, we observed inconsistency in two closed loops (Table S3). We performed a sensitivity analysis by excluding two studies (Momcilovic, 1998; Karimi et al., 2007), according to both global ($\chi^2$ (16)=12.23, p=0.728) and local approaches, the new model was consistent. In the sensitivity analysis, there were not any discrepancies neither in the differences between treatments nor in the hierarchy of treatments. Also another sensitivity analysis was performed by excluding those studies, or subgroups within studies, which are conducted with
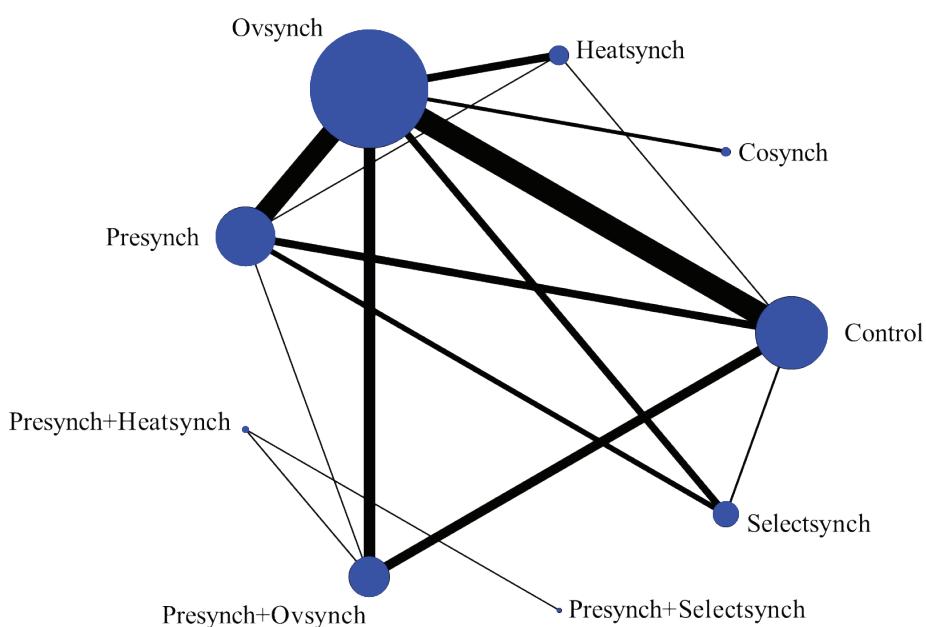


**Figure 3.** Network plot of synchronisation protocols in pregnancy rate
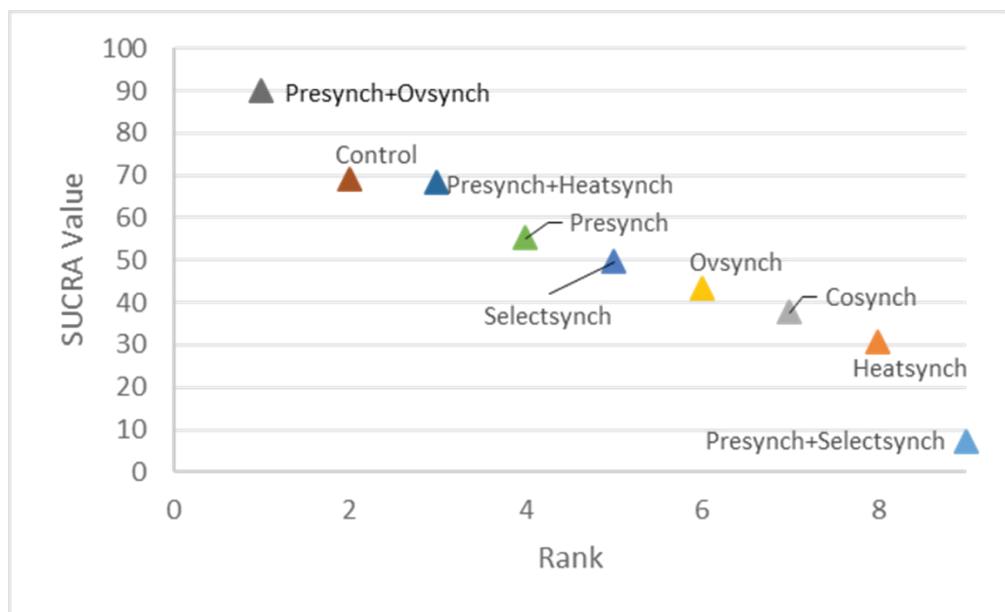
**Figure 4.** Ranking of synchronisation protocols in pregnancy rate

heifers and we did not observe any notable difference in results (King and Robertson, 1974; Stevenson et al., 2000, 2008; Iwakuma et al., 2008; Karakas et al., 2009; Nak et al., 2009; Gordon et al., 2010). In another sensitivity analysis that we performed excluding the studies published in Turkish journals in Turkish language, we did not encounter any different results compared to the primary model (Aksu, 2010; Bülbül and Ataman, 2005; Kaçar, 2008). Thus, we could achieve more evidence by including the studies published in Turkish language along with the studies published in English.

**DISCUSSION**

This study assessed the effectiveness of synchronisation protocols on pregnancy rate in dairy cows and heifers by using NMA. We systematically reviewed the trials comparing the synchronisation protocols and enrolled 48 trials involving 19.416 dairy cows and heifers for pregnancy rate. The major findings of this NMA suggested that Presynch+Ovsynch, control group, and Presynch+Heatsynch protocols were ranked as the best three protocols respectively in terms of pregnancy rate. It is crucial to state that the best three protocols were statistically comparable.

Although an NMA study does not exist to discuss the indirect evidence of our study, there is a small number of pair-wise meta-analysis that have considered the efficacy of synchronisation protocols in dairy cows (Rabiee et al., 2004, Rabiee et al., 2005, Bisinotto et al., 2015, Yan et al., 2016, Borchardt et al., 2017). In present NMA, the Presynch+Ovsynch protocol and control group was significantly superior to Ovsynch while other synchronisation protocols were comparable to Ovsynch in terms of pregnancy rate. In our study, the superiority of Presynch+Ovsynch to Ovsynch might be related to the advantage of presynchronisation in determination of the cows in herd are cyclic and also increasing the percentage of cows that ovulate responding to the first GnRH injection (Tibary et al., 2019). With the application of presynchronisation 12-14 days before the Ovsynch protocol, it can be ensured that the animals are on the most appropriate days of estrus cycle. In this way, all the animals in the herd can be available to start Ovsynch protocol on the 5-12th days of estrus cycle (Gumen et al., 2012). Thus, they are prevented to show estrus before planned time and the old follicles causing low fertility are expelled by ovulation in the animals which are enrolled to the protocol on the 0-5th days of the cycle (Gumen et al., 2012; Bisinotto et al., 2014). Although we found that Presynch+Ovsynch differed from Ovsynch, Rabiee et al., (2005) indicated no significant difference between Ovsynch and modified Ovsynch protocols which comprised of Presynch+Ovsynch and Cosynch in their Bayesian pair-wise meta-analysis. This variation might be due to the combination of two protocols (Presynch+Ovsynch and Cosynch) as a single group (modified Ovsynch) in the study of Rabiee et

al., (2005), however, we included in the protocols as separate two groups.

Pregnancy rate in dairy cows and heifers was observed significantly lower in Presynch+Selectsynch compared to Presynch+Ovsynch and Presynch+Heatsynch in this NMA. Yet, this finding was provided from only one trial between Presynch+Selectsynch and Presynch+Heatsynch (Cerri et al., 2004). Additionally, there was not any direct evidence between Presynch+Selectsynch and Presynch+Ovsynch. This situation causes hesitance on the reliability of this finding.

In addition to these findings, another remarkable evidence of this NMA was that none of the synchronisation protocols were significantly superior to the control group in pregnancy rate at first insemination. With this data, the suggestion of Rabiee et al., (2005) which referred to the possibility of reproductive management of dairy cows without using the Ovsynch protocol could be generalized for all of the synchronisation protocols included in this NMA. Furthermore, comparable effects of these protocols with control group is compatible with the findings of (Pursley et al., 1997) in which they indicated that synchronisation protocols allow effective management without estrus detection.

To the best of our knowledge, this is the first NMA that compares the efficacy of multiple synchronisation protocols on pregnancy rate. This NMA gave us the opportunity to rank the synchronisation protocols in terms of pregnancy rate evaluated in this study. We performed a series of sensitivity analyses to explore and conclude robustness of the NMA results

Besides these strengths, there are several limitations which should be taken into consideration in our study. First, we only included the conventionally used, timed AI-based protocols in which prostaglandin injections are applied. Unfortunately, we could not compare the programs applying ear implants or intravaginal devices, and also the programs becoming widespread in recent years such as PG-3-G or G-6-G. Second, because of the lack of information about the sources of bias in the individual trials included, most of the studies were under "unclear" risk of bias. Thus, the results should be interpreted considering the unclarity of risk of bias. Third, we observed that there was a deficiency in reporting confounders in the included studies. Therefore, we were not able to do the observational evaluation of the transitivity assumption of NMA. Nevertheless, this is common in most NMAs and we statistically confirmed consistency of direct and indirect evidence as a proxy for the transitivity assumption. Correspondingly, in the sensitivity analysis that we performed in this NMA, there was no information available that we could use to evaluate whether the excluded studies gave different results due to the presence of any type of effect modifiers. Fourth, we analyzed cows and heifers together in this NMA. But we did a sensitivity analysis that we excluded the studies or trials conducted only on heifers and had a model constructed with only the studies or trials conducted with cows, because it is known that cows and heifers have different reproductive characteristics (Abe et al., 2009). And consequently, we did not observe any significant differences in the results of the sensitivity analysis with the data of cows compared to the primary analysis with the data of both cows and heifers.

## CONCLUSIONS

In conclusion, it is clearly seen that the presynchronisation applications before Ovsynch and Heatsynch protocols were more efficient than other synchronisation programs on pregnancy rate at first insemination. However, it is challenging to advise using presynchronisation methods as in Presynch-Ovsynch, or Presynch-Heatsynch due to the extra requirements such as time, cost and labor. Moreover, the synchronisation of ovulation enables the overall herd management by eliminating the estrus detection, although the non-superiority of the synchronisation protocols against the control group with no hormonal treatment might be perceived as a disadvantage. In future studies, in addition to the determination of the effectiveness of reproduction performance, cost-effectiveness analysis might be performed in order to consider the profitability of dairy farms.

## CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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